MINISTRY OF HEALTH OF UKRAINE

ODESA NATIONAL MEDICAL UNIVERSITY

Faculty of **Dentistry**

Department of **Histology, Cytology, Embryology and Pathological Morphology with a course in Forensic Medicine**

APPROVED

Vice-rector for scientific and pedagogical work

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METHODOLOGICAL RECOMMENDATION FOR INDEPENDENT WORK OF STUDENTS OF HIGHER EDUCATION FROM EDUCATIONAL DISCIPLINE

Faculty of Dentistry, course 1, 2

Educational discipline - **"Histology, cytology and embryology."**

Approved:

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Step by Step – How to Use a Compound Microscope

1. Turn the Objective Lenses so that the longest lens (the lowest power one) is in viewing position. Be sure that there is room for it to move into place. Lower the Mechanical stage to make more room if needed.
2. Place the slide on the Mechanical stage and fasten it with the stage clips.
3. Look at the side of the microscope and turn the Adjustment knob until the lens is very close to, but not touching, the slide.
4. Look through the eyepiece and move the Adjustment knob so that the lens lifts away from the slide. The image should come into focus. Be careful not to drop the lens into the face of the slide, as this may cause damage to the lens.
5. The condenser can be adjusted to increase or decrease light intensity. You will usually want the most light possible for clearer viewing, but with low power objective lenses you may need to decrease the light.
6. The slide can be moved around to center the desired image in the field of view.
7. Once you have a clear image with the low-power objective, you may want to switch to a higher power one by switching the objective lens. Because they are shorter, you don't need to worry about turning them into the face of the slide and causing damage. This is one of the reasons we start with the lower ones.
8. You may need to slightly adjust the focus and centering of the object you are viewing. If you try this, and it doesn't seem to come into focus, then drop the lens to very near, but not touching, the slide (look from beside the microscope to do this, not through the eye piece) and then look through the eyepiece while you slowly raise the lens away from the slide. At some point, it will come into focus for you.
9. When you have finished viewing the slide, lower the Mechanical stage using the Adjustment knob, click the low power lens into viewing position (in preparation for next time), and remove the slide (by pressing on the ends of the clips to release it).

Steps on How to Use a Light Microscope

 Step 1: If your microscope uses a mirror instead of an illuminator, you can skip this step. Instead, find a place where natural light is easily accessible

 Step 2: Turn the revolving nosepiece so the lowest objective lens is in position.

 Step 3: Mount your specimen onto the stage. But before doing so, see to it that your specimen is adequately protected by placing a coverslip on top of it.

 Step 4: Use the metal clips to keep your slide in place. Make sure the specimen is positioned in the center, right under the lowest objective lens.

 Step 5: Look into the eyepiece and slowly rotate the coarse adjustment knob to bring your specimen to focus. See to it that the slide does not touch the lens.

 Step 6: Adjust the condenser for the maximum amount of light. Since you're on the low power objective, you may have to decrease the illumination. Use the diaphragm under the stage to adjust.

 Step 7: Now slowly rotate the fine adjustment knob until you obtain a clearer image of your specimen.

 Step 8: Examine your specimen.

 Step 9: After you're done viewing with the lowest power objective, switch to the medium power objective and re-adjust the focus with the fine adjustment knob.

 Step 10: Proceed to the high power objective once you have it focused.

THE METHODS OF INVESTIGATION IN HISTOLOGY.

HISTOLOGICAL TECHNIQUE

Histology

is the study of the structure and function of cells, tissues, and organs of the body at the microscopic level.

Histology involves all aspects of tissue biology, with the focus on how cells' structure and arrangement optimize functions specific to each organ.

Each of the fundamental tissues is formed by several types of cells and typically by specific associations of cells and extracellular matrix. Most organs are formed by an orderly combination of several tissues, except the central nervous system, which is formed almost solely by nervous tissue. The precise combination of these tissues allows the functioning of each organ and of the organism as a whole. The small size of cells and matrix components makes histology dependent on the use of microscopes. Advances in chemistry, molecular biology, physiology, immunology, and pathology—and the interactions among these fields—are essential for a better knowledge of tissue biology.

Knowledge of histology shows how different cells are organized to form tissues and how each cell and tissue show modification according to their functional demands. Knowledge of histology is gaining importance in diagnosis of certain diseases.

Methods of study

Microscopes allow researchers to see cellular and macromolecular details that are not visible to the naked eye.

1. Light microscopes employ transillumination and are used to examine living and prepared specimens and specimens with inherent or applied fluorescent properties.

2. Electron microscopes illuminate specimens with an electron beam. They have 1000 times the resolving power of light microscopes and provide resolution to the threshold of atomic detail.

a. Transmission electron microscopy (TEM) uses thin specimen sections and reveals detail of the cell's interior.

b. Scanning electron microscopy (SEM) provides a high resolution view of the cell surface and environment.

Light Microscopy

Conventional bright-field microscopy, as well as fluorescence, phasecontrast,

differential interference, confocal, and polarizing microscopy are all based on the interaction of light and tissue components and can be used to reveal and study tissue features. With the bright-field microscope, widely used by students of histology, stained preparations are examined by means of ordinary light that passes through the specimen. The microscope is composed of mechanical and optical parts. The optical components consist of three systems of lenses.

The condenser collects and focuses light, producing a cone of light that illuminates the object to be observed. The objective lenses enlarge and project the illuminated image of the object in the direction of the eyepiece. The eyepiece or ocular lens further magnifies this image and projects it onto the viewer's retina, photographic film, or (to obtain a digital image) a detector such as a chargecoupled device (CCD) camera.

The total magnification is obtained by multiplying the magnifying power of the objective and ocular lenses. For routine histological studies objectives having three different magnifications are generally used: x4 for low magnification observations of a large area (field) of the tissue; x10 for medium magnification of a smaller field; and x40 for high magnification of more detailed areas. The eyepiece or ocular has further magnifies this image another x10 and projects it onto the viewer's retina. The critical factor in obtaining a crisp, detailed image with a light microscope is its resolving power, defined as the smallest distance between two particles at which they can be seen as separate objects. The maximal resolving power of the light microscope is approximately 0.2 μm; this power permits good images magnified 1000–1500 times.

Objects smaller or thinner than 0.2 μm (such as a ribosome, a membrane, or a

filament of actin) cannot be distinguished with this instrument. Likewise, two objects

such as mitochondria will be seen as only one object if they are separated by less than

0.2 μm. The resolving power of a microscope depends mainly on the quality of its

objective lens. The eyepiece lens enlarges only the image obtained by the objective; it

does not improve resolution.

For this reason, when comparing objectives of different magnifications, those that

provide higher magnification also have higher resolving power.

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differential interference, confocal, and polarizing microscopy are all based on the

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Bright-Field Microscopy

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preparations are examined by means of ordinary light that passes through the

specimen.

Fluorescence Microscopy

When certain substances are irradiated by light of a proper wavelength, they emit

light with a longer wavelength. This phenomenon is called fluorescence. In

fluorescence microscopy, tissue sections are usually irradiated with ultraviolet (UV)

light and the emission is in the visible portion of the spectrum. The fluorescent

substances appear brilliant on a dark background. For this method, the microscope

has a strong UV light source and special filters that select rays of different

wavelengths emitted by the substances.

Phase-Contrast Microscopy

Some optical arrangements allow the observation of unstained cells and tissue

sections. Unstained biological specimens are usually transparent and difficult to view

in detail, because all parts of the specimen have almost the same optical density.

Phase-contrast microscopy, however, uses a lens system that produces visible images

from transparent objects. A related method of observing unstained cells or tissue

sections is the Nomarski differential interference microscopy, which produces an

image with a more apparent three-dimensional aspect than in routine phase-contrast

microscopy.

Polarizing Microscopy

Polarizing microscopy allows the recognition of structures made of highly organized

molecules. When normal light passes through a polarizing filter (such as a Polaroid),

it exits vibrating in only one direction. If a second filter is placed in the microscope

above the first one, with its main axis perpendicular to the first filter, no light passes

through. If, however, tissue structures containing oriented macromolecules are

located between the two polarizing filters, their repetitive structure rotates the axis of

the light emerging from the polarizer and they appear as bright structures against a

dark background.

Electron microscopy

Transmission and scanning electron microscopes are based on the interaction of

electrons and tissue components. The wavelength in the electron beam is much

shorter than of light, allowing a thousand-fold increase in resolution.

Steps needed to make and study a histological section

1. Fixation to prevent post-mortem decomposition, preserve structure, and intensify

subsequent staining. One of the best fixatives for routine light microscopy is

formalin, a buffered isotonic solution of 37% formaldehyde.

2. Dehydration. The fixed pieces is transferred through a series of increasingly more

concentrated alcohol solutions, ending in 100% which effectively removes all water

from the tissue. The ethanol is then replaced with a solvent miscible with both

alcohol and the embedding medium. As the tissues are infiltrated with this solvent, they generally become transparent (clearing).

3. Infiltration and embedding. Tissues are usually embedded in a solid medium to facilitate sectioning. Steps involved in embedding the tissue in a block of wax or plastic resin, or freezing of the material to a firm mass, for cutting into thin sections

on a microtome.

4. Sectioning. The hard blocks containing the tissues are then placed in an instrument

called a microtome and are sliced by the microtome's steel or glass blade into sections 1 to 10 micrometer thick. The sections are floated on water and then transferred to glass slides to be stained.

5. Staining. To be studied microscopically sections must typically be stained or dyed

because most tissues are colorless. Staining of the section with one or more reagents,

e.g., solutions of metallic salts, in one or more stages. The dyes stain tissue components more or less selectively. Most of these dyes behave like acidic or basic compounds and have a tendency to form electrostatic (salt) linkages with ionizable radicals of the tissues. Tissue components with a net negative charge (anionic) stain

more readily with basic dyes and are termed basophilic;cationic components, such as

proteins with many ionized amino groups, have affinity for acidic dyes and are termed acidophilic. Examples of basic dyes are toluidine blue, alcian blue, and methylene blue. Hematoxylin behaves like a basic dye, that is, it stains the basophilic

tissue components. The main tissue components that ionize and react with basic dyes

do so because of acids in their composition (nucleic acids, glycosaminoglycans, and

acid glycoproteins). Acid dyes (eg, orange G, eosin, acid fuchsin) stain the

acidophilic components of tissues such as mitochondria, secretory granules, and

collagen. Of all dyes, the simple combination of hematoxylin and eosin (H&E) is

used most commonly.

6. For light microscopy, the removal of surplus stain and water, and steps involved in

holding a thin glass coverslip to the section with a mounting medium having a

refractive index close to that of glass.

Histochemistry & Cytochemistry

The terms histochemistry and cytochemistry indicate methods for localizing cellular

structures in tissue sections using unique enzymatic activity present in those

structures.

Immunohistochemistry

A highly specific interaction between molecules is that between an antigen and its

antibody. For this reason, methods using labeled antibodies have become extremely

useful in identifying and localizing many specific proteins, not just those with

enzymatic activity that can be demonstrated by histochemistry. The body's immune

cells are able to discriminate its own molecules (self) from foreign ones.

When exposed to foreign molecules—called antigens—the body responds by

producing antibodies that react specifically and bind to the antigen, thus helping to

eliminate the foreign substance. Antibodies belong to the immunoglobulin family of

glycoproteins, produced by lymphocytes.

Autoradiography

Autoradiography is a method of localizing newly synthesized macromolecules (DNA,

RNA, protein, glycoproteins, and polysaccharides) in cells or tissue sections. Radioactively labeled metabolites (nucleotides, amino acids) incorporated into the macromolecules emit weak radiation that is restricted to the cellular regions where the molecules are located. Much information becomes available by autoradiography

of cells or tissues. Thus, if a radioactive amino acid is used, it is possible to know which cells in a tissue produce more protein and which cells produce less. If a radioactive precursor of DNA (such as tritium-labeled thymidine) is used, it is possible to know which cells in a tissue (and how many) are preparing to divide.

MECHANISMS OF RECEPTION

Some proprioceptors (internal receptors) for mechanical stimuli provide information

about posture and movements of parts of the body relative to each other; others contribute to an undisturbed course of coordinated muscular actions (e.g., in locomotion). Best known from studies of vertebrates and arthropods, some are tonic

proprioceptors (serving to maintain muscle tone in posture); others are of the phasic

type (serving movement); still others have a mixed phasic-tonic character. In principle, proprioceptors can be stimulated adequately by pressure or stretching during active movements of the animal (reafferent stimulation) as well as through passive external pushing and pulling (exafferent stimulation). One passive factor, particularly in land-inhabiting animals, is gravity as it acts on bodily tissues or organs. Proprioceptors thus not only serve reflex adjustments in posture and relatively automatic movements of parts of the body with respect to each other (as in

driving an automobile), but they also provide gravitational information about the positions of limbs or of the whole body in space. To the extent that they are gravity detectors, these sensory structures are properly called external receptors (exteroceptors instead of proprioceptors). For receptors that are diffusely located

within the body, a clean distinction between proprioceptive and possible exteroceptive function (gravity reception) is experimentally practicable only under conditions of weightlessness, as in space travel.

Well-known proprioceptors of all the four-limbed vertebrates studied are the muscle spindles occurring in the skeletal (striate) muscles; fish muscles show structurally simpler but functionally comparable receptors. Each muscle spindle in mammals consists of a few slender, specialized (intrafusal) muscle fibres that are surrounded by a sheath of connective tissue filled with lymph fluid. The muscle spindle itself is surrounded by and arranged parallel to the ordinary (extrafusal) muscle fibres. Each intrafusal fibre consists of contractile (motor) parts at both ends and a noncontractile sensory midsection that serves as a receptor for stretch (changes of length and tension). There is double (primary and secondary) sensory innervation in mammals, but the secondary endings are lacking in lower vertebrates. Even when the animal is at rest, both types of endings are active (under the tension of normal muscle tonus). Additional stretch (lengthening) of the intrafusal midsection increases the nerve impulse frequency, and relaxation (shortening) causes a decrease. The primary (phasic-tonic) ending responds quickly; responses of the secondary (tonic) endings are slower.

The length of the muscle spindle as a whole varies with the contraction phase and the length of the muscle to which it belongs. The length of the sensory midsection, however, may change more or less independently because its motor nerve endings function apart from the innervation of the extrafusal muscle fibres. Thus the ratio of extrafusal–intrafusal contraction determines whether or not a change of length in the midsection will occur during muscle activity. There are reasons to suppose that

midsection stretch remains more or less unchanged during self-initiated ("voluntary")

movements; reafferent stimulation of muscle spindles would be avoided in this way.

But as soon as an unexpected (exafferent) stretch of a muscle occurs—for example,

when a leg pushes against an obstacle during locomotion—the midsections stretch to

produce an increase of impulse frequency.

This neural activity elicits a compensatory reflex contraction of the stretched muscle,

as in the knee jerk during medical examinations: a blow beneath the kneecap causes

stretch of a thigh muscle, stimulation of its muscle spindles, and a compensatory

jerking contraction of the same muscle. Knee-jerk reflex and motor-neuron

connection.

Branched nerve endings on vertebrate tendons (not far from their point of attachment

to muscle) also respond to stretch; however, they are decidedly less sensitive than are

muscle spindles. These tendon organs produce no impulses under the stretch of

normal, resting muscle tonus. Neither is there a mechanism preventing reafferent

stimulation of tendon organs, nor does it make any difference whether the stretch is

brought about by active muscle contraction or passively following external influence.

In both cases tendon receptors respond according to the intensity of the stretch; their

response causes relaxation of the attached muscle and may serve (among other

functions) to prevent anatomical damage.

Human awareness of posture and movement of parts of the body with respect to each

other (kinesthetic sensations) is attributable neither to muscle spindles nor to tendon

organs. The sensations are based on stimulation of sensory nerve endings of various types at the joint capsules and of stretch receptors in the skin. There are also mechanoreceptors in the walls of some blood vessels (e.g., in the aorta and the carotid sinus); these are sensitive to blood-pressure changes and play a regulatory role in the circulatory system.

Invertebrates. Among invertebrates, the arthropods exhibit the most readily distinguished proprioceptors, called muscle-receptor organs and chordotonal proprioceptors. Both types of structure occur in crustaceans as well as in insects. Adequate stimuli are variations in length and tension (stretch).

Muscle receptor organs

Although they structurally and functionally resemble the muscle spindles of vertebrates, arthropod muscle receptor organs are always situated outside of the muscles proper. Numerous branches of multipolar primary nerve cells are connected with the noncontractile midsection of specialized muscle fibres, both ends of which are contractile and have an efferent (motor) innervation. In crustaceans, the muscle receptor organ contains two elements: a slowly contracting, nonadapting tonic fibre and a quickly contracting, rapidly adapting phasic element.

Chordotonal proprioceptors

Widely distributed among arthropods, chordotonal receptor organs are thin, elastic, innervated strands of connective tissue, stretched between adjacent segments of the body or of leg joints. The sensory endings of a few bipolar primary nerve cells, each provided with a spiny sensillum (scolopidium), are attached to the strand.

Chordotonal proprioceptor organs generate neural impulses that show them to contain both phasic movement receptors and tonic pressure receptors; sometimes two varieties of each. Thus there are receptors that selectively respond only during

flexion, only in the flexed position, only during stretch, or only in the stretched state

of the given strand. Several kinds of insects, apart from their clearly proprioceptive-

chordotonal functions, have other chordotonal elements that serve as typical

exteroceptors. Sense organs of this type (tympanic and subgenual organs in legs,

Johnston's organs in the antennae) may function in the reception of sound waves, of

vibrations in the ground, or of other external mechanical stimuli. Many insects also

have a special type of chordotonal-proprioceptor structure (campaniform sensilla) not

found in crustaceans. Sensory endings of primary nerve cells are connected with thin,

dome-shaped (campaniform) spots on the exoskeleton. These campaniform sensilla

respond to external stimuli such as local tensions and deformations of the body

surface. They function in the regulation of such movements as the beating of wings in

locusts. Similarly functioning proprioceptors (lyriform organs) are also observed

among spiders.

MITOSIS AND MEIOSIS.

Multiplication of cells takes place by division of pre-existing cells. Such

multiplication constitutes an essential feature of embryonic development. Cell

multiplication is equally necessary after birth of the individual for growth and for

replacement of dead cells.

We have seen that the chromosomes within of nuclei of cells carry genetic

information that call trolls the development and functioning of vat rows cells and

tissues — and, therefore, of the body as a whole. When a cell divides it is essential

that the whole of the genetic information within it be passed on to both the daughter

cells resulting from the division. In other words the daughter cells must have

chromosomes identical in number and in genetic content to those in the mother cell.

This type of cell division is called mitosis.

A different kind of cell division called meiosis occurs during the formation of gametes. This consists of two successive divisions called the first and second meiotic divisions. The cells resulting from these divisions (i.e., the gametes) differ from other cells in the body in that

(a) the number of chromosomes is reduced to half the normal number, and

(b) the genetic information in the various gametes produced is not identical.

Mitosis

Many cells of the body have a limited span of functional activity at the end of which they undergo division into two daughter cells. The daughters cells have their own span of activity followed by another division. The period during which the cell is actively dividing is the phase of mitosis. The period between two successive divisions is called the interphase.

Phases of mitosis

In prophase of mitosis the replicated chromatin condenses into discrete rodshaped bodies, the chromosomes, each consisting of duplicate sister chromatids closely associated longitudinally. The centrosomes with their centrioles separate and migrate to opposite poles of the cell. The duplication of the centrosomes and centrioles occurs during interphase. Simultaneously with the centrosomes the microtubules of the mitotic spindle appear between the two centrosomes and the nucleolus disappears as transcriptional activity there stops. Late in prophase, the nuclear envelope breaks of the nuclear lamina and inner membrane are 3–phosphorylated (PO4 groups added). The nuclear lamina and pore complexes disassemble.

During metaphase, the condensed chromosomes attach to microtubules of the mitotic spindle at large electron-dense protein complexes called kinetochores, which are

located at a constricted region of each chromatid called the centromere. The chromosomes are moved to the equatorial part of cell. Kinetochore microtubules bound to sister chromatids are continuous with centrosomes at opposite poles of the mitotic spindle.

In anaphase, the sister chromatids separate from each other and are slowly pulled at their kinetochores toward opposite spindle poles by kinesin motors moving along the microtubules. During spindle poles also move farther apart.

At telophase the two sets of chromosomes are at the spindle poles and begin reverting to their decondensed state. Microtubules of the spindle depolymerize and

the nuclear envelope reassembles. A belt-like contractile ring, containing actin filaments associated with myosins, develops in the peripheral cytoplasm at the equator of the parent cell. During the end of telophase, constriction of this ring produces a cleavage furrow and progresses until the cytoplasm and its organelles are divided in two daughter cells, each with one nucleus.

Cell Cycle

The cyclic alternation between mitosis and interphase, known as the cell cycle. The cell cycle has four distinct phases: mitosis, and three interphase periods termed G1 (the time gap between mitosis and DNA replication), S (the period of DNA synthesis), and G2 (the gap between duplication and the next mitosis). Cell cycle has two principal phases: mitosis (M phase) and interphase. Three other phases, gap1, (G,), synthesis phase (S), and gap2. Mitosis nearly always includes both karyokinesis (division of the nucleus into two daughter nuclei) and cytokinesis (division of the cell into two daughter cells) and lasts about 1 hour. It is usually followed by G1, a period in which no DNA synthesis occurs. G1, is usually a period of cell growth and may

last only a few hours in a rapidly dividing cell or may last a lifetime in a nondividing

cell. A cell that leaves the cycle in G1, to begin "terminal" differentiation enters the

G0 phase, "O" for "outside" the cycle. The S or DNA synthesis phase follows G1, and

usually lasts about 7 hours. The DNA of the cell is doubled during the S phase, and

new chromatids are formed that will become obvious at prophase or metaphase of the

next M phase. The brevity of the S phase allows the use of tritiated thymidine to label

only those cells engaged in DNA synthesis at the time the radioactively labeled

nucleotide is present. The S phase is also followed by a period in which no DNA

synthesis occurs, a second gap or G2 phase. G2 may be as short as 1 hour in rapidly

dividing cells or of nearly indefinite duration in some polyploid cells and in cells,

such as the primary oocyte, that are arrested in G2 for extended periods. Cells

identified as reserve stem cells may be thought of as G0 cells that may be induced to

reenter the cell cycle in response to injury of the cell populations within the tissues of

the body. Activation of these cells may occur in normal wound healing and in the

repopulation of the seminiferous epithelium after intense acute exposure of the testis

to x-irradiation or during regeneration of an organ, such as the liver, after removal of

a major portion. If the damage to the tissues is too severe, even the reserve stem cells

die, and there is no potential for regeneration.

Meiosis

As already stated meiosis consists of two successive divisions called the first and

second meiotic divisions. During the interphase preceding the first division

duplication of the DNA content of the chromosomes takes place as in mitosis.

First Meiotic Division

The prophase of the first meiotic division is prolonged and is usually divided into a number of stages as follows.

Leptotene: The chromosomes become visible (as in mitosis). Although each chromosome consists of two chromatids these cannot be distinguished at this stage. At first the chromosomes are seen as threads bearing bead-like thickenings (cliromomeres) along their length. One end of the thread is attached to the nuclear membrane. During leptotene the chromosomes gradually become thicker and shorter.

Zygotene: We have seen that the 46 chromosomes in each cell consist of pairs (the X and Y chromosomes of the male being taken as a pair). The two chromosomes of each pair come to lie parallel to each other, and are closely apposed. This pairing of chromosomes is also referred to as synopsis or conjugation The two chromosomes together constitute a bivalent.

Pachytene: The two chromatids of each chromosome become distinct. The bivalent now has four chromatids in it and is called a tetrad. There are two central and two peripheral chromatids, one from each chromosome. An important event now takes place. The two central chromatids (one belonging to each chromosome of the bivalent) become coiled over each other so that they cross at a number of points. This is called crossing over. At the site where the chromatids cross they become adherent:

the points of adhesion are called chiasmata.

Diplotene: The two chromosomes of a bivalent now try to move apart. As they do so the chromatids 'break' at the points of crossing and the 'loose' pieces become attached to the opposite chromatid. This results in exchange of genetic material between these chromatids. A study of that as a result of this crossing over of genetic material each of the four chromatids of the tetrad now has a distinctive genetic content.

The metaphase follows. As in mitosis the 46 chromosomes become attached to the spindle at the equator, the two chromosomes of a pair being close to each other.

The anaphase differs from that in mitosis in that there is no splitting of the centromeres. One entire chromosome of each pair moves to each pole of the spindle.

The resulting daughter cells, therefore, have 23 chromosomes, each made up of two chromatids.

The telophase is similar to that in mitosis. The first meiotic division is followed by a short interphase. This differs from the usual interphase in that there is no duplication of DNA. Such duplication is unnecessary as the chromosomes of the cells resulting from the first meiotic division already possess two chromatids each.

Second Meiotic Division

The second meiotic division is similar to mitosis. However, because of the crossing over that has occurred during the first division, the daughter cells are not identical in genetic content. This is the reason for regarding it as a meiotic division. At this stage it may be repeated that the 46 chromosomes of a cell consist of 23 pairs, one chromosome of each pair being derived from the mother and one from the father.

During the first meiotic division the chromosomes derived from the father and those derived from the mother are distributed between the daughter cells entirely at random. This, along with the phenomenon of crossing over, results in thorough shuffling of the genetic material so that the cells produced as a result of various meiotic divisions (i.e., ova and spermatozoa) all have a distinct genetic content. A third step in this process of genetic shuffling takes place at fertilization when there is a combination of randomly selected spermatozoa and ova. It is, therefore, not surprising that no two persons (except identical twins) are alike.

Amitosis.

Amitotic division at the person meets in cells of a liver and in the epithelium of urinary system. At this way of division does not occur spiralization of chromosomes and the genetic material is divided any way. Distinguish two stages of amitotic division – the kariotomia and the cytoplasmotomia.

THE REACTION OF CELLS TO STIMULI

Homeostatic imbalances

Homeostatic imbalances are the main driving force for changes in the body. These stimuli are monitored closely by receptors and sensors in different parts of the body.

These sensors are mechanoreceptors, chemoreceptors and thermoreceptors that, respectively, respond to pressure or stretching, chemical changes, or temperature changes. Examples of mechanoreceptors include baroreceptors which detect changes in blood pressure, Merkel's discs which detect sustained touch and pressure, and hair cells which detect sound stimuli. Homeostatic imbalances that can serve as internal stimuli include nutrient and ion levels in the blood, oxygen levels, and water levels.

Deviations from the homeostatic ideal may generate a homeostatic emotion, such as pain, thirst or fatigue, that motivates behavior that will restore the body to stasis (such as [1] withdrawal, drinking or resting).

Blood pressure

Blood pressure, heart rate, and cardiac output are measured by stretch receptors found in the carotid arteries. Nerves embed themselves within these receptors and when they detect stretching, they are stimulated and fire action potentials to the central nervous system. These impulses inhibit the constriction of blood vessels and lower the heart rate. If these nerves do not detect stretching, the body determines perceives

low blood pressure as a dangerous stimulus and signals are not sent, preventing the inhibition CNS action; blood vessels constrict and the heart rate increases, causing an increase in blood pressure in the [2] body. External stimuli

Touch and pain

Sensory feelings, especially pain, are stimuli that can elicit a large response and cause neurological changes in the body. Pain also causes a behavioral change in the body, which is proportional to the intensity of the pain. The feeling is recorded by sensory receptors on the skin and travels to the central nervous system, where it is integrated and a decision on how to respond is made; if it is decided that a response must be made, a signal is sent back down to a muscle, which acts [1] appropriately according to the stimulus. The postcentral gyrus is the location of the primary somatosensory area, the main sensory receptive area for the sense of [3] touch.

Pain receptors are known as nociceptors. Two main types of nociceptors exist, A-fiber nociceptors and C-fiber nociceptors. A-fiber receptors are myelinated and conduct currents rapidly. They are mainly used to conduct fast and sharp types of pain. Conversely, C-fiber receptors are unmyelinated and slowly transmit. These receptors conduct slow, burning, diffuse pain. The absolute threshold for touch is the minimum amount of sensation needed to elicit a response from touch receptors. This amount of sensation has a definable value and is often considered to be the force exerted by dropping the wing of a bee onto your cheek from a distance of one centimeter. This value will change based on the body part being touched.

Vision

Vision provides opportunity for the brain to perceive and respond to changes occurring around the body. Information, or stimuli, in the form of light enters the retina, where it excites a special type of neuron called a photoreceptor cell. A local

graded potential begins in the photoreceptor, where it excites the cell enough for the

impulse to be passed along through a track of neurons to the central nervous system.

As the signal travels from photoreceptors to larger neurons, action potentials must be

created for the signal to have enough strength to reach the CNS. If the stimulus does

not warrant a strong enough response, it is said to not reach absolute threshold, and

the body does not react. However, if the stimulus is strong enough to create an action

potential in neurons away from the photoreceptor, the body will integrate the

information and react appropriately. Visual information is processed in the occipital

lobe of the CNS, specifically in the primary visual cortex.

The absolute threshold for vision is the minimum amount of sensation needed to elicit

a response from photoreceptors in the eye. This amount of sensation has a definable

value and is often considered to be the amount of light present from someone holding

up a single candle 30 miles away, if one's eyes were adjusted to the dark.

Smell

Smell allows the body to recognize chemical molecules in the air through

inhalation. Olfactory organs located on either side of the nasal septum consist of

olfactory epithelium and lamina propria. The olfactory epithelium, which

contains olfactory receptor cells, covers the inferior surface of the cribiform plate,

the superior portion of the perpendicular plate, the superior nasal concha. Only

roughly two percent of airborne compounds inhaled are carried to olfactory organs

as a small sample of the air being inhaled. Olfactory receptors extend past the

epithelial surface providing a base for many cilia that lie in the surrounding mucus.

Odorant-binding proteins interact with these cilia stimulating the receptors.

Odorants are generally small organic molecules. Greater water and lipid solubility is related directly to stronger smelling odorants. Odorant binding to G protein coupled receptors activates adenylate cyclase, which converts ATP to camp. cAMP, in turn, promotes the opening of sodium channels resulting in a localized potential.

The absolute threshold for smell is the minimum amount of sensation needed to elicit a response from receptors in the nose. This amount of sensation has a definable value and is often considered to be a single drop of perfume in a sixroom house. This value will change depending on what substance is being smelled.

Taste

Taste records flavoring of food and other materials that pass across the tongue and through the mouth. Gustatory cells are located on the surface of the tongue and adjacent portions of the pharynx and larynx. Gustatory cells form on taste buds, specialized epithelial cells, and are generally turned over every ten days. From each cell, protrudes microvilli, sometimes called taste hairs, through

also the taste pore and into the oral cavity. Dissolved chemicals interact with these receptor cells; different tastes bind to specific receptors. Salt and sour receptors are chemically gated ion channels, which depolarize the cell. Sweet, bitter, and umami receptors are called gustducins, specialized G protein coupled receptors. Both divisions of receptor cells release neurotransmitters to afferent fibers causing action potential firing.

The absolute threshold for taste is the minimum amount of sensation needed to elicit a response from receptors in the mouth. This amount of sensation has a definable value and is often considered to be a single drop of quinine sulfate in 250

gallons of water.

Sound

Changes in pressure caused by sound reaching the external ear resonate in

the tympanic membrane, which articulates with the auditory ossicles, or the bones of the middle ear. These tiny bones multiply these pressure fluctuations as they pass the disturbance into the cochlea, a spiral-shaped bony structure within the inner ear. Hair cells in the cochlear duct, specifically the organ of Corti, are deflected as waves of fluid and membrane motion travel through the chambers of the cochlea. Bipolar sensory neurons located in the center of the cochlea monitor the information from these receptor cells and pass it on to the brainstem via the cochlear branch of cranial nerve VIII. Sound information is processed in the temportal lobe of the CNS, specifically in the primary auditory cortex.

The absolute threshold for sound is the minimum amount of sensation needed to elicit a response from receptors in the ears. This amount of sensation has a definable value and is often considered to be a watch ticking in an otherwise soundless environment 20 feet away.

Equilibrium

Semi circular ducts, which are connected directly to the cochlea, can interpret and convey to the brain information about equilibrium by a similar method as the one used for hearing. Hair cells in these parts of the ear protrude kinocilia and stereocilia into a gelatinous material that lines the ducts of this canal. In parts of these semi circular canals, specifically the maculae, calcium carbonate crystals known as statoconia rest on the surface of this gelatinous material. When tilting the head or when the body undergoes linear acceleration, these crystals move disturbing the cilia of the hair cells and, consequently, affecting the release of neurotransmitter to be taken up by surrounding sensory nerves. In other areas of the semi circular canal, specifically the ampulla, a structure known as the cupula—analogous to the gelatinous material in the maculae—distorts hair cells in a similar fashion when the fluid medium that surrounds it causes the cupula itself to move. The ampulla communicates to the brain information about the head’s horizontal rotation. Neurons of the adjacent vestibular ganglia monitor the hair cells in these ducts. These sensory fibers form the vestibular branch of the cranial nerve

Cellular response to stimuli

In general, cellular response to stimuli is defined as a change in state or activity of a cell in terms of movement, secretion, enzyme production, or gene expression.

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Receptors on cell surfaces are sensing components that monitorstimuli and respond to changes in the environment by relaying the signal to a control center for further processing and response. Stimuli are always converted into electrical signals via transduction. This electrical signal, or receptor potential, takes a specific pathway through the nervous system to initiate a systematic response. Each type of receptor is specialized to respond preferentially to only one kind of stimulus energy, called the adequate stimulus. Sensory receptors have a well-defined range of stimuli to which they respond, and each is tuned to the particular needs of the organism. Stimuli are relayed throughout the body by mechanotransduction or chemotransduction, depending on the nature of the

stimulus.

Mechanical

In response to a mechanical stimulus, cellular sensors of force are proposed to be extracellular matrix molecules, cytoskeleton, transmembrane proteins, proteins at the membrane-phospholipid interface, elements of the nuclear matrix, chromatin, and the lipid bilayer. Response can be twofold: the extracellular matrix, for example, is a conductor of mechanical forces but its structure and composition is also influenced by the cellular responses to those same applied or endogenously generated forces.

Mechanosensitive ion channels are found in many cell types and it has been shown that the permeability of these channels to cations is affected by stretch receptors and

mechanical stimuli. This permeability of ion channels is the basis for the conversion

of the mechanical stimulus into an electrical signal.

Chemical

Chemical stimuli, such as odorants, are received by cellular receptors that are often coupled to ion channels responsible for chemotransduction. Such is the case in olfactory cells. Depolarization in these cells result from opening of non-selective cation channels upon binding of the odorant to the specific receptor. G protein-coupled receptors in the plasma membrane of these cells can initiate second messenger pathways that cause cation channels to open.

In response to stimuli, the sensory receptor initiates sensory transduction by creating graded potentials or action potentials in the same cell or in an adjacent one. Sensitivity to stimuli is obtained by chemical amplification through second messenger pathways in which enzymatic cascades produce large numbers of intermediate products, increasing the effect of one receptor molecule.

Systematic response to stimuli

Nervous-system response

Though receptors and stimuli are varied, most extrinsic stimuli first generate localized graded potentials in the neurons associated with the specific sensory organ

or

tissue. In the nervous system, internal and external stimuli can elicit two different categories of responses: an excitatory response, normally in the form of an action potential, and an inhibitory response.

When a neuron is stimulated

by an excitatory impulse, neuronal dendrites are bound by neurotransmitters which cause the cell to become permeable to a specific type of ion; the type of neurotransmitter determines to which ion the neurotransmitter will become permeable. In excitatory postsynaptic potentials, an excitatory response is generated. This is caused by an excitatory neurotransmitter,

normally glutamate binding to a neuron's dendrites, causing an influx of sodium

ions through channels located near the binding site.

This change in membrane permeability in the dendrites is known as a local

graded potential and causes the membrane voltage to change from a negative resting potential to a more positive voltage, a process known as depolarization. The opening of sodium channels allows nearby sodium channels to open, allowing the change in permeability to spread from the dendrites to the cell body. If a graded

potential is strong enough, or if several graded potentials occur in a fast enough frequency, the depolarization is able to spread across the cell body to the axon hillock. From the axon hillock, an action potential can be generated and propagated down the neuron's axon, causing sodium ion channels in the axon to open as the
impulse travels. Once the signal begins to travel down the axon, the membrane potential has already passed threshold, which means that it cannot be stopped. This phenomenon is known as an all-or-nothing response. Groups of sodium channels opened by the change in membrane potential strengthen the signal as it travels away from the axon hillock, allowing it to move the length of the axon. As the depolarization reaches the end of the axon, or the axon terminal, the end of the neuron becomes permeable to calcium ions, which enters the cell via calcium ion channels. Calcium causes the release of neurotransmitters stored in synaptic vesicles, which enter the synapse between two neurons known as the presynaptic and postsynaptic neurons; if the signal from the presynaptic neuron is excitatory, it will cause the release of an excitatory neurotransmitter, causing a similar response [2]
in the postsynaptic neuron. These neurons may communicate with thousands of other receptors and target cells through extensive, complex dendritic networks. Communication between receptors in this fashion enables discrimination and the more explicit interpretation of external stimuli. Effectively, these localized graded potentials trigger action potentials that communicate, in their frequency, along nerve axons eventually arriving in specific cortexes of the brain. In these also highly specialized parts of the brain, these signals are coordinated with others to [6]
possibly trigger a new response.

If a signal from the presynaptic neuron is inhibitory, inhibitory
[2]
neurotransmitters, normally GABA will be released into the synapse. This neurotransmitter causes an inhibitory postsynaptic potential in the postsynaptic neuron. This response will cause the postsynaptic neuron to become permeable to chloride ions, making the membrane potential of the cell negative; a negative membrane potential makes it more difficult for the cell to fire an action potential and prevents any signal from being passed on through the neuron. Depending on the type of stimulus, a neuron can be either excitatory or
inhibitory. Muscular-system response
Nerves in the peripheral nervous system spread out to various parts of the
body, including muscle fibers. A muscle fiber and the motor neuron to which it is
[13]
connected. The spot at which the motor neuron attaches to the muscle fiber is known as the neuromuscular junction. When muscles receive information from internal or external stimuli, muscle fibers are stimulated by their respective motor neuron. Impulses are passed from the central nervous system down neurons until
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they reach the motor neuron, which releases the neurotransmitter acetylcholine (ACh) into the neuromuscular junction. ACh binds to nicotinic acetylcholine receptors on the surface of the muscle cell and opens ion channels, allowing sodium ions to flow into the cell and potassium ions to flow out; this ion movement causes a depolarization, which allows for the release of calcium ions

within the cell. Calcium ions bind to proteins within the muscle cell to allow for muscle contraction; the ultimate consequence of a
[2]
stimulus. Endocrine-system response
Vasopressin
The endocrine system is affected largely by many internal and external

stimuli. One internal stimulus that causes hormone release is blood pressure. Hypotension, or low blood pressure, is a large driving force for the release of vasopressin, a hormone which causes the retention of water in the kidneys. This process also increases an individuals thirst. By fluid retention or by consuming fluids, if an individual's blood pressure returns to normal, vasopressin release slows
and less fluid is retained by the kidneys. Hypovolemia, or low fluid levels in the [14]
body, can also act as a stimulus to cause this response.

Epinephrine

Epinephrine, also known as adrenaline, is also used commonly to respond to both internal and external changes. One common cause of the release of this hormone is the Fight-or-flight response. When the body encounters an external stimulus that is potentially dangerous, epinephrine is released from the adrenal glands. Epinephrine causes physiological changes in the body, such as constriction of blood vessels, dilation of pupils, increased heart and respiratory rate, and the metabolism of glucose. All of these responses to a single stimuli aid in protecting the individual, whether the decision is made to stay and fight, or run away and avoid danger.
[15] [16]

Digestive-system response

Cephalic phase

The digestive system can respond to external stimuli, such as the sight or smell of food, and cause physiological changes before the food ever enters the body. This reflex is known as the cephalic phase of digestion. The sight and smell of food are strong enough stimuli to cause salivation, gastric and pancreatic enzyme secretion, and endocrine secretion in preparation for the incoming nutrients; by starting the digestive process before food reaches the stomach, the body is able to more effectively and efficiently metabolize food into necessary [17]

nutrients. Once food hits the mouth, taste and information from receptors in the mouth add to the digestive response. Chemoreceptors and mechanorceptors, activated by chewing and swallowing, further increase the enzyme release in the [18] stomach and intestine.

Enteric nervous system

The digestive system is also able to respond to internal stimuli. The digestive tract, or enteric nervous system alone contains millions of neurons. These neurons act as sensory receptors that can detect changes, such as food entering the small intestine, in the digestive tract. Depending on what these sensory receptors detect, 23 certain enzymes and digestive juices from [th

2

e

]

pancreas and liver can be secreted to aid in metabolism and breakdown of food.

Methods and techniques

Clamping techniques

Intracellular measurements of electrical potential across the membrane can be obtained by microelectrode recording. Patch clamp techniques allow for the manipulation of the intracellular or extracellular ionic or lipid concentration while still recording potential. In this way, the effect of various conditions [2] on threshold and propagation can be assessed.

Noninvasive neuronal scanning

Positron emission tomography (PET) and magnetic resonance imaging (MRI) permit the noninvasive visualization of activated regions of the brain while the test subject is exposed to different stimuli. Activity is monitored in relation to

blood flow to a particular region of the

[2]

brain. Hindlimb withdrawal time

Sorin Barac et al. in a recent paper published in the Journal of

Reconstructive Microsurgery monitored the response of test rats to pain stimuli by inducing an acute, external heat stimulus and measuring hindlimb withdrawal

[19]

times (HLWT).

GENERAL PRINCIPLES OF TISSUE ORGANIZATION

The term tissue is used to describe a group of cells found together in the body. The cells within a tissue share a common embryonic origin. Microscopic observation reveals that the cells in a tissue share morphological features and are arranged in an orderly pattern that achieves the tissue's functions. From the evolutionary perspective, tissues appear in more complex organisms. For example, multicellular protists, ancient eukaryotes, do not have cells organized into tissues.

Although there are many types of cells in the human body, they are organized into four broad categories of tissues: epithelial, connective, muscle, and nervous. Each of

these categories is characterized by specific functions that contribute to the overall health and maintenance of the body. A disruption of the structure is a sign of injury or

disease. Such changes can be detected through histology, the microscopic study of tissue appearance, organization, and function.

The Four Types of Tissues

Epithelial tissue, also referred to as epithelium, refers to the sheets of cells that cover

exterior surfaces of the body, lines internal cavities and passageways, and forms certain glands. Connective tissue, as its name implies, binds the cells and organs of the body together and functions in the protection, support, and integration of all parts

of the body.Muscle tissue is excitable, responding to stimulation and contracting to

provide movement, and occurs as three major types: skeletal (voluntary) muscle, smooth muscle, and cardiac muscle in the heart. Nervous tissue is also excitable, allowing the propagation of electrochemical signals in the form of nerve impulses that communicate between different regions of the body.

The next level of organization is the organ, where several types of tissues come together to form a working unit. Just as knowing the structure and function of cells helps you in your study of tissues, knowledge of tissues will help you understand how organs function. The epithelial and connective tissues are discussed in detail in this chapter. Muscle and nervous tissues will be discussed only briefly in this chapter.

Embryonic Origin of Tissues

The zygote, or fertilized egg, is a single cell formed by the fusion of an egg and sperm. After fertilization the zygote gives rise to rapid mitotic cycles, generating many cells to form the embryo. The first embryonic cells generated have the ability to differentiate into any type of cell in the body and, as such, are called totipotent, meaning each has the capacity to divide, differentiate, and develop into a new organism. As cell proliferation progresses, three major cell lineages are established within the embryo. Each of these lineages of embryonic cells forms the distinct germ layers from which all the tissues and organs of the human body eventually form. Each germ layer is identified by its relative position: ectoderm(ecto- = “outer”), mesoderm (meso- = “middle”), and endoderm (endo- = “inner”). Figure 2 shows the types of tissues and organs associated with the each of the three germ layers. Note that epithelial tissue originates in all three layers, whereas nervous tissue derives primarily from the ectoderm and muscle tissue from mesoderm. Figure 1. Four Types of Tissue: Body. The four types of tissues are exemplified in nervous tissue, stratified

squamous epithelial tissue, cardiac muscle tissue, and connective tissue in small intestine. Clockwise from nervous tissue, LM × 872, LM × 282, LM × 460, LM × 800. (Micrographs provided by the Regents of University of Michigan Medical School © 2012)

Tissue Membranes

A tissue membrane is a thin layer or sheet of cells that covers the outside of the body (for example, skin), the organs (for example, pericardium), internal passageways that lead to the exterior of the body (for example, abdominal mesenteries), and the lining of the moveable joint cavities. There are two basic types of tissue membranes: connective tissue and epithelial membranes.

Connective Tissue Membranes

The connective tissue membrane is formed solely from connective tissue. These membranes encapsulate organs, such as the kidneys, and line our movable joints. A synovial membrane is a type of connective tissue membrane that lines the cavity of a freely movable joint. For example, synovial membranes surround the joints of the shoulder, elbow, and knee. Fibroblasts in the inner layer of the synovial membrane release hyaluronan into the joint cavity. The hyaluronan effectively traps available water to form the synovial fluid, a natural lubricant that enables the bones of a joint to move freely against one another without much friction. This synovial fluid readily exchanges water and nutrients with blood, as do all body fluids.

Epithelial Membranes

The epithelial membrane is composed of epithelium attached to a layer of connective tissue, for example, your skin. The mucous membrane is also a composite of connective and epithelial tissues. Sometimes called mucosae, these epithelial membranes line the body cavities and hollow passageways that open to the external environment, and include the digestive, respiratory, excretory, and reproductive tracts. Mucous, produced by the epithelial exocrine glands, covers the

epithelial layer. The underlying connective tissue, called the lamina propria (literally “own layer”), help support the fragile epithelial layer.

A serous membrane is an epithelial membrane composed of mesodermally derived epithelium called the mesothelium that is supported by connective tissue. These membranes line the coelomic cavities of the body, that is, those cavities that do not open to the outside, and they cover the organs located within those cavities. They are essentially membranous bags, with mesothelium lining the inside and connective tissue on the outside. Serous fluid secreted by the cells of the thin squamous mesothelium lubricates the membrane and reduces abrasion and friction between organs. Serous membranes are identified according locations. Three serous membranes line the thoracic cavity; the two pleura that cover the lungs and the pericardium that covers the heart. A fourth, the peritoneum, is the serous membrane in the abdominal cavity that covers abdominal organs and forms double sheets of mesenteries that suspend many of the digestive organs.

The skin is an epithelial membrane also called the cutaneous membrane. It is a stratified squamous epithelial membrane resting on top of connective tissue. The apical surface of this membrane is exposed to the external environment and is covered with dead, keratinized cells that help protect the body from desiccation and pathogens.

The human body contains more than 200 types of cells that can all be classified into four types of tissues: epithelial, connective, muscle, and nervous. Epithelial tissues act as coverings controlling the movement of materials across the surface. Connective tissue integrates the various parts of the body and provides support and protection to organs. Muscle tissue allows the body to move. Nervous tissues propagate information.

The study of the shape and arrangement of cells in tissue is called histology. All cells

and tissues in the body derive from three germ layers in the embryo: the ectoderm,

mesoderm, and endoderm. Different types of tissues form membranes that enclose

organs, provide a friction-free interaction between organs, and keep organs together.

Synovial membranes are connective tissue membranes that protect and line the joints.

Epithelial membranes are formed from epithelial tissue attached to a layer of

onnective tissue. There are three types of epithelial membranes: mucous, which

contain glands; serous, which secrete fluid; and cutaneous which makes up the skin.

EPITHELIAL STEM CELLS

Stem cells have the remarkable potential to develop into many different cell types in

the body during early life and growth. In addition, in many tissues they serve as a sort

of internal repair system, dividing essentially without limit to replenish other cells as

long as the person or animal is still alive. When a stem cell divides, each new cell has

the potential either to remain a stem cell or become another type of cell with a more

specialized function, such as a muscle cell, a red blood cell, or a brain cell. Stem cells

are distinguished from other cell types by two important characteristics. First, they

are unspecialized cells capable of renewing themselves through cell division,

sometimes after long periods of inactivity. Second, under certain physiologic or

experimental conditions, they can be induced to become tissue- or organ-specific

cells with special functions. In some organs, such as the gut and bone marrow, stem

cells regularly divide to repair and replace worn out or damaged tissues. In other

organs, however, such as the pancreas and the heart, stem cells only divide under

special conditions.

Until recently, scientists primarily worked with two kinds of stem cells from animals

and humans: embryonic stem cells and non-embryonic "somatic" or "adult" stem

cells. The functions and characteristics of these cells will be explained in this

document. Scientists discovered ways to derive embryonic stem cells from early

mouse embryos more than 30 years ago, in 1981. The detailed study of the biology of

mouse stem cells led to the discovery, in 1998, of a method to derive stem cells from

human embryos and grow the cells in the laboratory. These cells are called human

embryonic stem cells. The embryos used in these studies were created for

reproductive purposes through in vitro fertilization procedures. When they were no

longer needed for that purpose, they were donated for research with the informed

consent of the donor. In 2006, researchers made another breakthrough by identifying

conditions that would allow some specialized adult cells to be "reprogrammed"

genetically to assume a stem cell-like state. This new type of stem cell, called

induced pluripotent stem cells (iPSCs), will be discussed in a later section of this

document.

Stem cells are important for living organisms for many reasons. In the 3- to 5-day-old

embryo, called a blastocyst, the inner cells give rise to the entire body of the

organism, including all of the many specialized cell types and organs such as the

heart, lungs, skin, sperm, eggs and other tissues. In some adult tissues, such as bone

marrow, muscle, and brain, discrete populations of adult stem cells generate

replacements for cells that are lost through normal wear and tear, injury, or disease.

Given their unique regenerative abilities, stem cells offer new potentials for treating

diseases such as diabetes, and heart disease. However, much work remains to be done

in the laboratory and the clinic to understand how to use these cells for cell-based therapies to treat disease, which is also referred to as regenerative or reparative medicine.

Laboratory studies of stem cells enable scientists to learn about the cells'essential properties and what makes them different from specialized cell types. Scientists are already using stem cells in the laboratory to screen new drugs and to develop model systems to study normal growth and identify the causes of birth defects. Research on stem cells continues to advance knowledge about how an organism develops from a single cell and how healthy cells replace damaged cells in adult organisms. Stem cell

research is one of the most fascinating areas of contemporary biology, but, as with many expanding fields of scientific inquiry, research on stem cells raises scientific questions as rapidly as it generates new discoveries.

Stem cells differ from other kinds of cells in the body. All stem cells— regardless of their source—have three general properties: they are capable of dividing and renewing themselves for long periods; they are unspecialized; and they can give rise to specialized cell types.

Stem cells are capable of dividing and renewing themselves for long periods. Unlike muscle cells, blood cells, or nerve cells—which do not normally replicate themselves—stem cells may replicate many times, or proliferate. A starting population of stem cells that proliferates for many months in the laboratory can yield millions of cells. If the resulting cells continue to be unspecialized, like the parent stem cells, the cells are said to be capable of long-term self-renewal. Scientists are trying to understand two fundamental properties of stem cells that relate to their long-term self-renewal:

1. Why can embryonic stem cells proliferate for a year or more in the laboratory without differentiating, but most adult stem cells cannot; and 2. What are the factors in living organisms that normally regulate stem cellproliferation and self-renewal?

Discovering the answers to these questions may make it possible to understand how cell proliferation is regulated during normal embryonic development or during the abnormal cell division that leads to cancer. Such information would also enable scientists to grow embryonic and non-embryonic stem cells more efficiently in the laboratory.

The specific factors and conditions that allow stem cells to remain unspecialized are of great interest to scientists. It has taken scientists many years of trial and error to learn to derive and maintain stem cells in the laboratory without them spontaneously differentiating into specific cell types. For example, it took two decades to learn how to grow human embryonic stem cells in the laboratory following the development of conditions for growing mouse stem cells. Likewise, scientists must first understand the signals that enable a non-embryonic (adult) stem cell population to proliferate and remain unspecialized before they will be able to grow large numbers of unspecialized adult stem cells in the laboratory.

Stem cells are unspecialized. One of the fundamental properties of a stem cell is that it does not have any tissue-specific structures that allow it to perform specialized functions. For example, a stem cell cannot work with its neighbors to pump blood through the body (like a heart muscle cell), and it cannot carry oxygen molecules through the bloodstream (like a red blood cell). However, unspecialized stem cells can give rise to specialized cells, including heart muscle cells, blood cells, or nerve cells.

Stem cells can give rise to specialized cells. When unspecialized stem cells give rise

to specialized cells, the process is called differentiation. While differentiating, the cell

usually goes through several stages, becoming more specialized at each step.

Scientists are just beginning to understand the signals inside and outside cells that

trigger each step of the differentiation process. The internal signals are controlled by

a cell's genes, which are interspersed across long strands of DNA and carry coded

instructions for all cellular structures and functions. The external signals for cell

differentiation include chemicals secreted by other cells, physical contact with

neighboring cells, and certain molecules in the microenvironment. The interaction of

signals during differentiation causes the cell's DNA to acquire epigenetic marks that

restrict DNA expression in the cell and can be passed on through cell division.

Many questions about stem cell differentiation remain. For example, are the internal

and external signals for cell differentiation similar for all kinds of stem cells? Can

specific sets of signals be identified that promote differentiation into specific cell

types? Addressing these questions may lead scientists to find new ways to control

stem cell differentiation in the laboratory, thereby growing cells or tissues that can be

used for specific purposes such as cell-based therapies or drug screening.

Adult stem cells typically generate the cell types of the tissue in which they reside.

For example, a blood-forming adult stem cell in the bone marrow normally gives rise

to the many types of blood cells. It is generally accepted that a bloodforming cell in

the bone marrow—which is called a hematopoietic stem cell—cannot give rise to the

cells of a very different tissue, such as nerve cells in the brain. Experiments over the

last several years have purported to show that stem cells from one tissue may give

rise to cell types of a completely different tissue.

This remains an area of great debate within the research community. This controversy

demonstrates the challenges of studying adult stem cells and suggests that additional

research using adult stem cells is necessary to understand their full potential as future

therapies.

Embryonic stem cells, as their name suggests, are derived from embryos. Most

embryonic stem cells are derived from embryos that develop from eggs that have

been fertilized in vitro—in an in vitro fertilization clinic—and then donated for

research purposes with informed consent of the donors. They are not derived from

eggs fertilized in a woman's body.

Growing cells in the laboratory is known as cell culture. Human embryonic stem cells

(hESCs) are generated by transferring cells from a preimplantationstage embryo

into a plastic laboratory culture dish that contains a nutrient broth known asculture

medium. The cells divide and spread over the surface of the dish.

In the original protocol, the inner surface of the culture dish was coated with mouse

embryonic skin cells specially treated so they will not divide. This coating layer of

cells is called a feeder layer. The mouse cells in the bottom of the culture dish

provide the cells a sticky surface to which they can attach. Also, the feeder cells

release nutrients into the culture medium. Researchers have now devised ways to

grow embryonic stem cells without mouse feeder cells. This is a significant scientific

advance because of the risk that viruses or other macromolecules in the mouse cells

may be transmitted to the human cells.

The process of generating an embryonic stem cell line is somewhat inefficient, so

lines are not produced each time cells from the preimplantationstage embryo are placed into a culture dish. However, if the plated cells survive, divide and multiply enough to crowd the dish, they are removed gently and plated into several fresh culture dishes. The process of re-plating or subculturing the cells is repeated many times and for many months. Each cycle of subculturing the cells is referred to as a passage. Once the cell line is established, the original cells yield millions of embryonic stem cells. Embryonic stem cells that have proliferated in cell culture for six or more months without differentiating, are pluripotent, and appear genetically normal are referred to as an embryonic stem cell line. At any stage in the process, batches of cells can be frozen and shipped to other laboratories for further culture and experimentation.

At various points during the process of generating embryonic stem cell lines, scientists test the cells to see whether they exhibit the fundamental properties that make them embryonic stem cells. This process is called characterization.

Scientists who study human embryonic stem cells have not yet agreed on a standard battery of tests that measure the cells' fundamental properties. However, laboratories that grow human embryonic stem cell lines use several kinds of tests, including:

Growing and subculturing the stem cells for many months. This ensures that the cells are capable of long-term growth and self-renewal. Scientists inspect the cultures through a microscope to see that the cells look healthy and remainundifferentiated.

Using specific techniques to determine the presence of transcription factors that are typically produced by undifferentiated cells. Two of the most important transcription factors are Nanog and Oct4. Transcription factors help turn geneson and off at the right time, which is an important part of the processes of celldifferentiation and embryonic development. In this case, both Oct 4 and Nanog are associated with

maintaining the stem cells in an undifferentiated state, capable of self-renewal. Using

specific techniques to determine the presence of particular cell surface markers that

are typically produced by undifferentiated cells. Examining the chromosomes under a

microscope. This is a method to assess whether the chromosomes are damaged or if

the number of chromosomes has changed. It does not detect genetic mutations in the

cells.

Determining whether the cells can be re-grown, or subcultured, after freezing,

thawing, and re-plating.

Testing whether the human embryonic stem cells are pluripotent by 1)allowing the

cells to differentiate spontaneously in cell culture; 2) manipulating the cells so they

will differentiate to form cells characteristic of the three germ layers; or 3) injecting

the cells into a mouse with a suppressed immune system to test for the formation of a

benign tumor called a teratoma. Since the mouse's immune system is suppressed, the

injected human stem cells are not rejected by the mouse immune system and

scientists can observe growth and differentiation of the human stem cells. Teratomas

typically contain a mixture of many differentiated or partly differentiated cell

types—an indication that the embryonic stem cells are capable of differentiating into

multiple cell types.

As long as the embryonic stem cells in culture are grown under appropriate

conditions, they can remain undifferentiated (unspecialized). But if cells are allowed

to clump together to form embryoid bodies, they begin to differentiate spontaneously.

They can form muscle cells, nerve cells, and many other cell types.

Although spontaneous differentiation is a good indication that a culture of embryonic

stem cells is healthy, the process is uncontrolled and therefore an inefficient strategy

to produce cultures of specific cell types.

So, to generate cultures of specific types of differentiated cells—heart muscle cells,

blood cells, or nerve cells, for example—scientists try to control the differentiation of

embryonic stem cells. They change the chemical composition of the culture medium,

alter the surface of the culture dish, or modify the cells by inserting specific genes.

Through years of experimentation, scientists have established some basic protocols or

"recipes" for the directed differentiation of embryonic stem cells into some specific

cell types (Figure 1). (For additional examples of directed differentiation of

embryonic stem cells, refer to the 2006 NIH stem cell report.)

If scientists can reliably direct the differentiation of embryonic stem cells into

specific cell types, they may be able to use the resulting, differentiated cells to treat

certain diseases in the future. Diseases that might be treated by transplanting cells

generated from human embryonic stem cells include diabetes, traumatic spinal cord

injury, Duchenne's muscular dystrophy, heart disease, and vision and hearing loss.

An adult stem cell is thought to be an undifferentiated cell, found among

differentiated cells in a tissue or organ. The adult stem cell can renew itself and can

differentiate to yield some or all of the major specialized cell types of the tissue or

organ. The primary roles of adult stem cells in a living organism are to maintain and

repair the tissue in which they are found. Scientists also use the term somatic stem

cell instead of adult stem cell, where somatic refers to cells of the body (not the germ

cells, sperm or eggs). Unlike embryonic stem cells, which are defined by their origin

(cells from the preimplantation-stage embryo), the origin of adult stem cells in some

mature tissues is still under investigation.

Research on adult stem cells has generated a great deal of excitement. Scientists have

found adult stem cells in many more tissues than they once thought possible. This finding has led researchers and clinicians to ask whether adult stem cells could be used for transplants. In fact, adult hematopoietic, or blood-forming, stem cells from bone marrow have been used in transplants for more than 40 years.

Scientists now have evidence that stem cells exist in the brain and the heart, two locations where adult stem cells were not at first expected to reside. If the differentiation of adult stem cells can be controlled in the laboratory, these cells may

become the basis of transplantation-based therapies.

The history of research on adult stem cells began more than 60 years ago. In the 1950s, researchers discovered that the bone marrow contains at least two kinds of stem cells. One population, called hematopoietic stem cells, forms all the types of blood cells in the body. A second population, called bone marrow stromal stem cells(also called mesenchymal stem cells, or skeletal stem cells by some), were discovered a few years later. These non-hematopoietic stem cells make up a small proportion of the stromal cell population in the bone marrow and can generate bone,

cartilage, and fat cells that support the formation of blood and fibrous connective tissue.

In the 1960s, scientists who were studying rats discovered two regions of the brain that contained dividing cells that ultimately become nerve cells. Despite these reports,

most scientists believed that the adult brain could not generate new nerve cells. It was

not until the 1990s that scientists agreed that the adult brain does contain stem cells

that are able to generate the brain's three major cell types— astrocytes and oligodendrocytes, which are non-neuronal cells, and neurons, or nerve cells.

Adult stem cells have been identified in many organs and tissues, including brain, bone marrow, peripheral blood, blood vessels, skeletal muscle, skin, teeth, heart, gut, liver, ovarian epithelium, and testis. They are thought to reside in a specific area of each tissue (called a "stem cell niche"). In many tissues, current evidence suggests that some types of stem cells are pericytes, cells that compose the outermost layer of small blood vessels. Stem cells may remain quiescent (nondividing) for long periods

of time until they are activated by a normal need for more cells to maintain tissues, or by disease or tissue injury.

Typically, there is a very small number of stem cells in each tissue and, once removed from the body, their capacity to divide is limited, making generation of large quantities of stem cells difficult. Scientists in many laboratories are trying to find better ways to grow large quantities of adult stem cells in cell culture and to manipulate them to generate specific cell types so they can be used to treat injury or disease. Some examples of potential treatments include regenerating bone using cells derived from bone marrow stroma, developing insulin-producing cells for type 1 diabetes, and repairing damaged heart muscle following a heart attack with cardiac muscle cells.

Scientists often use one or more of the following methods to identify adult stem cells:

(1) label the cells in a living tissue with molecular markers and then determine the specialized cell types they generate; (2) remove the cells from a living animal, label

them in cell culture, and transplant them back into another animal to determine whether the cells replace (or "repopulate") their tissue of origin. Importantly, scientists must demonstrate that a single adult stem cell can generate a line of genetically identical cells that then gives rise to all the appropriate differentiated cell types of the tissue. To confirm experimentally that a putative adult stem cell is indeed a stem cell, scientists tend to show either that the cell can give rise to these genetically identical cells in culture, and/or that a purified population of these candidate stem cells can repopulate or reform the tissue after transplant into an animal.

As indicated above, scientists have reported that adult stem cells occur in many tissues and that they enter normal differentiation pathways to form the specialized cell types of the tissue in which they reside.

Normal differentiation pathways of adult stem cells. In a living animal, adult stem cells are available to divide for a long period, when needed, and can give rise to mature cell types that have characteristic shapes and specialized structures and functions of a particular tissue. The following are examples of differentiation pathways of adult stem cells (Figure 2) that have been demonstrated in vitro or in vivo.

Hematopoietic stem cells give rise to all the types of blood cells: red blood cells, B lymphocytes, T lymphocytes, natural killer cells, neutrophils, basophils, eosinophils, monocytes, and macrophages.

Mesenchymal stem cells have been reported to be present in many tissues. Those from bone marrow (bone marrow stromal stem cells, skeletal stem cells) give rise to a variety of cell types: bone cells (osteoblasts and osteocytes), cartilage cells (chondrocytes), fat cells (adipocytes), and stromal cells that support blood formation.

However, it is not yet clear how similar or dissimilar mesenchymal cells derived from

non-bone marrow sources are to those from bone marrow stroma.

Neural stem cells in the brain give rise to its three major cell types: nerve cells

(neurons) and two categories of non-neuronal cells—astrocytes and oligodendrocytes.

Epithelial stem cells in the lining of the digestive tract occur in deep crypts and give

rise to several cell types: absorptive cells, goblet cells, Paneth cells, and

enteroendocrine cells.

Skin stem cells occur in the basal layer of the epidermis and at the base of hair

follicles. The epidermal stem cells give rise to keratinocytes, which migrate to the

surface of the skin and form a protective layer. The follicular stem cells can give rise

to both the hair follicle and to the epidermis.

Transdifferentiation. A number of experiments have reported that certain adult stem

cell types can differentiate into cell types seen in organs or tissues other than those

expected from the cells' predicted lineage (i.e., brain stem cells that differentiate into

blood cells or blood-forming cells that differentiate into cardiac muscle cells, and so

forth). This reported phenomenon is called transdifferentiation.

Although isolated instances of transdifferentiation have been observed in some

vertebrate species, whether this phenomenon actually occurs in humans is under

debate by the scientific community. Instead of transdifferentiation, the observed

instances may involve fusion of a donor cell with a recipient cell.

Another possibility is that transplanted stem cells are secreting factors that encourage

the recipient's own stem cells to begin the repair process. Even when

transdifferentiation has been detected, only a very small percentage of cells undergo

the process.

In a variation of transdifferentiation experiments, scientists have recently demonstrated that certain adult cell types can be "reprogrammed" into other cell types in vivo using a well-controlled process of genetic modification (see Section VIfor a discussion of the principles of reprogramming). This strategy may offer a way to reprogram available cells into other cell types that have been lost or damaged due to disease. For example, one recent experiment shows how pancreatic beta cells, the insulin-producing cells that are lost or damaged in diabetes, could possibly be created by reprogramming other pancreatic cells. By "re-starting" expression of three critical beta cell genes in differentiated adult pancreatic exocrine cells, researchers were able to create beta cell-like cells that can secrete insulin. The reprogrammed cells were similar to beta cells in appearance, size, and shape; expressed genes characteristic of beta cells; and were able to partially restore blood sugar regulation in mice whose own beta cells had been chemically destroyed. While not transdifferentiation by definition, this method for reprogramming adult cells may be used as a model for directly reprogramming other adult cell types.

In addition to reprogramming cells to become a specific cell type, it is now possible to reprogram adult somatic cells to become like embryonic stem cells (induced pluripotent stem cells, iPSCs) through the introduction of embryonic genes. Thus, a source of cells can be generated that are specific to the donor, thereby increasing the chance of compatibility if such cells were to be used for tissue regeneration. However, like embryonic stem cells, determination of the methods by which iPSCs can be completely and reproducibly committed to appropriate cell lineages is still under investigation.

Human embryonic and adult stem cells each have advantages and disadvantages regarding potential use for cell-based regenerative therapies. One major difference between adult and embryonic stem cells is their different abilities in the number and type of differentiated cell types they can become. Embryonic stem cells can become all cell types of the body because they are pluripotent. Adult stem cells are thought to be limited to differentiating into different cell types of their tissue of origin.

Embryonic stem cells can be grown relatively easily in culture. Adult stem cells are rare in mature tissues, so isolating these cells from an adult tissue is challenging, and methods to expand their numbers in cell culture have not yet been worked out. This is an important distinction, as large numbers of cells are needed for stem cell replacement therapies.

Scientists believe that tissues derived from embryonic and adult stem cells may differ in the likelihood of being rejected after transplantation. We don't yet know for certain whether tissues derived from embryonic stem cells would cause transplant rejection, since relatively few clinical trials have tested the safety of transplanted cells derived from hESCS.

Adult stem cells, and tissues derived from them, are currently believed less likely to initiate rejection after transplantation. This is because a patient's own cells could be expanded in culture, coaxed into assuming a specific cell type (differentiation), and then reintroduced into the patient. The use of adult stem cells and tissues derived

from the patient's own adult stem cells would mean that the cells are less likely to be

rejected by the immune system. This represents a significant advantage, as immune rejection can be circumvented only by continuous administration of immunosuppressive drugs, and the drugs themselves may cause deleterious side effects. Induced pluripotent stem cells (iPSCs) are adult cells that have been genetically reprogrammed to an embryonic stem cell–like state by being forced to express genes and factors important for maintaining the defining properties of embryonic stem cells. Although these cells meet the defining criteria for pluripotent

stem cells, it is not known if iPSCs and embryonic stem cells differ in clinically significant ways. Mouse iPSCs were first reported in 2006, and human iPSCs were first reported in late 2007. Mouse iPSCs demonstrate important characteristics of pluripotent stem cells, including expressing stem cell markers, forming tumors containing cells from all three germ layers, and being able to contribute to many different tissues when injected into mouse embryos at a very early stage in development. Human iPSCs also express stem cell markers and are capable of generating cells characteristic of all three germ layers.

Although additional research is needed, iPSCs are already useful tools for drug development and modeling of diseases, and scientists hope to use them in transplantation medicine. Viruses are currently used to introduce the reprogramming

factors into adult cells, and this process must be carefully controlled and tested before

the technique can lead to useful treatment for humans.

In animal studies, the virus used to introduce the stem cell factors sometimes causes

cancers. Researchers are currently investigating non-viral delivery strategies. In any

case, this breakthrough discovery has created a powerful new way to "de-differentiate" cells whose developmental fates had been previously assumed to be

determined. In addition, tissues derived from iPSCs will be a nearly identical match

to the cell donor and thus probably avoid rejection by the immune system. The iPSC

strategy creates pluripotent stem cells that, together with studies of other types of pluripotent stem cells, will help researchers learn how to reprogram cells to repair damaged tissues in the human body.

There are many ways in which human stem cells can be used in research and the clinic. Studies of human embryonic stem cells will yield information about the complex events that occur during human development. A primary goal of this work is

to identify how undifferentiated stem cells become the differentiated cells that form

the tissues and organs. Scientists know that turning genes on and off is central to this

process. Some of the most serious medical conditions, such as cancer and birth defects, are due to abnormal cell division and differentiation. A more complete understanding of the genetic and molecular controls of these processes may yield information about how such diseases arise and suggest new strategies for therapy.

Predictably controlling cell proliferation and differentiation requires additional basic

research on the molecular and genetic signals that regulate cell division and specialization. While recent developments with iPS cells suggest some of the specific

factors that may be involved, techniques must be devised to introduce these factors safely into the cells and control the processes that are induced by these factors.

Human stem cells are currently being used to test new drugs. New medications are tested for safety on differentiated cells generated from human pluripotent cell lines. Other kinds of cell lines have a long history of being used in this way. Cancer cell lines, for example, are used to screen potential antitumor drugs. The availability of pluripotent stem cells would allow drug testing in a wider range of cell types.

However, to screen drugs effectively, the conditions must be identical when comparing different drugs. Therefore, scientists must be able to precisely control the differentiation of stem cells into the specific cell type on which drugs will be tested.

For some cell types and tissues, current knowledge of the signals controlling differentiation falls short of being able to mimic these conditions precisely to generate pure populations of differentiated cells for each drug being tested. Perhaps the most important potential application of human stem cells is the generation of cells and tissues that could be used for cell-based therapies. Today, donated organs and tissues are often used to replace ailing or destroyed tissue, but the need for transplantable tissues and organs far outweighs the available supply.

Stem cells, directed to differentiate into specific cell types, offer the possibility of a renewable source of replacement cells and tissues to treat diseases including macular degeneration, spinal cord injury, stroke, burns, heart disease, diabetes, osteoarthritis, and rheumatoid arthritis.

THE EPITHELIUM AS A MAIN COMPONENT OF HISTOHEMATIC BARRIER. (3 hours)

The barrier properties of endothelial cells are critical for the maintenance of water and protein balance between the intravascular and extravascular compartments. An impairment of endothelial barrier function has been implicated in the genesis and/or progression of a variety of pathological conditions, including pulmonary edema, ischemic stroke, neurodegenerative disorders, angioedema, sepsis and cancer. The altered barrier function in these conditions is often linked to the release of soluble mediators from resident cells (e.g., mast cells, macrophages) and/or recruited blood cells. The interaction of the mediators with receptors expressed on the surface of endothelial cells diminishes barrier function either by altering the expression of adhesive proteins in the inter-endothelial junctions, by

altering the organization of the cytoskeleton, or both. Reactive oxygen species (ROS), proteolytic enzymes (e.g., matrix metalloproteinase, elastase), oncostatin M, and VEGF are part of a long list of mediators that have been implicated in endothelial barrier failure. In this review, we address the role of blood borne cells, including, neutrophils, lymphocytes, monocytes, and platelets, in the regulation of endothelial barrier function in health and disease. Attention is also devoted to new targets for therapeutic intervention in disease states with morbidity and mortality related to endothelial barrier dysfunction.

Endothelial Barrier Function

An intact layer of healthy endothelial cells is essential for normal blood vessel function. The close apposition and alignment of endothelial cells in the vessel wall accounts for their ability to form a barrier that restricts the movement of water, proteins and blood cells between the intravascular and interstitial compartments. This barrier is formed by a layer of endothelial cells that are joined laterally by cell-cell junctions, while the basolateral aspect of this layer is attached to a basement membrane composed of collagen, fibronectin, laminin, and glycosaminoglycans (GAG). Cell-surface expressed integrins, which form regions called focal adhesions (FA), bind the endothelial cells to the extracellular matrix. The resulting barrier is semi-permeable to water and non-lipophilic molecules, and is both size- and charge-selective for solutes passage. Precise regulation of the restrictive properties of the endothelial barrier is essential for normal organ function. Indeed, diminished barrier function (and increasedvascular permeability) is associated with organ dysfunction and can lead to serious pathological consequences, as evidenced in diseases such as sepsis, as well as inflammatory and neurodegenerative diseases. Restoration of endothelial barrier integrity in these conditions can significantly impede disease progression.

Several different pools of proteins are assembled on endothelial cells to form membrane domains that create the cohesive structure that accounts for the barrier properties of the vessel wall. Among the barrier-forming adhesive structures, the most important are the adherens junctions (AJ), gap junctions (GJ), and tight

junctions (TJ). These domains collectively form the paracellular junctional

structure that regulates the partitioning of water and solutes between blood and interstitium, and an alteration in these membrane components often underlie the increased vascular permeability that accompanies inflammation.

In addition to their barrier function, signaling mediated through these adhesive membrane proteins contribute to a variety of endothelial cell processes, such as cell growth, cell polarity and their interactions with other cell types such as smooth muscle cells and pericytes.

Consequently, an alteration in endothelial adhesive proteins affects not only vascular permeability but also the vascular responses to changes in the perivascular environment.

Adherens junctions mediate cell-cell contact among endothelial cells in all types of blood vessels, and is composed mainly of VE-cadherin, a member of a $2+$ transmembrane Ca -dependent adhesion molecule family that regulatesvascular permeability. VE-cadherin on one cell strongly binds (homotypically) VE-cadherin on an adjacent cell, which leads to a reorganization of the cytoskeleton in both cells via an interaction with actin filaments after cadherin-catenin binding (β-catenin, p120 catenin and α-catenin). VE-cadherin recruits α-catenin, via β-catenin, to sites of adherens junction assembly. Other actin-binding, such as vinculin, α-actinin, and eplin are also recruited to the adherens junction by following conformational changes in α-catenin, which serve to reinforce the adherens junction.

The main function of VE-cadherin is to seal the paracellular space, but it also modulates angiogenesis, inhibits growth (cell contact inhibition), and protects cells from apoptosis.

Gap junctions allow for cell-cell communication via the formation of clusters of intercellular hemi-channels that link to each other to connect the

cytoplasm of the adjacent cells. Gap junctions are formed by proteins from the connexin family. Molecules less than 1000 daltons, such as ions, simple sugars, amino acids, nucleotides, and second messengers (cAMP, calcium, IP3) can move between cells via these channels. GJ are also involved in several cellular events, including metabolic transport, electrical coupling, apoptosis, differentiation, and tissue homeostasis, and phosphorylation of the inner tail of this junctional structure can affect these functions of GJ. In addition to allowing for communication between endothelial cells, GJ also allow for cross-talk between the endothelium and smooth muscle cells in the vessel wall.

The cerebral vasculature contains an additional component of the endothelial barrier called tight junctions (TJ), which closely fuses adjacent endothelial cells and further restricts the exchange of fluid and solutes through the paracellular spaces. TJ contribute to the highly selective properties of the blood brain barrier (BBB), which significantly limits the passage of substances from blood to brain interstitium. TJ are comprised of different proteins such as occludin, claudin family members, zonula occludens (ZO) family members and junctional adhesion molecules (JAM), which form a charge selective pore that only allows for the passage of small ions and uncharged molecules. Zonula occludens are scaffolding proteins that interact with intracellular components such as F-actin to influence

cytoskeleton mobility and other functions. The claudin family is comprised of more than 20 proteins and endothelial cells in the BBB are particularly rich in claudins 4, 5 and 16. TJ permeability is significantly influenced by the type(s) of

claudin present or absent in the endothelial cells. For example, in the absence of claudin-5 BBB permeability is profoundly comprised. Occludin is a phosphoprotein of 65-kDa located in the cytoplasmic membrane of endothelial cells in brain. Phosphorylation of occludin amino acid residues can strongly influence vascular barrier function. However, selective deletion of occludin has been shown not to affect vessel permeability, suggesting overlapping functions of

the different TJ proteins. JAM family members, including JAM-A, JAM-B and JAM-C, are also present in endothelial cells found in different vascular beds including liver, brain, intestine and lungs, and are expressed by circulating blood cells, includin gplatelets, lymphocytes and neutrophils. Known functions of JAMs include signaling to cytoskeletal proteins, assembly of TJ, and gathering cellpolarity

proteins to the TJ. Alterations in either of these TJ constituents members may result in endothelial barrier failure.

As a connective structure that links vascular endothelial cells to extracellular matrix proteins, focal adhesions (FA) are comprised of integrins, which participate in different cell functions such as adhesion, movement, and matrix remodeling. FA are connected to actomyosin bundles and serve as extracellular sensors. While FA do not directly form cell-cell junctions, these structures act as mechano- and chemo-sensors that modulate cytoskeleton tension. Intracellular signaling events associated with FA include the recruitment and activation of kinases that can modulate the binding affinity of integrins via phosphorylation. Immunoblockade of these integrins or interference with their binding to extracellular matrix constituents results in an increased vascular permeability, confirming the critical role of integrins in the regulation ofendothelial barrier function. The relative importance of integrins in modulatingendothelial barrier function appears to increase in conditions associated with angiogenesis or inflammation.

Another structural feature of endothelial cells that has been implicated in the modulation of vascular permeability is the glycocalyx, a 200–500 nm thick layer on the luminal surface of the cell that is comprised of proteoglycans with GAG side chains (e.g., heparan sulfates). A reduction in glycocalyx thickness caused by enzymatic degradation is associated with an increased transendothelial albumin flux, while stabilization of the glycocalyx with angiopoietin-1 reduces albumin permeability. The negative charge of GAGs in the glycocalyx is believed to impose a significant barrier to protein movement, while offering little resistance to the movement of water across the endothelial barrier.

While most attention devoted to vascular permeability has been given to modulation of the intercellular junctions (paracellular pathway), solutes and water can also cross the endothelial barrier via a transcellular pathway. Vesicules (or calveolae) have long been considered a pathway for the exchange of plasma proteins between the blood and interstitial compartment. The transcytosis process is regulated by different factors that target components of the vesicle, such as caveolin-1, which serves as a scaffold for albumin-binding proteins as well as

different signaling molecules that regulate transcytosis. In the cerebral microvasculature, with its tight junctions, the transcellular route is also important for the exchange of water. Aquaporins (AQP), cell membrane channels in vascular endothelium, have been shown to contribute to water exchange across the BBB

under both basal conditions and in certain pathological states. However, for most vascular beds, the quantitative importance of the transcellular pathway for the exchange of water and plasma protein exchange across endothelial cells appears small.

cAMP, a second messenger that is constantly formed in most cells, including endothelial cells, plays an important role in the modulation of endothelial barrierfunction. It is generated by the membrane-associated enzyme adenylyl cyclase following activation of G-protein coupled receptors (GPCR) by either endogenous (e.g., inflammatory mediators, hormones, neurotransmitters) or exogenous (e.g., drugs, xenobiotics, germs) stimuli. cAMP degradation is mediated byphosphodiesterase (PDE). The accumulation of cAMP in endothelial cells can result in either barrier-destabilization or -preservation, depending on the intracellular locus of cAMP generation, with cytosolic accumulation leading to increased vascular permeability, while increased cAMP in vacuoles appears to protect against barrier dysfunction. At least part of the endothelial barrierpreserving effect of cAMP reflects its influence on junctional proteins. cAMP-induced barrier preserving signaling includes: 1) activation of

cAMPdependentprotein
kinase A (PKA) and phosphorylation of downstream proteins,
such asERK1/2 and myosin light-chain kinase (MLCK), important modulators of vascular permeability; and 2) binding to intracellular proteins involved in inflammation, such as the exchange protein activated by cyclic AMP (EPAC1). EPAC1 is known to induce immunomodulatory genes such as suppressors of cytokine signaling 3 (SOCS-3) and to reduce integrin-mediated permeability responses. Furthermore, both PKA and EPAC1 are known to activate Rac1, a small GTPase involved in endothelial barrier protection via inhibition of RhoA, which regulates the MLCK, a protein whose activation leads to endothelial cell contraction. EPAC1 activation by cAMP also results in the activation of Rap1, via a PKA-independent pathway, and ultimately leads to enhanced endothelial barrier function by inducing the reorganization of cortical actin, redistribution of VEcadherin
and other junctional proteins to cell-cell contacts. Consequently, cellular events that alter the bioavailability of cAMP can exert a major influence on the barrier function of vascular endothelial cells.

A variety of chemical and physical factors (e.g., shear stress) act constantly on endothelial cells to influence its barrier properties. To some extent, the factors that act on endothelial cells are derived from other cell populations that comprise the vessel wall (e.g., podocytes, smooth muscle) or from neighboring cells that lie in the immediate perivascular space (e.g., mast cells, macrophages). Endothelial cells are also able to synthesize and release factors, such as adrenomedullin, that act to stabilize the endothelial barrier thereby opposing the actions of inflammatory mediators on vascular permeability. However, when mediator release from these other cell populations is excessive, endothelial barrier dysfunction or failure may

result. There is also mounting evidence that blood cells are capable of exerting a similar influence on endothelial barrierfunction, and may account for the barrier failure evidenced in different pathological conditions. In the following sections, we

briefly summarize evidence implicating different blood cell populations in the

modulation of endothelial barrier function, address their potential role in the vascular permeabilityresponses in different disease states, and discuss potential therapeutic targets for prevention of endothelial barrier dysfunction.

Pericytes, which heavily populate the vessel wall in some vascular beds, such as brain, lie in close contact with endothelial cells. The proximity between pericytes and endothelial cells allow for cross-talk between the 2 cell types, and accounts for the ability of pericytes to regulate the expression of junctional proteins. In the brain, pericytes also influences astrocyte cell organization/polarization, thereby maintaining the restrictive properties of the BBB. Some pericytes-derived mediators also exert a modulating influence on BBB function by regulating the expression of endothelial junction proteins. There include transforming growth factor-beta1 (TGF-beta1), glial cell-derived neurotrophic factor (GDNF), and angiopoietin 1 (ANG-1).

Leukocytes and Endothelial Barrier

Function Neutrophils

Neutrophils have been implicated as mediators of the increased vascular permeability that accompanies a variety of pathological conditions, including ischemia-reperfusion, sepsis, cancer nd neurological diseases. A role for neutrophils in these conditions is largely based on 2 observations: 1) neutrophils are recruited into the diseased/injured tissue, and 2) interfering with the neutrophil accumulation minimizes or prevents theendothelial barrier dysfunction. Activated neutrophils release an impressive mixture of chemicals that can impair endothelial barrier function, including reactive oxygen species (ROS), proteolytic enzymes, and cytokines These mediators and other products of neutrophil activation can alter barrier function by acting on the endothelial cell cytoskeleton, junctional proteins, and the endothelial glycocalyx. For example, endothelial cells exposed to ROS exhibit an increased permeability response that has been linked to disruption of the inter-endothelial junction, actomyosin contraction, gap formation, and an altered

expression and phosphorylation state of junctional adhesion molecules. Since superoxide is known to rapidly interact with (and inactivate) nitric oxide, some have attributed the effects of ROS on endothelial barrier function to an alteration in NO bioavailability. However, NO has been implicated as both a negative and a positive modulator of endothelial barrier function, with the protective role of NO attributed to its ability to inhibit leukocyte-endothelial cell adhesion. Nitric oxide synthase inhibition increases the permeability of endothelial cell monolayers, a response that is associated with the formation of stress fibers and disruption of adherens junctions.

Neutrophils are also able to enhance transendothelial protein exchange by releasing proteases, like elastase and matrix metalloproteinases (MMP), which appear to alter barrier function by disrupting junctional complexes and inducing endothelial cell retraction. Elastase has also been shown to promote the adhesion

and transendothelial migration of leukocytes in the microcirculation, suggesting that the permeability enhancing effect of the protease may also be related to an enhancement of neutrophil-endothelial cell adhesion. This possibility is supported by reports describing diminishedendothelial barrier function, resulting from junctional disassembly and cytoskeletal reorganization, following the ligation of

neutrophil adhesion molecules with their counter-receptors on endothelial cells, such as the binding of β-2 integrins with either ICAM-1 or VCAM-1. It has also been proposed that neutrophils can diminish barrier function due to physical disruption of the paracellular pathway caused by the passage of these cells through the junctions. This appears to occur despite the fact that endothelial cells can extend projections to envelop the migrating neutrophils, forming endothelial domes, with the leakage response resulting from the transfer of entrapped plasma proteins within the “dome.” It has also been reported that theendothelial barrier dysruption caused by transmigrating leukocytes are detected by the endothelial cells as a release of isometric tension, which results in protective actin remodeling

that is dependent on the production of reactive oxygen species.

Furthermore, the results of a recent study reveal that extravasating leukocytes deposit microparticles on the subendothelium during their passage through the junctions and that the microparticle deposition serves to maintain barrier function; inhibition of neutrophil-derived microparticle formation resulted in dramatically increased vascular leakage.

Another consequence of neutrophil activation within the microcirculation is capillary no-reflow, which is manifested as a reduced number of perfused capillaries and tissue hypoxia. The capillary malperfusion is worsened when neutrophil-dependent increases in vascular permeability lead to enhanced capillary fluid filtration and excessive accumulation of fluid in the interstitial compartment. The accompanying increase in interstitial fluid pressure leads to compression of the microvasculature, which further exacerbates the no-reflow response. This mechanism is supported by studies describing reductions invascular permeability and intersititial edema, and an improvement of capillary perfusion following neutrophil depletion or prevention of leukocyte-endothelial cell adhesion.

Lymphocytes

Less is known about the role of lymphocytes in the modulation of endothelial barrier function. Because T-cells are known to influence neutrophil function and to enhance the endothelial cell dysfunction mediated by neutrophils, it is often assumed that the contribution of T-cells to inflammation-induced vascular protein leakage largely reflects the ability of T-cells to enhance the recruitment and reactivity of neutrophils. However, studies in severe combined immunodeficient (SCID) mice, CD3+ T-cell deficient mice and wild type mice treated with CD4+ T-cell depleting antibody have revealed an important role for T-lymphocytes in mediating the increased vascular permeability induced by ischemia-reperfusion in the intestine, kidney and lung. T-cells have also been implicated in mediating the blood-brain barrier (BBB) disruption that is associated with experimental autoimmune encephalomyelitis(EAE). In this model of

neurological disease, CD4+ T cells appear to elicit changes in tight junction architecture and BBB permeability by inducing astrocytes to release vascular endothelial growth factor (VEGF). Studies of a CD8+ T-cell dependent model of BBB disruption that mimics multiple sclerosis have revealed that stimulation of CNS infiltrating CD8 T cells leads to astrocyte activation, alteration of BBB tight junction proteins and increased BBB permeability in a non-apoptotic manner, but these responses were not observed in perforin deficient mice. While other

lymphocyte-derived products, such as lymphotoxin, have been shown to increase the permeability of endothelial cell monolayers in vitro, the role of these products in T-cell dependent modulation of vascular permeability in vivo remains unclear.

Monocytes

Monocytes are known to produce and release a variety of mediators ofendothelial barrier dysfunction, notably factors such as oncostatin M (OSM) and VEGF. Oncostatin M (OSM), a member of the IL-6 superfamily, has been shown to reduce transendothelial electrical resistance (TEER) of monolayers comprised of cultured rat cerebral microvascular endothelial cells. OSM may also promote BBB dysfunction by stimulating brain cells to produce cytokines and prostaglandins, and to increase the expression of cell adhesion molecules on endothelial cells. While monocytes are the dominant source of OCM produced by blood cells, activated microglia and astrocytes are additional sources of OCM in the brain. Monocytes are also a rich source of VEGF. Monocyte-derived VEGF has been implicated in the enhanced vascular leakage that accompanies breast tumor metastasis to the lung. This mechanism may also contribute to the endothelial barrier dysfunction detected in other disease models that includes the recruitment of monocytes, such as atherosclerosis. Other monocyte-derived mediators that have been shown to increase vascular permeability include high mobility group box 1(HMGB-1), TNF-α and IL-1β.

The engagement of some inflammatory cells with integrins expressed on the endothelial cell surface can initiate a series of responses that will facilitate the

transendothelial migration of the attached blood cell. For example, the binding of integrins present on monocytic cells with adhesion molecules on endothelial cells induces HRas\Raf\MEK\ERK signaling, which leads to myosin light chain(MLC) activation. This results in the recruitment of Src to VE-cadherin and phosphorylation, the dissociation of VE-cadherin/β-catenin complex, and ultimately gap junction formation.

There is also evidence that supports a protective role for monocytes in the maintenance of endothelial barrier function. As described above for neutrophils, it has been reported that microparticles released from activated monocytes enhance the tightness of endothelial cell monolayers after exposure to bacterial endotoxin. While this microparticle mediated response was associated with inhibition of pSrc (tyr416) signaling, a cause-effect relationship with endothelial barrier function was not demonstrated. In another study, a different mechanism of monocyte-mediated protection was demonstrated. CD14+ peripheral monocytes, cultured under angiogenic conditions, were shown to acquire phenotypic and functional properties similar to cerebral microvascular endothelial cells. The features acquired by the

monocytes included the expression of tight junction proteins, high transcellular electrical resistance and low permeability to solutes. It was proposed that CD14+ blood monocytes may play an important role in repairing (sealing) the BBB after brain injury.

Platelets and endothelial barrier function

Recently, much attention has been devoted to addressing the role of platelets in inflammation, and the evolving consensus is that platelets tend to amplify diffrent components of the inflammatory response, most notably the expression of

endothelial cell adhesion molecules and the recruitment of leukocytes. While there are some reports that describe the ability ofplatelets to diminish endothelial barrier function, there is a larger body of evidence that supports an anti-permeability effect of platelets. For example, thrombocytopenia appears to elicit an increased vascular

permeability in resting microvessels and this response is reversed following the restoration of blood platelet count. Some of the beneficial effects of platelets in support of barrier function have been attributed to a purely physical effect resulting from adherent platelets covering gaps in the endothelial lining of injured blood vessels, however, soluble factors released by platelets are a more likely to explain the ability of these cells to maintain vascular wall integrity in the setting of inflammation or other pathological conditions. Platelet-conditioned media and different molecules released from platelets, including sphingosin-1-phosphate (S1P), serotonin, angiopoietin-and adenine nucleotides, have been shown to enhance the barrier properties of endothelial cells either in vivo or in vitro. S1P is believed to be continuously secreted into the blood stream by platelets as well as erythrocytesunder physiological conditions. The S1P subsequently binds to its receptor on the surface of endothelial cells thereby activating Rac1, which acts to preserveendothelial barrier function. The importance of platelet and erythrocytederived

S1P in modulating vascular permeability is evidenced by reports that describe a high basal leak of proteins in pulmonary microvessels of mutant mice that selectively lack S1P in plasma, and the observation that the increased permeability observed in intact microvessels perfused with an erythrocyte-free solution is reversed following the administration of exogenous S1P.

Platelets also hold the potential to influence endothelial barrier function by forming heterotypic aggregates with leukocytes. For example, platelet-neutrophil aggregates (PNA) have been implicated in the increased pulmonary vascular permeability in mice with sickle cell disease. n this model of human disease, interfering with PNA formation with a P-selectin blocking antibody decreased the lung vascular permeability response. While it is not clear how the aggregate formation leads to altered barrier function, the response may be related to the observation that neutrophils and monocytes with attached activated platelets produce more than twice the amount of superoxide than their platelet-free counterparts, and P-selectin mediated signaling underlies this response. Similarly,

it has been demonstrated that the generation ofplatelet activating factor (PAF) by the combination of platelets and neutrophils is 2-times higher than that detected in either cell activated separately, but this amplification

effect on PAF production results from transcellular phospholipid metabolism between the 2 cells, and does not require cell–cell adhesion. PAF, which is known to increase vascular permeability when engaged with its receptor on endothelial cells, disrupts the inter-endothelial junctions via Rac1-dependent relocation of junctional proteins (e.g., VE-cadherin, ZO-1) and actin polymerization.

Diseases associated with endothelial barrier dysfunction

An injured or dysfunctional endothelial barrier has the potential to significantly impact tissue function and viability. Discontinuities or breaks in the endothelial lining can impair blood flow regulation by interfering with

vasodilatory responses that are dependent on endothelial cell-cell communication (e.g., ascending vasodilation).

Clot formation can also result if the breach in the barrier is sufficient to expose platelets to the collagen layer that normally lies beneath the endothelial cell lining. However, endothelial barrier dysfunction is more commonly associated with subtle changes in the inter-endothelial junctions (discussed above) that can result in the excessive loss of water and proteins into the extravascular compartment. The magnitude of the leakage of fluid and protein that accompanies an increased vascular permeability can lead to edemagenic responses that range from small, reversible and without a long-lasting effect on tissue function to a severe and irreversible response that leads to tissue necrosis and organ failure. The entire range of permeability-dependent edemagenic responses is evidenced in human disease states. As noted in Table 1, increased vascular permeability has been implicated in a variety of pathological conditions, including both acute and chronic diseases. In some conditions, the permeability response is largely manifested in one organ system (e.g., COPD, nephrotic syndrome, Alzheimer

disease) while a more widespread (systemic) permeability response is noted in other diseases (e.g., sepsis, diabetes mellitus).

The contribution of the endothelial barrier dysfunction to disease morbidity and mortality appears to be condition- and organ-dependent. For example, while thevascular permeability increases that accompanies sickle cell disease and hypertension are not likely to contribute significantly to disease induction, progression and/or mortality, a significant contribution to disease outcome may be expected of the endothelial barrier failure that is associated with conditions such as sepsis, acute kidney injury, dengue hemorrhagic fever, and stroke. Two organs that appear to be most vulnerable to the deleterious consequences ofendothelial barrier dysfunction are the brain and lungs. In both tissues, excessive fluid loss across a leaky endothelial cell layer has the potential to profoundly impact organ function and/or viability. This is commonly manifested in the lungs as an accumulation of interstitial fluid in the alveolar spaces (pulmonary edema), which results when the alveolar membrane is ruptured due to excessive interstitial fluid accumulation (and an elevated interstitial pressure) secondary to capillary fluid leakage. A similar phenomenon has been described in the intestine, with excessive capillary fluid and protein leakage resulting in mucosal barrier disruption and the movement of interstitial fluid in the gut lumen. However, the response in gut is not as immediately life-threatening as pulmonary edema, which impairs gas exchange

and may cause respiratory failure. The rapidly evolving and often fatal (despite mechanical ventilation) pulmonary edema that is associated with Hantavirus infection likely results from endothelial barrier failure.

The structurally unique and highly restrictive endothelial barrier in the brain offers a level of tissue protection that is beyond that manifested in other organs. The BBB is largely impermeable to water, ions, plasma proteins, inflammatory mediators (e.g., cytokines), immune cells, and a variety of drugs. Consequently, BBB disruption in the brain can be associated with more profound local and systemic detrimental effects than observed in other tissues following endothelial

barrier failure. The fact that the brain is encased in a vault (the skull) results in

significantly larger increases in interstitial pressure when high fluid filtration rates result from BBB failure, which can result in blood vessel compression and blood flow restriction. Macrophages that normally reside in the brain, like microglia and astrocytes, no longer enjoy an “immunoprivileged” environment following BBB disruption. Consequently, inflammatory response elicited by a pathological insult is greatly amplified when the BBB loses its ability to impede the egress of immune cells and mediators. Many of these manifestations of BBB dysfunction are evidenced following an ischemic stroke and this response is believed to promote expansion of the infarcted area. While restoration of BBB function has gained attention as a potentially useful therapeutic goal in stroke patients, BBB disruption has also been exploited for enhanced delivery of imaging agents to optimize the detection and quantification of brain edema and infarct size following stroke.__

THE GLANDULAR EPITHELIUM. SECRETORY CYCLE. (3 hours)

This tissue has a free surface, which faces either a body fluid or the outside environment and thus provides a covering or a lining for some part of thebody. The cells are compactly packed with littleintercellular matrix. There are two types of epithelial tissues namely simple epithelium andcompound epithelium. Simple epithelium is composed of a single layer of cells and functions as a lining for body cavities, ducts, and tubes. The compound epithelium consists of two or more cell layers and has protective function as it does in our skin.On the basis of structural modification of the cells, simple epitheliumis further divided into three types. These are (i) Squamous, (ii) Cuboidal,(iii) Columnar (iv)Ciliated.

The squamous epithelium:it is made of a single thin layer of flattened cells with irregular boundaries. They are found in the walls of blood vessels and air sacs of lungs and are involved in a functions like forming a diffusionboundary.

The cuboidal epithelium:It is composed of a single layer of cube-like cells. This is commonly found in ducts of glands and tubular parts of nephrons in kidneys and its main functions are secretion and absorption. The epithelium of

proximal convoluted tubule (PCT) of nephron in the kidney has microvilli.

The columnar epithelium:It is composed of a single layer of tall and slender cells. Their nuclei are located at the base. Free surface may have microvilli. They are found in the liningof stomach and intestine and help in secretion and absorption.

Ciliated epithelium:If the columnar or cuboidal cells bear cilia on their free surface they are called ciliated epithelium.Their function is to move particles or mucus in a specific direction over the epithelium. They are mainly present in the inner surface of hollow organs like bronchioles and fallopian tubes.

Glandular epithelium tissue:Some of the columnar or cuboidal cells get specialised for secretion and are called glandular epithelium

GLANDS

In some organs the epithelium persists as such or as special structures called glands. When glands secrete, they usually produce an aqueous fluid which differs from bllod plasma or tissue fluid. This product of cellular activity is called the secretion. This difference in composition of the secretion and the tissue fluid may manifest itself in the production of new substances present only in the tissue fluid (insulin and other hormones, trypsin and other enzymes, mucin, milk).

Two types of glands exist: endocrine glands and exocrine glands. Glands are composed of single cells or groups of cells specialized for secretion.

All glands arise in early development from lining or covering epithelia.

Exocrine glands are those that keep their connection with the epithelium in the form of aduct. Endocrine glands (ductless glands) lose their connection with the surface and release their secretions into the bloodstream.

Classification of Exocrine Glands.

Exocrine glands may be classified according to their structure, secretory product, or mode of secretion.

1. By structure. Structural classification is based on the number of cells, the type of duct system, and the shape of the secretory portion of the gland.

2. By number of cells. Unicellular glands are single secretory cells

scattered among other cell types in epithelia (e.g., mucus-secreting goblet cells).Multicellular glands occur in 2 forms: Sheet glands, which empty their secretions directly into the lumen of a hollow organ (e.g., glands of the trachea), and solid glands, whose secretions are carried by ducts to the body surface (e.g., sweat glands) or to a lumen (e.g., salivary glands).

Formation of glands from covering epithelia. Epithelial cells proliferate and penetrate connective tissue. They may—or may not—maintain contact with the surface. When contact is maintained, exocrine glands are formed; without contact, endocrine glands are formed. The cells of endocrine glands can be arranged in cords or in follicles. The lumens of the follicles accumulate large quantities of secretions; cells of the cords store only small quantities of secretions in their cytoplasm.

Duct system. The duct system may be simple (unbranched), or compound (branched). Simple ducts may be straight or coiled.

Principal types of exocrine glands. The part of the gland formed by secretory cells is shown in black; the remainder shows the ducts. The compound glands have branching ducts.

Secretory portion The secretory portion of the gland may be tubular (test tube-shaped); alveolar, or acinar (flask-shaped); or tubuloacinar (with acini branching off the straight tubular portion).

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Examples of Structural Classifications of Multicellular Exocrine Glands

Duct System	Secretory Portion	Example
Simple	Tubular	Intestinal crypts of Lieberkuhn
Simple	Coiled tubular	Eccrine sweat glands of the skin
Simple	Branched tubular	Fundic glands of the stomach
Simple	Branched acinar	Sebaceous glands of the skin
Compound	Tubular	Cardiac glands of the stomach

Compound Tubuloacinar Submandibular salivary glands

Compound Acinar Exocrine pancreas

3. By secretory product.

Mucous secretion, or mucus, is a thick secretion containing proteins, chiefly highly glycosylated glycoproteins called mucins or mucin precursors calledmucinogens. Other glycoproteins (eg, membrane glycoproteins) commonly have short, N-linked oligosaccharides attached to asparagine. Mucous glycoproteins have longer, 0-linked oligosaccharide chains attached to hydroxyl groups of serine or threonine. This attachment is mediated by special glycosyltransferases in the Golgi complex of the mucus-secreting cells. Examples of mucus-secreting glands include goblet cells and the sublingual salivary glands.

Serous secretion is a watery secretion containing proteins and glycoproteins. The ex-ocrine pancreas and parotid salivary glands produce serous secretions.

Seromucous secretion is a mixed secretion of intermediate thickness. The submandib-ular salivary gland contains both serous and mucous secretory cells and produces sero-mucous secretions.

4. By mode of secretion

In merocrine secretion (eccrine secretion), the secretory product exits by

exocytosis, with no loss of cytoplasm or membrane. Most secretory cells release their products in this manner. Specific examples include the pancreas and thyroid gland.

In apocrine secretion, the secretory product collects in the cell apex, and the entire apex is released, with some loss of cytoplasm and membrane. Apocrine sweat glands of the skin and mammary glands both employ this type of secretion.

In holocrine secretion, storage of large amounts of secretory products in the cytoplasm is followed by cell lysis. The entire cell is released into the duct. The skin's sebaceous glands are the classic examples for this mode of secretion.

Some Comparisons of Exocrine and Endocrine Glands

Category	Exocrine Glands	Endocrine Glands
Transport of secretions	Typically by ducts. No ducts	Typically by the bloodstream
Cell number	May be unicellular (eg, goblet cells) or multicellular (eg, salivary glands)	May be unicellular (eg, DNES cells) or multicellular (eg, thyroid gland).
Secretory products	Include proteins (eg, digestive enzymes), glycoproteins (e.g., mucus),and some mixtures containing lipids (e.g., sebum, bile, apocrine sweat, and milk)	Hormones of 2 main types peptide hormones (eg, insulin) and steroid hormones (eg, adrenocorticoids). Plasma proteins produced by the liver (e.g., serum albumin and clotting factors) are also considered endocrine secretory products.
Mode of secretion	Merocrine (by exocytosis, no loss of cytoplasm), apocrine (loss of apical cytoplasm); holocrine (entire cell released into duct).	Merocrine only.

Steps of secretory process.

1. Accumulation of necessary precursors.
2. Synthesis and deposition.
3. Extrusion of secretory products.
4. Regeneration of glandular cell.

ENDOCRINE GLANDS

As the endocrine glands develop in the embryo, their connection with the surface epithilium is lost. In some cases the gland consists of sacs lined with epithilium and surrounded by connective tissue. In most cases the the invagination of the epithilium loses its lunen or solid from the very beginning. It is also effectively separated from the epithilial surface, and its cells from a compact mass thoroughly penetrated by a dense network of blood vessels and connective tissue.

As there no excretory ducts, all secretions find their way into the general circulation.

Other endocrine glands, which are not entirely dissociated from the excretory duct system, are called mixed glands. In the liver, for example, the hepatic cells that secrete bile into the duct system also eliminate internal secretions directly into the blood vessels. On the other hand, in the testis and pancreas, one group of cells secretes into the external duct system, while another group passes its internal secretion into the blood.

The endocrine glands secrete their specific products, called hormones,

directly into the blood stream. The endocrine glands are all circumscribed, with minor exceptions. They are thus set aside from numerous other structures believed to produce internal secretions and also important in coordination and integration within the organism. The circumscribed endocrine glands of man are the adrenal, hypophysis, thyroid, parathyroid, islets of Langerhans, and portions of the testis and ovary. Other glands, which resemble these morphologically in some respects, but do not produce any known secretion, are the pineal body and the paraganglia.

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Endocrine glands are subject to control by the central nervous system, by other endocrine glands, by certain metabolites, or by a combination of these factors.There is, then, a complicated series of endocrine interrelationships, which are highly important.

MAJOR TYPES OF EPITHELIAL

CELLS Epithelial Cells Specialized for Transport:

1. Ion-transporting cells. Some epithelial cells are specialized fortranscellular transport, ie, they can pump ions across their entire thickness, apex to base. Sheets of such cells form active barriers that control ion and water concentrations in body compartments. Tight junctions are often found between the cells and appear to prevent backflow. Ion-transporting cells typically have highly infolded basal plasma membranes that inter-digitate with numerous mitochondria.

+ +

Commonly, the ion pump is specific for sodium (ie, it is Na /K -ATPase), and chloride ions and water follow the sodium-ion flow passively. Some iontransporting

epithelia exploit this mechanism to concentrate other solutes by moving water from one compartment to another. Important ion-transporting epithelia are found in the kidney tubules, the striated ducts of the salivary glands, the gallbladder, the choroid plexus and the ciliary body of the eye.

2. Cells that transport by pinocytosis. Epithelial cells specialized for pinocytosis have tight junctions and abundant pinocytotic vesicles. The vesicles transport substances across the cell from the luminal surface to the basal surface, where the vesicle contents are released. The best example is the endothelial cells lining the blood vessels, where transcellular transport is rapid (2-3 minutes).

Epithelial Cells Specialized for Absorption:

Specialized absorptive cells lining the digestive tract (especially the small intestine) have numerous microvilli on their apical surfaces to increase the exposed area. Small nutrient molecules diffuse into the microvilli, and contraction of the microfilaments shortens the microvilli, bringing the nutrients into the cytoplasm. Other nutrients are pinocytosed between microvilli. Absorptive cells with similarspecializations occur in the proximal tubules of the kidney.

Epithelial Cells Specialized for Secretion:

1. Protein-secreting cells. Cells that synthesize proteins for segregation and secretion have abundant basophilic rough endoplasmic reticulum, a well-developed Golgi complex, and, frequently, an accumulation of secretory granules in the cell apex. Proteins secreted by epithelial cells include the digestive enzymes, produced by pancreatic acinar cells and the chief cells of the stomach; serum albumin, produced by liver hepatocytes; and protein hormones, eg, parathyroid hormone produced by the chief cells of the parathyroid gland.

2. Polypeptide-secreting cells. Secreted polypeptides have fewer amino

acids than the secreted proteins just mentioned. Polypeptide-secreting cells have a small amount of rough endoplasmic reticulum, a supranuclear Golgi complex, and an accumulation of 100- to 400-nm secretory granules in their bases. These APUD cells (amine precursor uptake and decarboxylation) characteristically concentrate important bioactive amines such as epinephrine, norepinephrine, and serotonin in

their cytoplasm. They may absorb these amines from the bloodstream or synthesize them from amino acid precursors by means of amino acid decarboxylases, also found in high concentrations in these cells. Most APUD cells are unicellular glands scattered among other epithelial cells. The number, variety, and wide distribution of cells with these characteristics has generated the concept of the diffuse neuroffndocrine system (DNES). DNES is becoming the preferred designation, but DNES and APUD refer to the same polypeptide-secreting cells. Some APUD polypeptide hormone cells have paracrine effects on neighboring cells; others are released into the bloodstream and have endocrine effects on distant cells. Some important APUD polypeptides are glucagon, from pancreatic islet A cells; insulin, from pancreatic islet B cells; gastrin, from the stomach, small intestine, and pancreatic islet G cells; and somatostatin, from the stomach, small intestine, and pancreatic islet D cells. Tumors composed of APUD cells are called apudomas.

3. Mucous cells. Mucus-secreting cells occur as unicellular, sheet, or solid glands. His-tologic features of these cells include a light-staining, foamy appearance caused by numerous large mucus-containing vesicles concentrated near the cell apex; PAS-positive staining from an abundance of oligosaccharide residues; predominantly acidophilic staining with H&E; a large supranuclear Golgi
complex with distinctive glycosyltransferases; and nuclei and sparse rough endoplasmic reticulum in the base of the cell. Goblet cells and mucous cells of the sublingual salivary glands are examples of unicellular and multicellular mucous glands, respectively.

4. Serous cells. Cells that produce serous secretions have characteristics of

protein-secreting cells. They are usually smaller, darker-staining, and more basophilic than mucus-secreting cells. Serous cells include pancreatic acinar cells and secretory cells of the parotid salivary glands.

5. Steroid-secreting cells. Endocrine cells specialized to secrete steroid hormones are polygonal or rounded, with a central nucleus and pale-staining, acidophilic cytoplasm that often contains numerous lipid droplets. Their abundant smooth endoplasmic reticulum contains enzymes for cholesterol synthesis and for converting steroid hormone precursors (eg, pregnenolone) into specific hormones (eg, androgens, estrogens, and progesterone). Their numerous mitochondria typically have tubular rather than shelflike cristae and contain enzymes that convert cholesterol to pregnenolone. Steroid hormones include testosterone, produced by interstitial cells of the testes; estrogen, from follicle cells of the ovaries; progesterone, from granulosa lutein cells of the corpus luteum; and cortisone and aldosterone, from cells of the adrenal cortex.

THROMBOSIS. STAGES AND MECHANISMS

Blood is fluid tissue of human body, classified as a tissue of inner environment. However, its intercellular substance is a liquid, and its cells are not in a fixed position as is the case in other tissues. The blood of adult vertebrates is a red liquid, which circulates in a closed system of tubes, the blood vessels. It is pumped from the heart into arteries, from the arteries into capillaries, and from the capillaries it flows into veins for return to the heart. Each system of the human body plays an important part in maintaining homeostasis in the internal cellular environment, but the movement of the blood through the circulatory system is of fundamental importance. The chief function of the blood is to maintain normal cell function by the constant exchange of nutrients and wasted with all cells. The blood must also maintain optimum pH and temperature of the intracellular fluid if the cells' enzyme systems are to work

efficiently. The blood transports oxygen from the lungs to the tissues, and carbon dioxide to the lungs for elimination. It transport nutrients from the intestine to all parts of the body, and it carries certain waste products to the kidneys for excretion. The blood distributes the heat produced in active muscles and thus aids in the regulation of body temperature. It transports internal secretions from the glands in which they are produced to the tissues on which each exerts its effects.

The buffers in the blood help to maintain acid – base balance. The blood also is involved in immunity to disease and in protecting the body against invading bacteria.

Platelets: Platelets, or thrombocytes, the smallest formed elements in the blood, are dislike cell fragments that vary in diameter from 2 to 5 μm. In humans, they lack nuclei and originate by budding from large cells in the bone marrow called megakaryocytes. They range in number from 150,000 to 300,000 microliter of blood

and have a lifespan of about 10 days. In blood smears they appear in clumps. Each platelet has a peripheral hyalomere region that stains a faint blue and a dense central

granulomere that contains a few mitochondria and glycogen granules and a variety of

purple granules. Dense bodies, or delta granules, are 250- 300 μm in diameter and contain calcium ions, pyrophosphate, ADP, and ATP; they take up and store serotonin. Alpha granules are 300-500 μm in diameter and contain fibrinogen, platelet-derived growth factor, and other platelet-specific proteins. Lambda granules

(platelet lysosomes) are 175-200 μm in diameter and contain only lysosomal enzymes. The hyalomere contains a marginal bundle of microtubules that helps to maintain the platelet's discoid shape. The glycocalyx is unusually rich in glycosaminoglycans and is associated with adhesion, the major functional characteristic of platelets. Platelets have an important physical role in plugging wounds. They promote blood clotting and help repair gaps in the walls of blood vessels, preventing loss of blood. Electron micrograph of human platelets. x40,740.

The Role of Platelets is controlling hemorrhage can be summarized as follows.

1. Primary aggregation – Discontinuities in the endothelium, produced by blood vessel lesions, are followed by absorption of plasma proteins on the subjacent collagen. Platelets immediately aggregate on this damaged tissue, forming a platelet plug.

2. Secondary aggregation – Platelets in the plug release the contents of their alpha and delta granules. ADP is a potent inducer of platelet aggregation.

3. Blood coagulation – Platelets release fibrinogen in addition to that normally found in the plasma. The fibrinogen is converted by the clotting factor cascade into fibrin, which forms a dense fibrous mat to which more platelets and other blood cells attach to form blood clot or thrombus.

4. Clot Retraction: The clot (thrombus) that initially bulges into the blood vessel lumen contracts because of the interaction of platelet actin, myosin, and ATP.

5. Clot Removal: Protected by the clot, the vessel wall is restored by new tissue formation. The clot is then removed, mainly by the proteolytic enzyme plasmin, formed, through the activation of the plasma proenzyme plasminogen activators. Enzymes released from platelet lambda granules also contribute to clot removal. A marked reduction in the number of blood platelets is called thrombocytopenia, and a marked increase in the number of blood platelets is calledthrombocytosis.

WHITE BLOOD CELLS. THE MECHANISMS OF ADHESION, MIGRATION AND KILLING MICROORGANISMS.

White blood cells (abbreviated WBCs, also called leukocytes from leuko = white +cyte=cell) comprise several distinct cell types, neutrophils, eosinophils, basophils, lymphocytes andmonocytes. Certain developmental and morphological similarities permit the first three these cells to be usefully grouped together as granulocytes or polymorphonuclear leukocytes. The latter two types are then categorized as mononuclear leukocytes.

Granulocytes, also called polymorphonuclear leukocytes, have grainy cytoplasm and

elongated or lobed nuclei. They arise in bone marrow from cells called myeloblasts

and pass through stages called myelocytes and metamyelocytes. The granulocytes

include neutrophils, eosinophils, and basophils.

Neutrophils (also called neutrophilic granulocytes, or

polymorphonuclear neutrophilic leukocytes, PMNs, or polys) are the most

numerous of the leukocytes, about 60% of the white blood cell count. They are about

12 ��m in diameter in blood smear preparations (about twice the size of red blood

cells). Neutrophils take their name from the staining properties of their cytoplasmic

lysosomal granules (vesicles containing stored lysosomal enzymes). These granules

are neutrophilic, meaning they show no special affinity for either acidic or basic

stains but are stained mildly by both. (This is in contrast to the specific granules of

eosinophils, which stain red with acidic stains such as eosin, and those of basophils,

which stain with basic stains.)

The nuclei of mature neutrophils are elongated and pinched into several distinct

lobes, hence the term polymorphonuclear. This characteristic nuclear structure

confers a distinctive appearance on neutrophils, both in blood smears and in tissue

sections. Immature neutrophils have a band-shaped nucleus and are hence sometimes

called "bands". Mature neutrophils, in contrast, are called "segs", in reference to the

segmented nucleus.

Neutrophils are anti-bacterial cells which lyse (break down) bacterial cells by

releasing the lysosomal enzymes which are stored in their specific granules.

Neutrophils gather rapidly in peripheral tissue during acute inflammation, by

emigrating from the blood. There they recognize bacteria as foreign by the antibodies

which have attached to the bacterial surface. [Antibodies are molecules found in

blood plasma and interstitial fluid which bind to specific foreign antigens.]

Neutrophils are only occasionally seen in normal tissue sections outside blood. Here

they may be most easily recognized by their lobed nuclei. One neutrophil nucleus

might be mistaken for a cluster of very small nuclei, but each of the lobes is much too

small to be an entire nucleus -- only two or three ��m across, much smaller than the

nuclei of lymphocytes which are among the smallest of our cells.

Eosinophils (eosinophilic granulocytes) normally comprise less than two to four

percent of the peripheral leukocytes. Their specific granules are intense eosinophilic

(stained by eosin), hence the name. Eosinophils are about the same size as

neutrophils. Their nuclei are typically band shaped (elongated) or twolobed. The

function of eosinophils remains obscure, although they are known to proliferate in

association with allergies and parasites.

Basophils (basophilic granulocytes) normally comprise less than 1 % of the

peripheral leukocytes. Their specific granules are intense basophilic, hence the name.

Like eosinophils, basophils are similar in size to neutrophils. Their nuclei may be

band shaped or segmented. Basophils seem to be functionally similar to tissue mast

cells, involved in triggering inflammation.

Mononuclear leukocytes comprise both lymphocytes and monocytes. Both cell

types work together in immune responses.

Monocytes are the largest of the leukocytes, and constitute about 5 % of the WBC

population in peripheral blood. In blood smears, their nuclei are typically indented,

sometimes deeply so, with a kidney-bean or bent-horseshoe shape. Monocytes belong

to the same functional population as tissue macrophages. Monocytes/macrophages

engulf and digest foreign microorganisms, dead or wornout cells, and other tissue

debris. They interact closely with lymphocytes to recognize and destroy foreign

substances. Most ordinary connective tissues contain resident macrophages which normally remain at rest in the tissue. But the normal number of fixed macrophages is supplemented during inflammation by the influx of many monocytes from the blood.

Lymphocytes are small cells, 7-9 ��m in diameter in blood smears, and are the second most common white blood cell type, comprising about 30 % of the leukocyte population in peripheral blood. Lymphocytes have a round heterochromatic (deeply staining) nucleus surrounded by a relatively thin rim of cytoplasm. (Example fromWebPath; another example from WebPath; electron micrograph at WebPath.) Lymphocytes travel in the blood, but they routinely leave capillaries and wander through connective tissue. Therefore, lymphocytes may be normally encountered at any time in any location. They even enter epithelial tissue, crawling between the epithelial cells. They reenter circulation via lymphatic system channels (hence their name).

Lymphocytes also emigrate from blood in response to inflammation, but they accumulate somewhat later during the inflammatory process than neutrophils. Their presence in large numbers indicates the continuing presence of antigen. Lymphocytes produce the multitude of diverse antibody molecules (one specific type of antibody per lymphocyte) which provide the mechanism for chemical recognition of foreign materials (distinguishing between self and non-self) and so for mediating and regulating immune responses.

Lymphocytes are most easily recognized in histological sections as small "naked" nuclei (the cytoplasm is usually inconspicuous) which occur here and there in most connective tissues, especially commonly near mucous membranes. Lymphocytes are found densely packed in lymphoid tissue--spleen, lymph nodes, and lymph nodules in

mucous membranes (e.g., tonsils, appendix), where they proliferate.

Plasma cells are lymphocytes which are specialized for mass production and secretion of circulating antibodies. Plasma cells have more extensive cytoplasm filled with rough endoplasmic reticulum (for synthesizing protein, specifically antibody molecules). This cytoplasm is distinctly basophilic, a consequence of the large numbers of ribosomes associated with the rER, and typically forms a lopsided bulge on one side of the nucleus. The heterochromatin of plasma cells is typically clumped in a characteristic "spoke-wheel" arrangement which also aids plasma cell recognition.

Wandering leukocytes and mast cells

One of the greatest deficiencies in histological training cited by pathologists is the inability of students to identify wandering leukocytes (leukocytes outside of the the bloodstream). Of course, when pathologists view wandering leukocytes it is usually under pathological conditions and these vagabond cells often appear in great numbers. Identifying wandering leukocytes under normal conditions is much more challenging.

Leukocytes outside of the blood stream can appear quite different than in blood smears. This is partly because of the different techniques used to prepare the tissue: Blood smears are simply air-dried, stained with alcohol-based dyes (typically a variety of the Romanovsky stains), and, ideally, are spread to a one cell thick layer. In contrast, tissue sections are formalin fixed, dehydrated with alcohol (significantly shrinking the cells), stained with H&E (which does not optimally differentiate cytoplasmic granules), and are typically more than one cell layer thick. Additionally, monocytes and B-lymphocytes undergo a radical metamorphosis after exiting the

blood stream to become macrophages and plasma cells, respectively. All of these factors combine to require different criteria for identifying leukocytes outside of the blood stream.

Lymphocytes appear as small (7-8 microns), round, heterochromatic nuclei with little or no cytoplasm. They can occur in large clusters as lymphatic nodules. Plasma cells (transformed B lymphocytes) have a basophilic cytoplasm (ergastoplasm) and an eccentric nucleus with heterochromatin distributed around the perimeter (i.e., a clock-face nucleus). Adjacent to the nucleus a Golgi shadow can often be observed. The amount of cytoplasm (and hence size and shape of the cell) is variable, depending upon the level of synthetic activity. These cells are very common next to secretory epithelia. Eosinophils have bi-lobed nuclei and strongly eosinophilic granules. These cells are often found in conjunction with plasma cells.

Neutrophils are distinguishable by their polymorphic (multi-lobed, but beware of orientation!) nuclei and distinctly pink staining cytoplasm. In many tissues macrophages have a characteristic position which makes their identification somewhat simpler (e.g., alveolar macrophages of the lung, hepatic sinusoidal macrophages of the liver, subcapsular macrophages of the lymph nodes). In other tissues, macrophages have an extremely variable appearance depending upon their location (loose versus dense connective tissue) and state of activity (dormant versus active; empty versus debris-filled). In loose connective tissue, active macrophages appear as plumb cells with a faint eosinophilic cytoplasm and amoeboid borders (due to filipodia and lamellipodia). The nucleus is large and often irregularly shaped. Debris can sometimes be observed within the cell. Mast cells technically are not wandering leukocytes although they share a common lineage with basophils. No anatomist in his right mind would attempt to quantify mast cells using H&E staining

(we leave such folly to pathologists). Mast cells are shown to much better advantage

when stained with metachromatic dyes such as toluidine blue which as seen here renders the histamine granules a striking purple and all other non-metachromatic tissue blue. Contrast this image with the corresponding field of view stained with H&E. These sections are from the mouse pinna (external ear) whose dermis for

reasons unknown contains a high density of mast cells and macrophages.

Demonstration slides of this tissue with both stains is available for evaluation. Like macrophages, the shape of mast cells can be

constrained by their location. When unconstrained, the cells have an oval to round shape with an oval- to round-shaped central nucleus as seen here in the mammary gland (s78). The cytoplasmic granules can be difficult to observe in H&E stained

sections and the cytoplasm can range from darkly eosiniophilic to slightly basophilic.

COOPERATION OF BLOOD CELLS AND CELLS OF CONNECTIVE TISSUE DURING INFLAMMATION

Inflammation, a response triggered by damage to living tissues. The inflammatory response is a defense mechanism that evolved in higher organisms to protect them from infection and injury. Its purpose is to localize and eliminate the injurious agent

and to remove damaged tissue components so that the body can begin to heal. The response consists of changes in blood flow, an increase in permeability of blood essels, and the migration of fluid, proteins, and white blood cells (leukocytes) from the circulation to the site of tissue damage. An inflammatory response that lasts only

a few days is called acuteinflammation, while a response of longer duration is referred to as chronic inflammation.

Although acute inflammation is usually beneficial, it often causes unpleasant sensations, such as the pain of a sore throat or the itching of an insect bite.

Discomfort is usually temporary and disappears when the inflammatory response has

done its job. But in some instancesinflammation can cause harm. Tissue destruction

can occur when the regulatory mechanisms of the inflammatory response are

defective or the ability to clear damaged tissue and foreign substances is impaired. In

other cases an inappropriate immune response may give rise to a prolonged and

damaging inflammatory response. Examples includeallergic, or hypersensitivity,

reactions, in which an environmental agent such as pollen, which normally poses no

threat to the individual, stimulates inflammation, and autoimmune reactions, in which

chronicinflammation is triggered by the body's immune response against its own

tissues.

The factors that can stimulate inflammation include microorganisms, physical agents,

chemicals, inappropriate immunological responses, and tissue death. Infectious

agents such as viruses and bacteria are some of the most common stimuli of

inflammation. Viruses give rise toinflammation by entering and destroying cells of

the body; bacteria release substances called endotoxins that can initiate inflammation.

Physical trauma, burns, radiation, and frostbite can damage tissues and also bring

about inflammation, as can corrosive chemicals such as acids, alkalis, and oxidizing

agents. As mentioned above, malfunctioning immunological responses can incite an

inappropriate and damaging inflammatory response. Inflammation can also result

when tissues die from a lack of oxygen or nutrients, a situation that often is caused by

loss of blood flow to the area.

The four cardinal signs of inflammation—redness (Latin rubor), heat(calor), swelling

(tumor), and pain (dolor)—were described in the 1st century AD by the Roman

medical writer Aulus Cornelius Celsus. Redness is caused by the dilation of small blood vessels in the area of injury. Heat results from increased blood flow through the area and is experienced only in peripheral parts of the body such as the skin. Fever is brought about by chemical mediators of inflammation and contributes to the rise in temperature at the injury. Swelling, calledедема, is caused primarily by the accumulation of fluid outside the blood vessels. The pain associated with inflammation results in part from the distortion of tissues caused by edema, and it also is induced by certain chemical mediators of inflammation, such as bradykinin,serotonin, and the prostaglandins.

A fifth consequence of inflammation is the loss of function of the inflamed area, a feature noted by German pathologist Rudolf Virchow in the 19th century. Loss of function may result from pain that inhibitsmobility or from severe swelling that prevents movement in the area.

The Acute Inflammatory Response.

Vascular changes

When tissue is first injured, the small blood vessels in the damaged area constrict momentarily, a process called vasoconstriction. Following thistransient event, which is believed to be of little importance to the inflammatory response, the blood vessels dilate (vasodilation), increasing blood flow into the area. Vasodilation may last from 15 minutes to several hours. Next, the walls of the blood vessels, which normally allow only water and salts to pass through easily, become more permeable. Protein-rich fluid, called exudate, is now able to exit into the tissues. Substances in the exudate include clotting factors, which help prevent the spread of infectious agents throughout the body. Other proteins include antibodies that help destroy invading microorganisms.

As fluid and other substances leak out of the blood vessels, blood flow becomes more

sluggish and white blood cells begin to fall out of the axial stream in the centre of the

vessel to flow nearer the vessel wall. The white blood cells then adhere to the blood

vessel wall, the first step in their emigration into the extravascular space of the tissue.

Cellular changes

The most important feature of inflammation is the accumulation of white blood cells

at the site of injury. Most of these cells are phagocytes, certain “celleating”

leukocytes that ingest bacteria and other foreign particles and also clean up cellular

debris caused by the injury. The main phagocytes involved in acute inflammation are

the neutrophils, a type of white blood cell that contains granules of cell-destroying

enzymes and proteins. When tissue damage is slight, an adequate supply of these

cells can be obtained from those already circulating in the blood.

But, when damage is extensive, stores of neutrophils—some in immature form—

are released from the bone marrow, where they are generated. To perform their tasks,

not only must neutrophils exit through the blood vessel wall but they must actively

move from the blood vessel toward the area of tissue damage. This movement is

made possible by chemical substances that diffuse from the area of tissue damage and

create a concentration gradient followed by the neutrophils. The substances that

create the gradient are called chemotactic factors, and the one-way migration of cells

along the gradient is called chemotaxis.

Large numbers of neutrophils reach the site of injury first, sometimes within an hour

after injury or infection. After the neutrophils, often 24 to 28 hours after

inflammation begins, there comes another group of white blood cells, the monocytes,

which eventually mature into cell-eating macrophages. Macrophages usually become

more prevalent at the site of injury only after days or weeks and are a cellular

hallmark of chronic inflammation.

Chemical mediators of inflammation

Although injury starts the inflammatory response, chemical factors released upon this

stimulation bring about the vascular and cellular changes outlined above. The

chemicals originate primarily from bloodplasma, white blood cells (basophils,

neutrophils, monocytes, and macrophages), platelets, mast cells, endothelial cells

lining the blood vessels, and damaged tissue cells.

One of the best-known chemical mediators released from cells duringinflammation is

histamine, which triggers vasodilation and increases vascular permeability. Stored in

granules of circulating basophils and mast cells, histamine is released immediately

when these cells are injured. Other substances involved in increasing vascular

permeability are lysosomal compounds, which are released from neutrophils.

Manycytokines secreted by cells involved in inflammation also have vasoactive and

chemotactic properties.

The prostaglandins are a group of fatty acids produced by many types of cells. Some

prostaglandins increase the effects of other substances that promote vascular

permeability. Others affect the aggregation of platelets, which is part of the clotting

process. Prostaglandins are associated with the pain and fever of inflammation. Anti-

inflammatorydrugs, such as aspirin, are effective in part because they inhibit

anenzyme involved in prostaglandin synthesis. Prostaglandins are synthesized from

arachidonic acid, as are the leukotrienes, another group of chemical mediators that

have vasoactive properties.

The plasma contains four interrelated systems of proteins—complement, the kinins,

coagulation factors, and the fibrinolytic system—that generate various mediators of

inflammation. Activated complement proteins serve as chemotactic factors for neutrophils, increase vascular permeability, and stimulate the release of histamine from mast cells. They also adhere to the surface of bacteria, making them easier targets for phagocytes. The kinin system, which is activated by coagulation factor XII, produces substances that increase vascular permeability.

The most important of the kinins is bradykinin, which is responsible for much of the

pain and itching experienced with inflammation. The coagulation system converts the

plasma protein fibrinogen into fibrin, which is a major component of the fluid exudate. The fibrinolytic system contributes to inflammation primarily through the formation of plasmin, which breaks down fibrin into products that affect vascular permeability.

Once acute inflammation has begun, a number of outcomes may follow. These include healing and repair, suppuration, and chronicinflammation. The outcome depends on the type of tissue involved and the amount of tissue destruction that has occurred, which are in turn related to the cause of the injury.

Healing and repair

During the healing process, damaged cells capable of proliferation regenerate. Different types of cells vary in their ability to regenerate. Some cells, such as epithelial cells, regenerate easily, whereas others, such as liver cells, do not normally

proliferate but can be stimulated to do so after damage has occurred. Still other types

of cells are incapable of regeneration. For regeneration to be successful, it is also necessary that the structure of the tissue be simple enough to 64 reconstruct. For example, uncomplicated structures such as the flat surface of the skin are easy to rebuild, but the complex architecture of a gland is not. In some cases, the failure to

replicate the original framework of an organ can lead to disease. This is the case in cirrhosis of the liver, in which regeneration of damaged tissue results in the construction of abnormal structures that can lead to hemorrhaging and death.

Repair, which occurs when tissue damage is substantial or the normal tissue architecture cannot be regenerated successfully, results in the formation of a fibrous scar. Through the repair process, endothelial cells give rise to new blood vessels, and

cells called fibroblasts grow to form a loose framework of connective tissue. This delicate vascularized connective tissue is called granulation tissue. It derives its name from the small red granular areas that are seen in healing tissue (e.g., the skin beneath a scab). As repair progresses, new blood vessels establish blood circulation in the healing area, and fibroblasts produce collagen that imparts mechanical strength to the growing tissue. Eventually a scar consisting almost completely of densely packed collagen is formed. The volume of scar tissue is usually less than that of the tissue it replaces, which can cause an organ to contract and become distorted. For example, scarring of the intestines can cause the tubular structure to become obstructed through narrowing. The most dramatic cases of scarring occur in response to severe burns or trauma.

The process of pus formation, called suppuration, occurs when the agent that provoked the inflammation is difficult to eliminate. Pus is a viscous liquid that consists mostly of dead and dying neutrophils and bacteria, cellular debris, and fluid leaked from blood vessels. The most common cause of suppuration is infection with the pyogenic (pus-producing) bacteria, such as Staphylococcus and Streptococcus.

Once pus begins to collect in a tissue, it becomes surrounded by a membrane, giving rise to a structure called an abscess. Because an abscess is virtually inaccessible to antibodies and antibiotics, it is very difficult to treat.

Sometimes a surgical incision is necessary to drain and eliminate it. Some abscesses, such as boils, can burst of their own accord. The abscess cavity then collapses, and the tissue is replaced through the process of repair.

Chronic inflammation

If the agent causing an inflammation cannot be eliminated, or if there is some interference with the healing process, an acute inflammatory response may progress to the chronic stage. Repeated episodes of acuteinflammation also can give rise to chronic inflammation. The physical extent, duration, and effects of chronic inflammation vary with the cause of the injury and the body's ability to ameliorate the damage.

In some cases, chronic inflammation is not a sequel to acuteinflammation but an independent response. Some of the most common and disabling human diseases, such as tuberculosis,rheumatoid arthritis, and chronic lung disease, are characterized by this type of inflammation. Chronic inflammation can be brought about by infectious organisms that are able to resist host defenses and persist in tissues for an extended period. These organisms include Mycobacterium tuberculosis (the causative agent of tuberculosis), fungi, protozoa, and metazoal parasites. Other inflammatory agents are materials foreign to the body that cannot be removed by phagocytosis or enzymatic breakdown. These include substances that can be inhaled, such as silica dust, and materials that can gain entry to wounds, such as metal or wood splinters. In autoimmune reactions the stimulus to chronic inflammation is a normal component of

the body to which the immune system has become sensitized. Autoimmune reactions

give rise to chronic inflammatory diseases such as rheumatoid arthritis.

The hallmark of chronic inflammation is the infiltration of the tissue site by

macrophages, lymphocytes, and plasma cells (mature antibody-producing B

lymphocytes). These cells are recruited from the circulation by the steady release of

chemotactic factors. Macrophages are the principal cells involved in chronic

inflammation and produce many effects that contribute to the progression of tissue

damage and to consequent functional impairment. Granulomatous inflammation is a

distinct type of chronic inflammation. It is marked by the formation of granulomas,

which are small collections of modified macrophages called epithelioid cells and are

usually surrounded by lymphocytes.

Granulomas often contain giant, or Langhans, cells that form from the coalescence of

epithelioid cells. A classic example of granulomatous inflammation is tuberculosis,

and the granulomas formed are called tubercles. Granulomas also typically arise from

fungal infections, and they are present in schistosomiasis, syphilis, and rheumatoid

arthritis.

REPARATION OF LOOSE CONNECTIVE TISSUE. REGULATION

OF VOLUME AND STRUCTURE OF THE MATRIX OF CONNECTIVE

TISSUE

Connective tissues (including cartilage and bone are derived from mesoderm or

mesectoderm (for the head) of the embryo, via an intermediate stage called

mesenchyme.

Mesenchyme consists of pale cells, with extended processes, lying in jellylike matrix.

In later development, the cells and extracellular matrix (ECM) become specialized

for various tasks, and the matrix comprises amorphous 'ground substance' reinforced

to greater or lesser extent by specialized fibres. The various cells, fibres, and ground

substances will be discussed, followed by a treatment of the tissues that they combine

to build. Connective tissue from hereon may be abbreviated to CT.

Cells of Connective Tissues

1. Mesenchymal cell

1. Has a similar appearance to a small, young fibroblast, but is far more

multipotential in what cell types it can turn into.

2. In adult tissues, two views are:

o a few are present and can explain such findings as the formation of ectopic (out of

its expected place) bone in soft CT, otherwise difficult to account for unless

differentiated cells such as fibroblasts can dedifferentiate and change their role; o

mesenchymal cells all differentiate early in life and thereafter are not present, and

fibroblasts or other cells can de- and redifferentiate and become osteoblasts.

2. Fibroblast

1. Occurs in young active, and adult quiescent/less active forms.

2. Young has abundant, basophil cytoplasm, with a well-developed Golgi complex

and GER for protein and proteoglycan synthesis.

3. Nucleus is ovoid, with weakly staining chromatin granules.

4. The cell is elongated, and often sends out processes to take on a more elongated or

stellate form.

5. Adult fibroblasts (fibrocytes) have smaller, darker nuclei, and very little cytoplasm.

They remain fixed and squashed into a spindle/cigar form amongst the fibres that

they formed.

6. Function - forming and remodelling collagen, reticular and elastic fibres, and the

ground substances. The remodelling requires the production of destructive enzymes,

and inhibitors to help restrain their action. TIMPs - Tissue Inhibitors of MetalloProteinases - are an example. In some sites, e.g., the periodontal ligament holding the teeth in place, the fibroblasts more aggressively destroy fibres, in the process of matrix turnover.

7. Young fibroblasts, aside from making fibres, may in some circumstances (e.g., wound repair) take on some smooth-muscle characteristics, and become contractile myofibroblasts, which contribute to the disabling contractures of some scar tissue.

3. Reticular/reticulum cells

1. Immunocytochemistry, EM, and enzymatic analysis distinguish at least three kinds

of reticular cell: fibroblastic, and two phagocytic kinds -interdigitating (T-zone:) and

dendritic (B-zone: antigen-presenting).

2. The supporting reticular fibres of lymphoid tissues and bone marrow are presumed

to be produced by the fibroblastic variety.

3. Caution! The principal reticular cell in the thymus is an epithelial kind, although extending cell processes to build a reticulum.

4. Fat cell/adipocyte

1. A genuinely fattened cell, initially resembling a fibroblast with a few droplets in the cytoplasm.

2. For the white or yellow unilocular fat seen in adult man, the droplets (mainly glycerides of fatty acids) coalesce and more fat is added, until the nucleus is bulged to one side of a spheroid cell up to 200 μm in diameter, distended by a huge droplet.

3. Cytoplasm, with a Golgi complex, ER and mitochondria, is present as an attenuated peripheral shell.

4. The cell is static, but its content is not. The stored fat is participating in the body's

carbohydrate and fat metabolism.

5. Fat in the usual wax-imbedded section is dissolved out, but with osmium tetroxide

fixation it remains and is black. Some dyes will colour it, if it is preserved by frozen

sectioning. Besides a number of adipocyte-specific enzymes for fat metabolism, fat cells secrete leptin, which helps control energy balance and body fat mass.

5. Macrophage/histiocyte

1. An ovoid or spheroid cell, which may change its shape while lying alongside fibres, or when extending pseudopodia to move and ingest materials.

2. Phagocytoses dead cells, cell detritis, live and inert foreign bodies.

3. Coordinates the inflammatory response and healing by means of signalling peptides and proteins - cytokines, e.g., IL-1, TGF-beta.

4. Nucleus is smaller and more condensed than that of the active fibroblast.

5. Cytoplasm is pale with little GER, but has many lysosomes, when digesting phagocytosed material.

6. Macrophages may fuse to become foreign-body gigant cells with many nuclei, when faced with a large object for digestion. More on macrophages.

6. Macrophage/reticuloendothelial/mononuclear phagocyte system (MPS)

1. Comprises cells related directly to blood monocytes, or derived from the same precursor in marrow.

2. A tentative division of the macrophage-system cells recognizes: Phagocytic antigen-presenters

o Macrophages of connective tissues and serous cavities. o Alveolar macrophages/lung dust cells.

o Macrophages of lymph nodes, spleen and bone marrow.

o Kupffer sinusoid-lining cells of liver.

Weakly phagocytic antigen-presenters

o Dendritic and interdigitating reticulum cells of lymphoid

tissues. o Langerhans cells of epidermis and other epithelia.

o Foreign-body giant cells.

o Microglia cells of CNS.

o Synovial A cells lining joints.

o Osteoclasts resorbing bone.

3. The phagocytic group (i.e., the original reticulo-endothelial series) can be revealed

by vital injection (into the living animal) of colloidal or particulate coloured matter,

e.g., Trypan blue or India ink, which the phagocytic cells of the system preferentially

accumulate in their cytoplasm, thereby identifying themselves. Nowadays, MPS cells

are distinguished by their cell-surface glycoprotein profiles, e.g., CD antigens.

7. Mast cell

1. A 'watchdog' cell starting the inflammatory response to noxious intruders.

2. From the German verb, mästen, it meant a 'fattened' cell.

3. Spheroid or ovoid with a small central nucleus, and its cytoplasm packed with dense basophil granules.

4. Granules give a metachromatic staining reaction with thionine or toluidine blue, i.e., a reddish-purple colour, because they contain a sulphated polysaccharide - heparin.

5. Heparin is an anticoagulant for blood, first obtained from the liver (hepar), but it also inhibits vascular smooth muscle proliferation and some immune complement reactions. As a polyanion, it cans complex materials, e.g., the trypsin-like enzyme, tryptase, in the granules.

6. Histamine, increasing capillary permeability, is also present in the granules. The chemokines also released can then more easily attract white blood cells out of the vessels.

7. Many stimuli (e.g., antigens and agents released by lymphocytes during an immune response) activate a release of the granule contents, from this 'mobilepharmacy' cell, with its many chemical mediators.

8. Mast cells favour positions in CT close to veins (MCt subtype), and at dermal and

mucosal interfaces with the hostile environments of the skin, airway, and gut (MCtc

subtype).

9. The mast cell subtypes in man differ in the proteases that they contain:

o MCt cells have mast-cell tryptase and are involved directly with defence.

o MCtc cells contain, in addition to tryptase, chymase, cathepsin G, and other

proteases, and are more concerned with adaptive and remodelling responses of blood

vessels and CT.

7. Melanophore/CT pigment cell/CT melanocyte

1. A process-bearing cell with melanin pigment granules in its cytoplasm.

2. Found in the skin's dermis, brain's pia matter and the scleral and choroid coats of

the eye.

8. Plasma cell

1. Many tissues, particularly those lining tracts open to outside the body, are not immunologically virgin, but have been exposed to foreign organisms that have provoked immune responses by local CT plasma cells and lymphocytes. A lamina propria may have many of both and some eosinophils, e.g., in the gut.

2. Plasma cells are ovoid, roughly 10 μm in length, with an eccentrically placed nucleus having its denser chromatin granules clumped regularly around the nuclear membrane (clock-face appearance).

3. Cytoplasm is deeply basophilic from the rich GER, except for a pale central region

where the Golgi complex lies.

4. Proteins synthesized by plasma cells in lymphoid organs reach the plasma as immunoglobulins/ antibodies, inactivating foreign invaders, e.g., viruses.

5. Plasma cells in CT make antibodies for local use, e.g., in the airway or gut, to counter toxins and control microbial populations.

Connective Tissue Cells

Cell Type

Characteristics Useful for Light Microscopic

Major Functions

Identification

Fixed*

Fibroblast

Fusiform cell with cytoplasm that is usually indistinguishable from the surrounding matrix. Tapering processes are present but are difficult to visualize in most sections.

Some very active cells have basophilic cytoplasm. Elliptical nucleus, sometimes slightly folded, with sparse heterochromatin that presents a speckled appearance. One to two nucleoli. Make fibers and ground substance.

Adipocyte

Large cell (up to 200um in diameter) with a flattened nucleus due to the presence of a large flat droplet. TEM shows an external lamina. Lipid reserve.

Macrophage

A cell that can be difficult to distinguish from a fibroblast without the presence of phagocytosed material. Frequently displays a kidney shaped nucleus. Phagocytose, store and present antigens to antibody producing cells.

Mast cell

A relatively large cell frequently found near blood vessels. Round small nucleus (as compared to cell size). Numerous granules show metachromasia with some stains. Release histamine and heparin.

Plasma cell

Eccentrically placed nucleus with round shape. The distinct pattern or heterochromatin frequently presents a cartwheel appearance. Negative Golgi and basophilic cytoplasm are evident. Secretes antibodies.

Reticular cell

This cell has long processes which are sometimes hard to see. The processes cover reticular fibers which are the collagenous stroma of the lymph node.Supporting meshwork in lymph nodes and bone marrow.

Monocytes Indented nucleus frequently lacking nucleoli. Large cell, Precursor to Release some histaminase and phagocytose antigen-antibody complex.

Eosinophil

Phagocyte and antibacterial.

Multilobed nucleus with heterochromatin but no visible cleolus. Some granules in cytoplasm may stain light blue to pink depending on conditions.

Neutrophil nu Active cell of immune system. clear cytoplasm with some vacuoles.

Lymphocyte Small round cell with dark nucleus and only a thin rim of cytoplasm.

macrophage.

Bilobed nucleus in eosinophils from humans. Rodent eosinophils have an annular shaped nucleus and the monkey eosinophils contain a multilobed nucleus. Red granules in cytoplasm.

Fibres of Connective Tissues

1. Collagen fibres

1. Fibres

o Fibres are long, wavy or straight, and colourless.

o They have great tensile strength and resistance to stretching, whilst

retaining considerable flexibility.

o Fibres are made up of finer fibrils packed together.

2. Fibrils

o Fibrils show a characteristic, complex cross-banding pattern regularly along their length with a periodicity of 67 nm (64 nm when shrunken).

o Chemical dissolution of collagen followed by chemical manipulation results in a reforming of fibrils displaying a longer periodicity – a long-spacing collagen, believed to comprise rearranged collagen molecules.

o The fundamental unit is a sequence of 234 amino acids, 67 nm long.

These units in differently staggered arrays account for the various banding patterns of

artificially and naturally occurring collagens, including the main natural collagen

molecule with a length of 300 nm.

o In the natural shorter-spacing fibril, these 300 nm-long units lie end to end, side by

side, but staggered in such a way as to produce regions of greater and lesser density

with the 67 nm periodicity.

3. Collagen molecule and fibril-formation T

o he collagen molecule is made up of three intertwined and cross-linked helical

chains of amino acids, with glycine, proline and hydroxyproline prominent amongst

them. Dependent on the amino-acids, a chain may be alpha 1, types I or II, or alpha 2,

etc. Triplet combinations of these and other chains furnish around 17 types of

collagen.

o Procollagen is made in the GER of the fibroblast, osteoblast, etc. and cleaved to

300 nm collagen at its release from the cell.

o Fibril-formation takes place immediately outside the cell, by self-assembly

followed by a crosslinking crucial to strength.

o Fibrils farther from the cells are thicker (30-300 nm), so presumably the initially

thin fibrils can thicken by additions from soluble collagen to the orderly crystalline

array.

o Various enzymes, e.g., collagenase, break down collagen molecules.

o Man can achieve man-made fibrous strength by repeated spinning, e.g., for hawsers

to tow ships: cells can only spin inside themselves at the molecular level of trimers,

thereafter strength comes solely from crosslinking the molecules and the diverse

glycoprotein and

FACIT-collagen binders.

4. Collagen types: distribution and use

1. All collagen molecules are trimers of helical alpha chains (with two-number

designations) intertwined together, mostly as a robust super-helix, e.g., [alpha-1(II)]3

in cartilage, [alpha1(IV)2alpha2(IV)] in basement membrane.

2. Collagen molecules differ in their amounts of helical versus globular shapes along

the molecule. The ones (fibrillar) that are cleaved to be only helical assemble into

fibrils, the others (non-fibrillar) attach to and space the fibrils, in scaffolds of various

patterns, fibril-widths, densities and strengths, appropriate to the mechanics of the

tissue. Some of the scaffold-glueing ones, e.g., types IX, XII, and XIV, are termed

Fibril-Associated Collagens with InterrupTed helices - FACIT.

3. The types are relatively tissue-specific, but not absolutely so as once was thought.

4. Of the seventeen types, some important ones are:

Type I in bone, fibrocartilage, and established soft connective tissues Type II in

hyaline cartilage

Type III in these same tissues as embryonic or reparative forerunners (and as a minor

mature component) Type IV in basement membranes

Type VII to anchor BMs, and

Type VIII from endothelium lining vessels.

5. Collagen staining

o Collagen (type I) often is present in bulk, and is stained selectively by: aniline blue

in Mallory's method, light green in Masson's, or red acid fuchsin in van Gieson's.

(Eosin stains it orange.)

o Mallory's, Masson's and van Gieson's trichrome methods distinguish collagen from

muscle, and also react with the nuclei and cytoplasm of other cells.

2. Reticular fibres

1. Collagen fibres, running parallel to one another, do not join up with others running

differently. Such an arrangement is seen, however, with reticular fibres, which form a

network or reticulum.

2. Reticular fibres stain black with reduced silver methods, hence their other names -

argyrophil or argentophil. H and E and some trichrome stains leave them unstained.

3. X-ray diffraction and EM show them to be like fine collagen fibres, having the

same 67 nm-repeating crossbanding. Furthermore, they appear first at many sites, as

in mesenchyme and healing wounds, where collagen fibres will later form. Thus

reticular fibres are an immature, fine kind of collagen fibre, mostly of type III

collagen.

4. They persist into the adult in several organs, where a fine fibrous support is needed

that does not interfere with a close relatiom between fixed cells and blood or lymph,

e.g., in endocrine glands.

5. Reticular fibres fasten to the underside of basal laminae of epithelia and

endothelium, and bind and secure muscle and nerve fibres, using their external

laminae.

3. Elastic fibres

1. May be fine, single and branching in areolar CT, or thick and parallel in elastic

ligaments. Walls of blood vessels have incomplete elasticmembranes.

2. The elastic nature of the fibres is shown by the spiralling and kinking of their

recoiled broken ends, in spread preparations.

3. Elastic fibres and membranes, if thick, stain pink with eosin, or red with Masson's

method; otherwise, they remain unseen, unless elastic stains, e.g., orcein or

Verhoeff's, are used.

4. In bulk, unstained, they appear yellow to the naked eye.

5. Formation and nature - fibroblasts and vascular smooth muscle cells form and release two components: (a) fine protein microfibrils thought to orient (b) tropoelastin as it polymerizes into amorphous elastin. With little structure in EM, elastin is a network of long protein chains held in a springy arrangement crosslinked by desmosines, each derived from four lysines of the protein amino-acid chains.

Ground Substances

1. Location

In interstitial/tissue spaces, cartilage and bone matrices, under basal laminae, on and between CT fibres. Ground substance(s) is the extracellular matrix, less the fibrous and fibrillar elements.

2. Nature

Large negatively charged proteoglycan molecules (polyanionic macromolecules) bind to a varying degree water, electrolytes, and other macromolecules, such as collagen, and the glycoproteins, fibronectin and tenascin.

3. Proteoglycan chemistry

From a long protein backbone molecule, many long sugar side chains stick out, because negative charges along each chain repel adjacent chains and each other. The chains are composed of repeating pairs of sugar/saccharide units. Each pair has an hexosamine and a uronic acid. The loss of hydrogen ions from the many acids in the chain of glycosaminoglycans (GAGs) leaves negative charges, only + some of which are neutralized by counterions such as Na .

4. Nomenclature

The many linked sugars of the side-chains are polysaccharides, hence with the protein

backbone the general name - 'protein-polysaccharide'. However, this also describes

glyoproteins, for example, mucoproteins and mucopolysaccarides. Proteoglycans

differ from glycoproteins in: their core proteins; the use of fewer species of sugar;

lack of branching of the sugar chains; and usually their longer sugar chains, and more

acidic/negative character

5. Proteoglycan varieties

Dependent on the specific sugars, and the sites of sulphation, if any:

Hyaluronate - soft connective tissues; synovial fluid; vitreous humour;

Dermatan sulphate (chondroitin sulphate B) - skin and corneal CT;

Keratan suphate - cartilage matrix;

Chondroitin-4-sulphate (A) - cartilage matrix;

Chondroitin-6-sulphate (C) - cartilage matrix:

Heparin (also sulphated) - granules of mast cell and basophil.

6. Staining

The failure of counterions to neutralize all anions leaves regions of high negative

charge density. If the proteoglycan is prevented from dissolving out, its reactions are:

basophil with basic stains, e.g. in hyaline cartilage;

positive with Alcian blue and Hale's iron;

metachromatic, e.g., with toluidine blue, where the blue molecules, in binding to the

closely spaced anions, interfere with each other, so that their effect on light becomes

a red one, e.g., in mast cell granules.

7. Physical properties

The high negative charge:

attracts cations, and restricts the movement of water, ions and molecules (and

bacteria), thus controlling transport through the CT to the cells and other tissues;

structures the matrix, making it gel-like, or even firmer in cartilage, where the

proteoglycans are themselves strung along a hyaluronic acid backbone; binds CT

fibers to one another, influencing their strength and functioning; acts as a reservoir for growth factors and other agents controlling cell behaviour.

8. Overview of proteoglycans (PGs) and glycoproteins in connective tissues

1. The large PG monomer molecules may be aggregated by being strung along a hyaluronate backbone, by means of a link protein for the core protein-HA attachment. PG aggregation produces huge molecules extending over micrometres, and visible with conventional TEM. Proteoglycans amenable to such assembly are aggrecans, susceptible to breakdown by aggrecanase. However, the chemical nature and heterogeneity of monomers and their aggregates make study of these important matrix constituents difficult. Note that proteoglycans are also kept within some cells to work with other molecules.

2. The glycosaminoglycan side chains of proteoglycans vary in number, nature and length. Combinations of sulphated and non-sulphated hexosamines, and relatively tissue-specific core proteins, yield a diversity of PGs, crudely classifiable by molecular size into large and small:

Large

o Chondroitin-6-sulphate, skeletal keratan sulphates - Cartilage

o Versican/Fibroblast PG - Soft CTs

o Cell-surface-associated, e.g., the membrane-attached PGs syndecans, with heparan-sulphate and chondroitin-sulphate chains, and the HSPGs - glypicans - on epithelial and other cells

o Basement-membrane heparan-sulphate PGs - basement membranes

Small

o Decorin/PGII (chondroitin/dermatan sulphates) - extracellular matrix

o Biglycan/PG-S1 (") - associated with a variety of cells including non-CT ones

o Fibromodulin (keratan sulphate)

o Dermatan sulphate

o Small bone proteoglycans I & II

3. Non-collagenous glycoproteins of connective tissues include: Fibronectin, Tenascin, Thrombospondin, Bone sialoprotein/BSPII, Osteopontin/BSPI, Osteonectin/Bone Gla protein, Cartilage-matrix protein, Alkaline phosphatase, Chondronectin, and Fibrillin.

They interact with other macromolecules and influence cell behaviour. One clinical aspect is their use as urinary or serum markers of excessive turnover, e.g., Gla protein

for bone disease. Fibrillin is a crucial component of elastic fibres and other structures

in CTs; and genetic defects in its formation result in the weak arterial walls, poorly suspended eye lens, lax ligaments, etc. of Marfan's syndrome.

4. Fibronectin and Tenascin

o Forms of the glycoprotein, fibronectin, occur in CT matrices, basal laminae and blood plasma.

o It is a multiple adhesive, since various domains of the molecule bind glycosaminoglycans, collagen, fibrin, and some cells.

o Made by fibroblasts and available from blood, it helps in the scaffoldbuilding, and

cellular migrations and attachments, which give tissues their microarchitecture during embryogenesis and wound repair.

o Tenascin shares some structure with fibronectin, but plays its part more during development, e.g., at sites of epithelial-mesenchymal interaction. It reappears in malignant tumours.

Anti-adhesive macromolecules provide another control on cell interactions with the ECM. Tenascin and decorin are glycoprotein and proteoglycan examples.

ARTICULAR CARTILAGE

Articular cartilage is the highly specialized connective tissue of diarthrodial joints. Its

principal function is to provide a smooth, lubricated surface for articulation and to

facilitate the transmission of loads with a low frictional coefficient. Articular cartilage is

devoid of blood vessels, lymphatics, and nerves and is subject to a harsh biomechanical

environment. Most important, articular cartilage has a limited capacity for intrinsic healing

and repair. In this regard, the preservation and health of articular cartilage are paramount

to joint health.

Structure and Composition of Articular Cartilage

Articular cartilage is hyaline cartilage and is 2 to 4 mm thick. Unlike most tissues,

articular cartilage does not have blood vessels, nerves, or lymphatics. It is composed of a

dense extracellular matrix (ECM) with a sparse distribution of highly specialized cells

called chondrocytes. The ECM is principally composed of water, collagen, and

proteoglycans, with other noncollagenous proteins and glycoproteins present in lesser

amounts. Together, these components help to retain water within the ECM, which is

critical to maintain its unique mechanical properties.

Along with collagen fiber ultrastructure and ECM, chondrocytes contribute to the various

zones of articular cartilage—the superficial zone, the middle zone, the deep zone, and the

calcified zone. Within each zone, 3 regions can be identified—the pericellular region, the

territorial region, and the interterritorial region.

Zones

The thin superficial (tangential) zone protects deeper layers from shear stresses and makes

up approximately 10% to 20% of articular cartilage thickness. The collagen fibers of this

zone (primarily, type II and IX collagen) are packed tightly and aligned parallel to the

articular surface. The superficial layer contains a relatively high number of flattened

chondrocytes, and the integrity of this layer is imperative in the protection and

maintenance of deeper layers. This zone is in contact with synovial fluid and is responsible

for most of the tensile properties of cartilage, which enable it to resist the sheer, tensile,

and compressive forces imposed by articulation. Immediately deep to the superficial zone

is the middle (transitional) zone, which provides an anatomic and functional bridge

between the superficial and deep zones. The middle zone represents 40% to 60% of the

total cartilage volume, and it contains proteoglycans and thicker collagen fibrils. In this

layer, the collagen is organized obliquely, and the chondrocytes are spherical and at low

density. Functionally, the middle zone is the first line of resistance to compressive forces.

The deep zone is responsible for providing the greatest resistance to compressive forces,

given that collagen fibrils are arranged perpendicular to the articular surface. The deep

zone contains the largest diameter collagen fibrils in a radial disposition, the highest

proteoglycan content, and the lowest water concentration. The chondrocytes are typically

arranged in columnar orientation, parallel to the collagen fibers and perpendicular to the

joint line. The deep zone represents approximately 30% of articular cartilage volume.

The tide mark distinguishes the deep zone from the calcified cartilage. The deep zone is

responsible for providing the greatest amount of resistance to compressive forces, given

the high proteoglycan content. Of note, the collagen fibrils are arranged perpendicular to

the articular cartilage. The calcified layer plays an integral role in securing the cartilage to

bone, by anchoring the collagen fibrils of the deep zone to subchondral bone. In this zone,

the cell population is scarce and chondrocytes are hypertrophic.

Regions

In addition to zonal variations in structure and composition, the matrix consists of several

distinct regions based on proximity to the chondrocytes, composition, and collagen fibril

diameter and organization. The ECM can be divided into pericellular, territorial, and

interterritorial regions. The pericellular matrix is a thin layer adjacent to the cell

membrane, and it completely surrounds the chondrocyte. It contains mainly proteoglycans,

as well as glycoproteins and other noncollagenous proteins. This matrix region may play a

functional role to initiate signal transduction within cartilage with load bearing. The

territorial matrix surrounds the pericellular matrix; it is composed mostly of fine collagen

fibrils, forming a basketlike network around the cells. This region is thicker than the

pericellular matrix, and it has been proposed that the territorial matrix may protect the

cartilage cells against mechanical stresses and may contribute to the resiliency of the

articular cartilage structure and its ability to withstand substantial loads. The interterritorial

region is the largest of the 3 matrix regions; it contributes most to the biomechanical

properties of articular cartilage. This region is characterized by the randomly oriented

bundles of large collagen fibrils, arranged parallel to the surface of the superficial zone,

obliquely in the middle zone, and perpendicular to the joint surface in the deep zone.

Proteoglycans are abundant in the interterritorial zone.

Chondrocytes

The chondrocyte is the resident cell type in articular cartilage. Chondrocytes are highly

specialized, metabolically active cells that play a unique role in the development,

maintenance, and repair of the ECM. Chondrocytes originate from mesenchymal stem

cells and constitute about 2% of the total volume of articular cartilage. Chondrocytes vary

in shape, number, and size, depending on the anatomical regions of the articular cartilage.

The chondrocytes in the superficial zone are flatter and smaller and generally have a

greater density than that of the cells deeper in the matrix. Each chondrocyte establishes a

specialized microenvironment and is responsible for the turnover of the ECM in its

immediate vicinity. This microenvironment essentially traps the chondrocyte within its

own matrix and so prevents any migration to adjacent areas of cartilage. Rarely do

chondrocytes form cell-to-cell contacts for direct signal transduction and communication

between cells. They do, however, respond to a variety of stimuli, including growth factors,

mechanical loads, piezoelectric forces, and hydrostatic pressures. Unfortunately,

chondrocytes have limited potential for replication, a factor that contributes to the limited

intrinsic healing capacity of cartilage in response to injury. Chondrocyte survival depends

on an optimal chemical and mechanical environment.

Extracellular Matrix

In normal articular cartilage, tissue fluid represents between 65% and 80% of the total

weight. Collagens and proteoglycans account for the remaining dry weight. Several other

classes of molecules can be found in smaller amounts in the ECM; these include lipids,

phospholipids, noncollagenous proteins, and glycoproteins.

Water

Water is the most abundant component of articular cartilage, contributing up to 80% of its

wet weight. Approximately 30% of this water is associated with the intrafibrillar space

within the collagen, although a small percentage is contained in the intracellular space.

The remainder is contained in the pore space of the matrix. Inorganic ions such as sodium,

calcium, chloride, and potassium are dissolved in the tissue water. The relative water

concentration decreases from about 80% at the superficial zone to 65% in the deep zone.

The flow of water through the cartilage and across the articular surface helps to transport

and distribute nutrients to chondrocytes, in addition to providing lubrication. Much of the

interfibrillar water appears to exist as a gel, and most of it may be moved through the

ECM by applying a pressure gradient across the tissue or by compressing the solid matrix.

Frictional resistance against this flow through the matrix is very high; thus, the

permeability of the tissue is very low. It is the combination of the frictional resistance to

water flow and the pressurization of water within the matrix that forms the 2 basic

mechanisms by which articular cartilage derives its ability to withstand significant loads,

often multiple times one’s body weight.

Collagens

Collagen is the most abundant structural macromolecule in ECM, and it makes up about

60% of the dry weight of cartilage. Type II collagen represents 90% to 95% of the

collagen in ECM and forms fibrils and fibers intertwined with proteoglycan aggregates.

Collagen types I, IV, V, VI, IX, and XI are also present but contribute only a minor

proportion. The minor collagens help to form and stabilize the type II collagen fibril

network.

There are at least 15 distinct collagen types composed of no fewer than 29 polypeptide

chains. All members of the collagen family contain a region consisting of 3 polypeptide

chains (α-chains) wound into a triple helix. The amino acid composition of polypeptide

chains is primarily glycine and proline, with hydroxyproline providing stability via

hydrogen bonds along the length of the molecule. The triple helix structure of the

polypeptide chains provides articular cartilage with important shear and tensile properties,

which help to stabilize the matrix.

Proteoglycans

Proteoglycans are heavily glycosolated protein monomers. In articular cartilage, they

represent the second-largest group of macromolecules in the ECM and account for 10% to

15% of the wet weight. Proteoglycans consist of a protein core with 1 or more linear

glycosaminoglycan chains covalently attached. These chains may be composed of more

than 100 monosaccharides; they extend out from the protein core, remaining separated

from one another because of charge repulsion. Articular cartilage contains a variety of

proteoglycans that are essential for normal function, including aggrecan, decorin, biglycan,

and fibromodulin. The largest in size and the most abundant by weight is aggrecan, a

proteoglycan that possesses more than 100 chondroitin sulfate and keratin sulfate chains.

Aggrecan is characterized by its ability to interact with hyaluronan (HA) to form large

proteoglycan aggregates via link proteins Aggrecan occupies the interfibrillar space of the

cartilage ECM and provides cartilage with its osmotic properties, which are critical to its

ability to resist compressive loads.

The nonaggregating proteoglycans are characterized by their ability to interact with

collagen. Although decorin, biglycan, and fibromodulin are much smaller than aggrecan,

they may be present in similar molar quantities. These molecules are closely related in

protein structure; however, they differ in glycosaminoglycan composition and function.

Decorin and biglycan possess 1 and 2 dermatan sulfate chains, respectively, whereas

fibromodulin possesses several keratin sulfate chains. Decorin and fibromodulin interact

with the type II collagen fibrils in the matrix and play a role in fibrillogenesis and

interfibril interactions. Biglycan is mainly found in the immediate surrounding of the

chondrocytes, where they may interact with collagen VI.

Noncollagenous Proteins and Glycoproteins

Although a number of noncollagenous proteins and glycoproteins are found within

articular cartilage, their specific function has not been fully characterized. Some of these

molecules (such as fibronectin and CII, a chondrocyte surface protein) likely play a role in

the organization and maintenance of the macromolecular structure of the ECM.

Metabolism

In adults, the articular cartilage matrix is separated from the subchondral vascular spaces

by the subchondral plate. Nutrition of the articular cartilage occurs by diffusion from the

synovial fluid. The cartilage matrix restricts materials by size, charge, and molecular

configuration. It is estimated that the average pore size within the ECM is approximately

6.0 nm. Without a direct supply of nutrients from blood vessels or lymphatics,

chondrocytes depend primarily on anaerobic metabolism.

Chondrocytes are responsible for the development, maintenance, and repair of the ECM

via a group of degradative enzymes. Chondrocytes synthesize matrix components,

including proteins and glycosaminoglycan side chains. The metabolic activity of the

chondrocytes can be altered by a variety of factors within their surrounding chemical and

mechanical environment. Proinflammatory cytokines (such as interleukin-1 and tumor

necrosis factor−α) have catabolic and anabolic effects that play a role in the degradation

and synthesis of matrix macromolecules.

Proteoglycans are synthesized, maintained, and secreted into the ECM by chondrocytes. A

number of growth factors and regulatory peptides have been implicated in the regulation

of proteoglycan metabolism, including insulin-likegrowth factors, transforming growth

factor−β, interleukin-1, and tumor necrosis factor−α. Very little is known about the

molecular mechanism by which these growth factors and peptides elicit their effects on

proteoglycan metabolism.

Chondrocytes are protected from the potentially damaging biomechanical forces by the

surrounding ECM. A homeostasis of ECM metabolism balances the degradation of the

different macromolecules with their replacement by newly synthesized products.

Proteoglycan turnover can take up to 25 years, whereas the half-life of collagen is

estimated to range from several decades to up to 400 years.

The primary proteinases involved in cartilage turnover include the metalloproteinases

(collagenase, gelatinase, and stromelysin) and the cathepsins (cathepsin B and D).

Collagenase degrades native helical collagen fibrils at a single site. Gelatinase degrades

denatured type II and type IV collagen; it also has significant activity against fibronectin,

elastin, and collagen types V, VII, X, and XI. The role of stromelysin is to degrade the

protein core of aggrecan. All metalloproteinases are secreted as latent proenzymes that

require activation extracellularly. Cathepsins are active in the degradation of aggrecan.

Joint motion and load are important to maintain normal articular cartilage structure and

function. Inactivity of the joint has also been shown to lead to the degradation of cartilage.

Regular joint movement and dynamic load is important for the maintenance of healthy

articular cartilage metabolism. The development of disease such as osteoarthritis is

associated with dramatic changes in cartilage metabolism. This occurs when there is a

physiological imbalance of degradation and synthesis by chondrocytes.

Biomechanical Function

Articular cartilage is a thin layer of specialized connective tissue with unique viscoelastic

properties. Its principal function is to provide a smooth, lubricated surface for low friction

articulation and to facilitate the transmission of loads to the underlying subchondral bone.

Articular cartilage is unique in its ability to withstand high cyclic loads, demonstrating

little or no evidence of damage or degenerative change.

The biomechanical behavior of articular cartilage is best understood when the tissue is

viewed as a biphasic medium. Articular cartilage consists of 2 phases: a fluid phase and a

solid phase. Water is the principal component of the fluid phase, contributing up to 80% of

the wet weight of the tissue. Inorganic ions such as sodium, calcium, chloride, and

potassium are also found in the fluid phase. The solid phase is characterized by the ECM,

which is porous and permeable. The relationship between proteoglycan aggregates and

interstitial fluid provides compressive resilience to cartilage through negative electrostatic

repulsion forces.

The initial and rapid application of articular contact forces during joint loading causes an

immediate increase in interstitial fluid pressure. This local increase in pressure causes the

fluid to flow out of the ECM, generating a large frictional drag on the matrix. When the

compressive load is removed, interstitial fluid flows back into the tissue. The low

permeability of articular cartilage prevents fluid from being quickly squeezed out of the

matrix. The 2 opposing bones and surrounding cartilage confine the cartilage under the

contact surface. These boundaries are designed to restrict mechanical deformation.

Articular cartilage is viscoelastic and exhibits time-dependent behavior when subjected to a constant load or deformation. Two types of mechanisms are responsible for viscoelasticity in articular cartilage: flow dependent and flow independent. The flow-dependent mechanism depends on interstitial fluid and the frictional drag associated with this flow. The drag resulting from the interstitial fluid is known as biphasic viscoelastic behavior. The flow-independent component of viscoelasticity is caused by macromolecular motion—specifically, the intrinsic viscoelastic behavior of the collagen-proteoglycan matrix. As a result, the fluid pressure provides a significant component of total load support, thereby reducing the stress acting upon the solid matrix. Articular cartilage also exhibits a creep and stress-relaxation response. When a constant compressive stress is applied to the tissue, its deformation increases with time, and it will deform or creep until an equilibrium value is reached. Similarly, when cartilage is deformed and held at a constant strain, the stress will rise to a peak, which will be followed by a slow stress-relaxation process until an equilibrium value is reached. Because articular cartilage tends to stiffen with increased strain, it cannot be described by a single Young's modulus. Rather, the modulus of the tissue depends on the time at which the force measurement was taken during a stress-relaxation test, which was common practice in the preliminary studies of mechanical testing on articular cartilage. The current method is to apply a known strain, which is immediately followed by a peak in measured force and

a slow stress-relaxation process; the force/stress value is recorded when it has reached

equilibrium. This process is repeated across a range of strain values, and the equilibrium

modulus is calculated as the slope of the stress-strain curve. The complex composition and

organization of cartilage through the middle zones of cartilage contributes significantly to

its shear-resistant properties.

Stretching of the randomly distributed collagen fibrils provides cartilage with its shear

stress response . The tensile force-resisting properties derive from the precise molecular

arrangement of collagen fibrils. The stabilization and ultimate tensile strength of the

collagen fiber are thought to result from the intra- and intermolecular cross-links .

Age and Development

Age determines the composition of the ECM as well as the organization of chondrocytes

and their response to external factors such as cytokines. With increasing age, there are

zonal changes in the distribution of chondrocytes; however, the total number of

chondrocytes remains essentially unchanged.

Chondrocytes begin to dissipate in the superficial region, whereas the deeper layers have

an increased number of cells. With increasing age, there is a decrease in the hydration of

the matrix, with a corresponding increase in compressive stiffness. This may have

implications for the underlying subchondral bone, which may see increased forces as the

cartilage loses its ability to undergo reversible deformation. Such changes may be noted on

magnetic resonance imaging (MRI) as consolidation of trabeculae and subchondral

sclerosis, which is often seen in association with a bone marrow edema pattern in the

setting of cartilage degeneration. Preservation of a homeostatic ECM environment is

critical to the maintenance of articular cartilage. The size of proteoglycan aggregates

within the ECM decreases with age.

This may occur as a result of a decrease in the available binding sites of the HA chain or

as the result of proteolytic damage to link proteins and their glycosaminoglycan chains.

Aggregation may also affect pore size distribution and solute permeability. There is also

an increased ratio of keratin sulfate to chondroitin sulfate. The concentration of

hyaluronan increases with age, but this results from the gradual accumulation of partially

degraded hyaluronan rather than 3 increased synthesis.

BONE AS AN ORGAN

Bone is often stereotyped as simply a protective and supportive framework for the body.

Though it does perform these functions, bone is actually a very dynamic organ that is

constantly remodeling and changing shape to adapt to the daily forces placed upon it.

Moreover, bone stores crucial nutrients, minerals, and lipids and produces blood cells that

nourish the body and play a vital role in protecting the body against infection. All these

functions make the approximately 206 bones of the human body an organ that is essential

to our daily existence.

The skeletal system consists of bones, cartilage, and the membranes that line the bones.

Each bone is an organ that includes nervous tissue, epithelial tissue (within the blood

vessels), and connective tissue (blood, bone, cartilage, adipose, and fibrous connective

tissue).

Bones have many functions, including the following:

Support: Bones provide a framework for the attachment of muscles and other tissues.

Protection: Bones such as the skull and rib cage protect internal organs from injury.

Movement: Bones enable body movements by acting as levers and points of attachment for

muscles.

Mineral storage: Bones serve as a reservoir for calcium and phosphorus, essential

minerals for various cellular activities throughout the body.

Blood cell production: The production of blood cells, or hematopoiesis, occurs in the red

marrow found within the cavities of certain bones.

Energy storage: Lipids, such as fats, stored in adipose cells of the yellow marrow serve as

an energy reservoir.

As the main constituent of the adult skeleton, bone tissue supports fleshy structures,

protects such vital organs as those in the cranial and thoracic cavities, and harbors the bone

marrow, where blood cells are formed. Bone also serves as a reservoir of calcium,

phosphate, and other ions that can be released or stored in a controlled fashion to maintain

constant concentrations of these important ions in body fluids. In addition to these

functions, bones form a system of levers that multiply the forces generated during skeletal

muscle contraction and transform them into bodily movements.

Bone is a specialized connective tissue composed of intercellular calcified material, the

bone matrix, and three cell types: osteocytes, which are found in cavities (lacunae) within

the matrix; osteoblasts, which synthesize the organic components of the matrix; and

osteoclasts, which are multinucleated giant cells involved in the resorption and remodeling

of bone tissue.

Since metabolites are unable to diffuse through the calcified matrix of bone, the exchanges

between osteocytes and blood capillaries depend on communication through the canaliculi

– thin, cylindrical spaces that perforate the matrix.

All bones are lined on both internal and external surfaces by layers of tissue containing

osteogenic cells—endosteum on the internal surface and periosteum on the external

surface.

Osteoblasts are responsible for the synthesis of the organic components of bone matrix

(type I collagen, proteoglycans, and glycoproteins). Osteoblasts are exclusively located at

the surfaces of bone tissue, side by side, in a way that resembles simple epithelium. When

they are actively engaged in matrix synthesis,

osteoblasts have a cuboidal to columnar shape and basophilic cytoplasm. When their

synthesizing activity declines, they flatten, and cytoplasmic basophilia declines. Some

osteoblasts are gradually surrounded by newly formed matrix and become osteocytes.

During this process a space called a lacuna is formed. Lacunae are occupied by osteocytes

and their extensions, along with a small amount of extracellular noncalcified matrix.

During matrix synthesis, osteoblasts have the ultrastructure of cells actively synthesizing

proteins for export. Osteoblasts are polarized cells. Matrix omponents are secreted at the

cell surface, which is in contact with older bone matrix, producing a layer of new (but not

yet calcified) matrix, called osteoid,between the osteoblast layer and the previously

formed bone. This process, bone apposition, is completed by subsequent deposition of

calcium salts into the newly formed matrix.

Osteocytes, which derive from osteoblasts, lie in the lacunae situated between lamellae of

matrix. Only one osteocyte is found in each lacuna. The thin, cylindrical matrix canaliculi

house cytoplasmic processes of osteocytes. Processes of adjacent cells make contact via

gap junctions, and molecules are passed via these structures from cell to cell. Some

molecular exchange between osteocytes and blood vessels also takes place through the

small amount of extracellular substance located between osteocytes (and their processes)

and the bone matrix.

This exchange can provide nourishment for a chain of about 15 cells.

Osteoclasts are very large, branched motile cells. Dilated portions of the cell body contain

from 5 to 50 (or more) nuclei. In areas of bone undergoing resorption, osteoclasts lie

within enzymatically etached depressions in the matrix known as Howship's lacunae.

Osteoclasts are derived from the fusion of bone marrow-derived cells, and belong to the

mononuclear phagocyte system. When compared with osteoblasts, the flat, almond-shaped

osteocytes exhibit a significantly reduced rough endoplasmic reticulum and Golgi complex

and more condensed nuclear chromatin. These cells are actively involved in the

maintenance of the bone matrix, and their death is followed by resorption of this matrix.

In active osteoclasts, the surface-facing bone matrix is folded into irregular, often subdivided projections, forming a ruffled border. Surrounding the ruffled border is a cytoplasmic zone—the clear zone—that is devoid of organelles, yet rich in actin microfilaments. This zone is a site of adhesion of the osteoclast to the bone matrix and creates a microenvironment in which bone resorption occurs. The osteoclast secretes collagenase and other enzymes and pumps protons into a subcellular pocket (the microenvironment referred to above), promoting the localized digestion of collagen and dissolving calcium salt crystals. Osteoclast activity is controlled by cytokines (small signaling proteins that act as local mediators) and hormones. Osteoclasts have receptors for calcitonin, a thyroid hormone, but not for parathyroid hormone. However, osteoblasts have receptors for parathyroid hormone and, when activated by this hormone, produce a cytokine called osteoclast stimulating factor. Lysosomal enzymes packaged in the Golgi complex and hydrogen ions produced are released into the confined microenvironment created by the attachment between bone matrix and the osteoclast's peripheral clear zone.

The acidification of this confined space facilitates the dissolution of calcium phosphate from bone and is the optimal pH for the activity of lysosomal hydrolases. Bone matrix is thus removed and the products of bone resorption are taken up by the osteoclast's cytoplasm, probably digested further, and transferred to blood capillaries.

BONE MATRIX

Inorganic matter represents about 50-70% of the dry weight of bone matrix. Calcium and

phosphorus are especially abundant, but bicarbonate, citrate, magnesium, potassium, and

sodium are also found. In electron micrographs, hydroxyapatite crystals of bone appear as

plates that lie alongside the collagen fibrils but are surrounded by ground substance. The

surface ions of hydroxyapatite are hydrated, and a layer of water and ions forms around

the crystal. This layer, the hydration shell, facilitates the exchange of ions between the

crystal and the body fluids.

The organic matter in bone matrix is type I collagen and ground substance, which contains

proteoglycan aggregates and several specific structural glycoproteins.Some of the

glycoproteins are produced by osteoblasts and demonstrate affinity for both

hydroxyapatite and the cell membrane; they might be involved in binding osteoblasts or

osteoclasts to bone matrix. Bone glycoproteins may also be responsible for promoting

calcification of bone matrix. Other tissues containing type I collagen are not normally

calcified and do not contain these glycoproteins. Because of its high collagen content,

decalcified bone matrix intensely binds stains for collagen fibers. The association of

hydroxyapatite with collagen fibers is responsible for the hardness and resistance of bone

tissue. After a bone is decalcified, its shape is preserved, but it becomes as flexible as a

tendon. Removal of the organic part of the matrix—which is mainly collagenous—also

leaves the bone with its original shape; however, it becomes fragile, breaking and

crumbling easily when handled.

PERIOSTEUM & ENDOSTEUM

External and internal surfaces of bone are covered by layers of bone-forming cells and

connective tissue called periosteum and endosteum.

The periosteum consists of an outer layer of collagen fibers and fibroblasts. Bundles of

periosteal collagen fibers, called Sharpey's fibers, penetrate the bone matrix, binding the

periosteum to bone. The inner, more cellular layer of the periosteum is composed of

fibroblast-like cells called osteoprogenitor cells, with the potential to divide by mitosis and

differentiate into osteoblasts. Osteoprogenitor cells play a prominent role in bone growth

and repair.

The endosteum lines all internal cavities within the bone and is composed of a single

layer of flattened osteoprogenitor cells and a very small amount of connective tissue. The

endosteum is therefore considerably thinner than the periosteum. The principal functions

of periosteum and endosteum are nutrition of osseous tissue and provision of a continuous

supply of new osteoblasts for repair or growth of bone.

TYPES OF BONE

Gross observation of bone in cross section shows dense areas without

cavities—corresponding to compact bone—and areas with numerous interconnecting

cavities—corresponding to cancellous (spongy) bone.

Under the microscope, however, both compact bone and the trabeculae separating the

cavities of cancellous bone have the same basic histologic structure. In long bones, the

bulbous ends—called epiphyses—are composed of spongy bone covered by a thin layer of

compact bone. The cylindrical pan— diaphysis (a growing between)—is almost totally

composed of compact bone, with a small component of spongy bone on its inner surface

around the bone marrow cavity. Short bones usually have a core of spongy bone

completely surrounded by compact bone. The flat bones that form the calvaria have two

layers of compact bone calledplates (tables), separated by a layer of spongy bone called

the diploe.

Microscopic examination of bone shows two varieties: primary, immature, or woven bone

and secondary, mature, or lamellar bone. Primary bone is the first bone tissue to appear in

embryonic development and in fracture repair and other repair processes. It is

characterized by random disposition of fine collagen fibers, in contrast to the organized

lamellar disposition of collagen in secondary bone.

Primary bone tissue is usually temporary and is replaced in adults by secondary bone

tissue except in a very few places in the body, eg, near the sutures of the flat bones of the

skull, in tooth sockets, and in the insertions of some tendons. In addition to the irregular

array of collagen fibers, other characteristics of primary bone tissue are a lower mineral

content (it is more easily penetrated by xrays) and a higher proportion of osteocytes than

that in secondary bone tissue.

Secondary bone tissue is the variety usually found in adults. It characteristically shows

collagen fibers arranged in lamellae that are parallel to each other or concentrically

organized around a vascular canal. The whole complex of concentric lamellae of bone

surrounding a canal containing blood vessels, nerves, and loose connective tissue is called

a haversian system, or osteon.

Lacunae containing osteocytes are found between and occasionally within the lamellae. In

each lamella, collagen fibers are parallel to each other. Surrounding each haversian system

is a deposit of amorphous material called the cementing substance that consists of

mineralized matrix wiih few collagen fibers. In compact bone (eg, the diaphysis of long

bones), the lamellae exhibit a typical organization consisting of haversian systems, outer

circumferential lamellae, inner circumferential lamellae, and interstitial lamellae. Inner

circumferential lamellae are located around the marrow cavity, and outer circumferential

lamellae are located immediately beneath the periosteum. There are more outer than inner

lamellae. Between the two circumferential systems are numerous haversian systems,

including triangular or irregularly shaped groups of parallel lamellae called interstitial (or

intermediate) lamellae. These structures are lamellae left by haversian systems destroyed

during growth and remodeling of bone. Each haversian system is a long, often bifurcated

cylinder parallel to the long axis of the diaphysis. It consists of a central canal surrounded

by 4-20 concentric lamellae. Each endosteum-lined canal contains blood vessels, nerves,

and loose connective tissue. The haversian canals communicate with the marrow cavity,

the periosteum, and one another through transverse or oblique Volkmann's canals.

Volkmann's canals do not have concentric lamellae; instead, they perforate the

lamellae.All vascular canals found in bone tissue come into existence when matrix is laid

down around preexisting blood vessels.

The protruding haversian system on the left shows the orientation of collagen fibers in

each lamella. At the right is a haversian system showing lamellae, a central blood capillary

(there are also small nerves, not shown), and many osteocytes with their processes.

Examination of haversian systems with polarized light shows bright anisotropic layers

alternating with dark isotropic layers. When observed under polarized light at right angles

to their length, collagen fibers are birefringent (anisotropic). The alternating bright and

dark layers are due to the changing orientation of collagen fibers in the lamellae. In each

lamella, fibers are parallel to each other and follow a helical course. The pitch of the helix

is, however, different for different lamellae, so that at anygiven point, fibers from adjacent

lamellae intersect atapproximately right angles. Collagen fibers of contiguous lamellae are

sectioned at different angles. Note the numerous canaliculi that permit communication

between lacunae and with the haversian canals. Although it is not apparent in this

simplified diagram,

each lamella consists of multiple parallel arrays of collagen fibers. In adjacent lamellae,

the collagen fibers are oriented in different directions. The presence of large numbers of

lamellae with differing fiber orientations provides the bone with great strength, despite its

light weight. There is great variability in the diameter of haversian canals. Each system is

formed by successive deposits of lamellae, starting inward from the periphery, so that

younger systems have larger canals. In mature haversian systems, the most recently

formed lamella is the one closest to the central canal.

BONE REMODELING . REGENERATION OF BONE

replacement of old bone tissue by new bone tissue. It involves the processes of bone

deposition or bone production done by osteoblasts and bone resorption done by

osteoclasts, which break down old bone. Normal bone growth requires vitamins D, C,

and A, plus minerals such as calcium, phosphorous, and magnesium. Hormones such

as parathyroid hormone, growth hormone, and calcitonin are also quite high, with

five to seven percent of bone mass being recycled every week. Differences in

turnover rates exist in different areas of the skeleton and in different areas of a bone.

For example, the bone in the head of the femur may be fully replaced every six

months, whereas the bone along the shaft is altered much more stronger when

subjected to stress. Bones that are not subject to normal everyday

Stages of fracture repair

The healing of a bone fracture follows a series of progressive steps: (a) A fracture

hematoma forms. (b) Internal and external calli form. (c) Cartilage of the calli is

replaced by trabecular bone. (d) Remodeling occurs.

A fractured or broken bone undergoes repair through four stages:

hemorrhage, resulting in the formation of clotted blood, or a hematoma, at the site of

the break. The severed blood vessels at the broken ends of the bone are sealed

hematoma, while phagocytic cells begin to clear away the dead cells. Though

fragments of the blood clot may remain, fibroblasts and osteoblasts enter the area and

begin to reform bone. Fibroblasts produce collagen fibers that connect the broken

bone ends, while osteoblasts start to form spongy bone. The repair tissue between the

broken bone ends, the fibrocartilaginous callus, is composed of both into a bony

callus of spongy bone. It takes about two months for the broken bone ends to be

firmly joined together after the fracture. This is similar to the endochondral formation

of bone when cartilage becomes ossified; osteoblasts, and osteoblasts, with excess

material on the exterior of the bone and within the medullary cavity being removed.

Compact bone is added to create bone tissue that is similar to the original, unbroken

bone. This remodeling can take many months; osteoclasts, and bone matrix are

present.

4. Bone remodeling: The bony callus is then remodelled by osteoclasts stress (for

example, when a limb is in a cast) will begin to lose mass. Bone renewal continues

after birth into adulthood. Bone remodeling is the required for proper bone growth

and maintenance. Bone turnover rates, the rates at which old bone is replaced by new

bone, are slowly.

Bone remodeling allows bones to adapt to stresses by becoming thicker and

1. Hematoma formation: Blood vessels in the broken bone tear and by the clotting

process. Bone cells deprived of nutrients begin to die.

2. Bone generation: Within days of the fracture, capillaries grow into the hyaline and

fibrocartilage . Some bone spicules may also appear at this point.

3. Bony callous formation: The fibrocartilaginous callus is converted the bone may

remain uneven for years.

MUSCLE AS AN ORGAN. REGENERATION OF MUSCLES. (3 hours)

Muscle function:

contraction for locomotion and skeletal movement

contraction for propulsion

contraction for pressure regulation

Muscle classification: muscle tissue may be classified according to a morphological classification or a functional classification.

Morphological classification (based on structure)

There are two types of muscle based on the morphological classification system

1. Striated

2. Non striated or smooth.

Functional classification

There are two types of muscle based on a functional classification system

1. Voluntary

2. Involuntary.

Types of muscle: there are generally considered to be three types of muscle in the human body.

Skeletal muscle: which is striated and voluntary

Cardiac muscle: which is striated and involuntary

Smooth muscle: which is non striated and involuntary

Characteristics of skeletal muscle

Skeletal muscle cells are elongated or tubular. They have multiple nuclei and these nuclei are located on the periphery of the cell. Skeletal muscle is striated. That is, it has an alternating pattern of light and darks bands that will be described later.

Characteristics of Cardiac muscle

Cardiac muscle cells are not as long as skeletal muscles cells and often are branched cells. Cardiac muscle cells may be mononucleated or binucleated. In either case the nuclei are located centrally in the cell. Cardiac muscle is also striated. In addition cardiac muscle contains intercalated discs.

Characteristics of Smooth muscle

Smooth muscle cell are described as spindle shaped. That is they are wide in the middle and narrow to almost a point at both ends. Smooth muscle cells have a single centrally located nucleus. Smooth muscle cells do not have visible striations although they do contain the same contractile proteins as skeletal and cardiac muscle, these proteins are just laid out in a different pattern.

For the purposes of this class we will focus mainly on skeletal muscle.

Shapes of skeletal muscles:

1. Parallel or fusiform: as their name implies their fibers run parallel to each other. These muscles contract over a great distance and usually have good endurance but are not very strong. Examples: Sartorius muscle and rectus abdominus muscle.

2. Convergent: the muscle fibers converge on the insertion to maximize the force of muscle contraction. Examples: Deltoideus muscle and Pectoralis Major muscle.

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3. pennate: many fibers per unit area. These types of muscles are strong but they tie or quickly. There are three types of pennate muscle.

unipennate

bipennate

multipennete

4. Circular: the muscle fibers surrounded opening to act as a sphincter. Examples: Orbicularis oris and Orbicularis oculi muscles.

5. fusiform: some texts classify parallel muscles that are slightly wider in their middle (spindle shaped) as fusiform. This term will not be used in this course.

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Muscle terminology

myofiber or myocyte: a muscle cell

sarcolemma: the plasma membrane of a muscle cell sarcoplasm: the cytoplasm of the
muscle
cell
sarcoplasmic reticulum: the endoplasmic reticulum of a muscle cell sarcosome: the mitochondria of a muscle cell
sarcomere: the contractile or functional unit of muscle

For the purposes of this class we will focus mainly on skeletal muscle.

Muscles have three major areas:

1. a belly or Gaster
2. an origin: a tendinous connection of the muscle to a bone, usually the bone that is stabilized.
3. an insertion: a tendinous connection of the muscle to a bone, usually the bone to be moved.

Skeletal muscle is designed as a bundle within a bundle arrangement. We will start with a whole muscle and then work our way down to the microscopic level of the muscle

The entire muscle is surrounded by a connective tissue called the epimysium.

The muscle is made up of smaller bundles known as fascicles. Fascicles are actually bundles of individual muscle cells (myofibers or myocytes). These bundles are surrounded by a connective tissue sheath called the perimysium.

Each fascicle is made up of several muscle cells known as myocytes. They may also be called myofibers or muscle fibers. Each muscle cell is surrounded by a connective tissue sheath known as the endomysium. This sheath is very important

in the physiology of muscle contraction because it electrically insulates the individual muscle cells from each other.

At the ends of the muscle all of the connective tissue sheaths (epimysium, perimysium, and endomysium) converge to form a tendon which will connect the

muscle to its attachment site.
Each muscle fiber (muscle cell) contains all of the organelles that we find in other cell types.
Although these organelles are the same as in other cells they are given special names. Note that the prefixes sarco and myo both refer to muscle.

Therefore if you see a word with either of these prefixes you should immediately think MUSCLE.
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The nucleus contains the genetic material of the muscle cell.
The sarcolemma is the name given to the plasma membrane of the muscle cell. There are specialized invaginations of the sarcolemma that run transversely across the cell. These invaginations are known as T tubules (short for transverse tubules). The T tubules are essential for carrying the depolarization brought to the cell by a motor nerve impulse down into the muscle cell where it can have an affect on the terminal cisternae. We will cover more about this in the unit on the physiology of muscle contraction.
The cytosol is the cytoplasm of the muscle cell.
The sarcoplasmic reticulum is the endoplasmic reticulum of the muscle cell. There are sac-like regions of the sarcoplasmic reticulum known as terminal cisternae. The terminal cisternae act as calcium storage sites. The calcium ions stored in the terminal cisternae are essential in muscle contraction. We will cover more about this in the unit on the physiology of muscle contraction. NOTE: this is not calcium storage for use in general body physiology as we would see with bone tissue, but rather is calcium storage for muscle contraction.
In skeletal muscle two terminal cisternae are associated with a T tubule to form a structure known as a triad. This differs from cardiac muscle where one terminal cisternae associates with one T tubule to form a diad.
Mitochondria are sites of energy production (ATP synthesis) in the muscle cell as in all other cells of the body, except for mature red blood cells.

A myofibril is a cylindrical bundle of contractile proteins found within the muscle cell. Note that there are several myofibrils within each muscle cell. It is the arrangement of the contractile proteins within the myofibril that cause the striated appearance of skeletal and cardiac muscle.

Myofibrils are composed of individual contractile proteins called myofillaments. These myofilaments are generally divided into thick and thin myofilaments.

The thin myofilaments are composed mainly of a protein known as actin. Actin filaments are anchored into the z-line of a sarcomere.

The thick myofilaments are composed mainly of the protein myosin.

It is the orderly overlapping of the actin and myosin filaments that give cardiac and skeletal muscle their striated appearance (light and dark bands).

The A band is the dark band and corresponds to the length of a bundle of myosin filaments. Because muscle contraction is a sliding of the myofilaments past each other we do not see any of the myofilaments actually shorten. However the width of the banding patterns change as the degree of overlap changes. Because the A band corresponds to the length of the myosin filaments, and these filaments do not shorten, the width of the A band also does not shorten.

The light bands are known as I bands. The I bands are composed mainly of actin filaments. Each I band is bisected by a protein disc known as the Z-line. Actin filaments are anchored into the Z-line. During muscle contraction the actin filaments slide over the myosin filaments which results in a shortening of the I

band.

In the middle of the A band is a somewhat lighter area known as the H zone. This zone corresponds to the area where we have myosin not overlapped by actin (the area between the thin filaments). During muscle contraction the actin sliding over the myosin encroaches into this area so that the H zone shortens. In the middle of the H zone we see a dark band known as the M line. The M line is

comprised of protein fibers that function to anchor the myosin filaments.
The area between two Z lines is known as a sarcomere. The sarcomere is the functional or contractile unit of muscle.
To recap, a whole muscle if made up of many smaller bundles known as fascicles. Each fascicle is made up of many muscle cells (myofibers). Myofibers contain cylindrical bundles of myofibrils which in turn contain many smaller bundles of myofilaments.
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Muscles contract when they receive a motor impulse from a motor nerve.
These nerve impulses serve only a limited number of muscle fibers. The muscle fibers served by a single motor neuron make up a structure known as a motor unit. Motor units allow for selective contraction of muscle fibers so that we may control the strength and extent of muscle contraction. Without motor units a nerve impulse to the muscle would result in the entire muscle contracting to its full extent. That would make every motion that we make an “all or none” motion. This type of movement would make life nearly impossible.
Note that this diagram shows a neuromuscular junction of one motor neuron with one muscle fiber. In a motor unit the motor neuron branches to form neuromuscular junctions with several muscle fibers. To repeat, a motor neuron and all of the muscle fibers it supplies is called a MOTOR UNIT.
MUSCULAR REGENERATION
l Skeletal muscle
l Some regeneration occurs at the cut ends of fibres. (A cut is insufficient injury to kill the cell throughout its length.)
2 The end-piece reverts to the narrow myotube stage, seen in embryonic growth.
3 Just outside the sarcolemma of intact muscle fibres lie satellite cells that act as residual, peripheral myoblasts, able to respond to injury by becoming active myoblasts.
4 The end grows out a little way into the defect, then increases in thickness.

If the cut is not wide, myotubes regenerating from each side may fuse and restore the fibres.

5 A deep cut may sever nerves disturbing regeneration in two ways:

(a) Denervation of muscle fibres reduces their regenerative response.

(b) Dense fibrous CT then fills the gap and obstructs reinervation of the muscle.

2 Smooth and cardiac muscle

l Radioautographic studies indicate that smooth muscle cells, e.g., in the gut, are capable of some proliferation to replace damaged cells and partially restore continuity in a muscular tunic.

2 Cardiac muscle is at a disadvantage, because it cannot relax and rest for a

period to permit cell division and muscle reorganization; and there may be an early and unfavourable response from its CT.Lung, likewise, is prevented by its elasticity and motion, and other factors, from effective regeneration, despite its epithelial content. 3 Cuts into cardiac muscle fill quickly with collagenous CT, but muscle fibres injured by infections can regenerate.In general then, a large lesion in muscle will be filled with C.T. Only a little new muscle tissue forms to replace that lost or to fill gaps. Surviving muscle fibres may hypertrophy in an attempt to restore the power of the muscle as a whole.

DEVELOPMENT OF DIGESTIVE GLANDS (2 hours)

Early development review

INTRODUCTION: the entoderm appears about day 8 and rapidly forms the yolk sac and the epithelial lining of the digestive tract and its associated glands. The process of body cylinder formation divides the yolk sac into 2 parts toward the end of month 1

Part 1 is extraembryonic and is the yolk sac itself, which regresses early and

disappears at about 3 months. The head, tail, and lateral folds incorporate the dorsal part of the yolk sac and the allantois into the embryo

Part 2 is intraembryonic and is the primitive gut which is the early origin of the epithelium of the digestive tube and its accessory glands, the liver, the pancreas, and the biliary apparatus

The epithelium at the cranial and caudal ends of the tract is derived from ectoderm of the stomodeum (primitive mouth) and the proctodeum (anal pit), respectively

THE SPLANCHNIC MESODERM, which is formed about day 15 during gastrulation, surrounds the entoderm and provides the digestive tract with its connective tissue, muscle, and serous (peritoneal) coverings

Major or principal stages of development

GENERAL INFORMATION

The digestive tube is initially closed cephalically by the buccopharyngeal or oropharyngeal membrane (a bilaminar membrane: ectoderm externally and entoderm internally) and caudally by the cloacal membrane

The buccopharyngeal membrane is resorbed at the beginning of week 4, connecting the amniotic cavity with the digestive tube

The derivatives of the cloacal membrane open at the end of

week 9 The development of the digestive system consists of

A very complex anterior pharyngeal portion, the foregut

The foregut extends from the buccopharyngeal membrane to the duodenum where the liver bud arises (the anterior intestinal portal)

A very extensive growth in length of its middle or abdominal portion, themidgut

The middle portion of the midgut remains connected to the yolk sac via the vitelline or omphalomesenteric duct

The midgut extends from just caudal to the liver bud (the anterior intestinal portal) to a point where, in the adult, the right two-thirds and left one-third of the transverse colon are found (the posterior intestinal portal in the embryo)

An intermingling and very close association with the urogenital system is found in relation to its terminal portion, the hindgut

The hindgut extends from the posterior intestinal portal to the cloacal membrane

The foregut, midgut, and hindgut are supplied by the celiac artery, the superior mesenteric artery, and the inferior mesenteric artery, respectively

The liver primordium appears in the middle of the third week as an outgrowth of the endodermal epithelium at the distal end of the forgut.

This outgrowth, the hepatic diverticulum, or liver bud, consists of rapidly proliferating cells that penetrate the septum transversum, that is, the mesodermal plate between the pericardial cavity and the stalk of the yolk sac. While hepatic cells continue to penetrate the septum, the connection between the hepatic diverticulum and the foregut (duodenum) narrows, forming the bile duct. A small ventral outgrowth is formed by the bile duct, and this outgrowth gives rise to the gallbladder and the cystic duct. During further development, epithelial liver cords intermingle with the vitelline and umbilical veins, which form hepatic sinusoids. Liver cords differentiate into the parenchyma (liver cells) and form the lining of the biliary ducts. Hematopoietic cells, Kupffer cells, and connective tissue cells are derived from mesoderm of the septum transversum. When liver cells have invaded the entire septum transversum, so that the organ bulges caudally into the abdominal cavity, mesoderm of the septum transversum lying between the liver and the foregut and the liver and the ventral abdominal wall becomes membranous, forming the lesser omentum and falciform ligament, respectively. Together, having formed the peritoneal connection between the foregut and the ventral abdominal wall, they are known as the ventral mesentery. Mesoderm on the surface of the liver differentiates into visceral peritoneum except on its cranial surface. In this region, the liver remains in contact with the rest of the original septum transversum. This portion of the septum, which consists of densely packed

mesoderm, will form the central tendon of the diaphragm. The surface of the liver that is in contact with the future diaphragm is never covered by peritoneum; it is the bare area of the liver. In the 10th week of development, the weight of the liver is approximately 10% of the total body weight. Although this may be attributed partly to the large numbers of sinusoids, another important factor is its hematopoietic function. Large nests of proliferating cells, which produce red and white blood cells, lie between hepatic cells and walls of the vessels. This activity gradually subsides during the last 2 months of intrauterine life, and only small hematopoietic islands remain at birth. The weight of the liver is then only 5% of the total body weight. Another important function of the liver begins at approximately the 12th week, when bile is formed by hepatic cells. Meanwhile, since the gallbladder and cystic duct have developed and the cystic duct has joined the hepatic duct to form the bile duct, bile can enter the gastrointestinal tract. As a result, its contents take on a dark green color. Because of positional changes of the duodenum, the entrance of the bile duct gradually shifts from its initial anterior position to a posterior one, and consequently, the bile duct passes behind the duodenum.

All of the foregut endoderm has the potential to express liver-specifi c genes and to differentiate into liver tissue. However, this expression is blocked by factors produced by surrounding tissues, including ectoderm, noncardiac mesoderm, and particularly the notochord. The action of these inhibitors is blocked in the prospective hepatic region by fi broblast growth factors (FGF2) secreted by cardiac mesoderm and by blood vessel-forming endothelial cells adjacent to the gut tube at the site of liver bud outgrowth. Thus, the cardiac mesoderm together with neighboring vascular endothelial cells “instructs” gut endoderm to express liver137 specifi c genes by inhibiting an inhibitory factor of these same genes. Other factors participating in this “instruction” are bone morphogenetic proteins (BMPs) secreted by the septum transversum. BMPs appear to enhance the competence of

prospective liver endoderm to respond to FGF2. Once this “instruction” is

received, cells in the liver fi eld differentiate into both hepatocytes and biliary cell lineages, a process that is at least partially regulated by hepatocyte nuclear transcription factors (HNF3 and 4)

The pancreas makes its appearance in week 5 as 2 buds originating from the entodermal epithelium of the duodenum, namely, a dorsal bud (opposite and slightly above the hepatic diverticulum) and aventral bud (in the angle below the hepatic rudiment)

THE LARGER DORSAL BUD appears first and rapidly grows into the dorsal mesentery. The dorsal bud forms the major portion of the pancreas, namely, the upper half of the head, the isthmus, the body, and the tail. THE VENTRAL BUD develops near the entry of the common bile duct into the duodenum

When the duodenum grows and rotates to the right (clockwise) and becomes C-shaped, the ventral pancreatic bud is carried dorsally along with the common bile duct and comes to lie in the mesoduodenum immediately below and behind the dorsal bud

The parenchyma and the duct systems of both buds then fuse

The ventral bud forms the uncinate process and the inferior part of the head of the pancreas. AS THE PANCREATIC BUDS FUSE, their ducts anastomose

The main pancreatic duct (of Wirsung) forms from the duct of the ventral bud and the distal part of the duct of the dorsal bud

The proximal portion of the duct of the dorsal part often persists as the accessory pancreatic duct (of Santorini), that opens just above the main duct

The common bile duct and the duct of Wirsung open into the ampulla of Vater in the second part of the duodenum, either together or separately, with the bile duct above the pancreatic duct

PANCREATIC PARENCHYMA is of entodermal origin and forms a tubular primitive duct network

Acini, early in the fetal period, develop from cell clusters around the ends of the tubules

The islets of Langerhans develop in month 3 from the parenchymatous

pancreatic tissue that separates from the tubules and lies between the acini
Insulin secretion begins at about month 5
Since fetal insulin levels are independent from maternal insulin levels, it is unlikely that insulin crosses the placenta
The connective tissue covering and the pancreatic septa form from the surrounding splanchnic mesenchyme
DEVELOPMENT OF THE EAR (2 hours)
The ear, or organ of hearing, is divisible into three parts: the external ear, the middle ear ortympanic cavity, and the internal ear or labyrinth.
FIG. 898– Section through the head of a human embryo, about twelve days old, in the region of the hind-brain. (Kollmann.)
FIG. 899– Section through hind-brain and auditory vesicles of an embryo more advanced than that of Fig. 898. (After His.)
FIG. 900– Lateral views of membranous labyrinth and acoustic complex. X 25 dia. (Streeter.)
absorpt. focu, area of wall where absorption is complete; amp., ampulla membranacea; crus,crus commune; d. sc. lat., ductus semicircularis lateralis; d. sc. post., ductus semicircularis posterior; d. sc. sup., ductus semicircular superior; coch. or cochlea, ductus cochlearis; duct. endolymph, ductus endolymphaticus; d. reuniens, ductus reuniens Henseni; endol. or endolymphs appendix endolymphaticus; rec. utr., recessus utriculi; sacc.,

sacculus; sac. endol., saccus endolymphaticus; sinus utr. lat., sinus utriculi lateralis; utric., utriculus; vestib. p.,vestibular pouch.
The Development of the Ear.—The first rudiment of the internal ear appears shortly after that of the eye, in the form of a patch of thickened ectoderm, the auditory plate, over the region of the hind-brain. The auditory plate becomes depressed and converted into the auditory pit (Fig. 898). The mouth of the pit is then closed, and thus a shut sac, the auditory vesicle, is formed(Fig. 899); from it the epithelial lining of the membranous labyrinth is derived. The vesicle becomes

pear-shaped, and the neck of the flask is obliterated (Fig. 900). From the vesicle certain diverticula are given off which form the various parts of the membranous labyrinth. One from the middle part forms the ductus and saccus endolymphaticus, another from the anterior end gradually elongates, and, forming a tube coiled on itself, becomes the cochlear duct, the vestibular extremity of which is subsequently constricted to form the canalis reuniens. Three others appear as disk-like evaginations on the surface of the vesicle; the central parts of the walls of the disks coalesce and disappear, while the peripheral portions persist to form the semicircular ducts; of these the superior is the first and the lateral the last to be completed (Fig. 902). The central part of the vesicle represents the membranous vestibule, and is subdivided by a constriction into a smaller ventral part, the saccule, and a larger dorsal and posterior part, the utricle. This subdivision is effected by a fold which extends deeply into the proximal part of the ductus endolymphaticus, with the result that the utricle and saccule ultimately communicate with each other by means of a Y-shaped canal. The saccule opens into the cochlear duct, through the canalis reuniens, and the semicircular ducts communicate with the utricle.

FIG. 901– Median views of membranous labyrinth and acoustic complex in human embryos. X 25 dia. (Streeter.)

FIG. 902– Transverse section through head of fetal sheep, in the region of the labyrinth. X 30. (After Boettcher.)

The mesodermal tissue surrounding the various parts of the epithelial

labyrinth is converted into a cartilaginous ear-capsule, and this is finally ossified to form the bony labyrinth. Between the cartilaginous capsule and the epithelial structures is a stratum of mesodermal tissue which is differentiated into three layers, viz., an outer, forming the periosteal lining of the bony labyrinth; an inner, in direct contact with the epithelial structures; and an intermediate, consisting of gelatinous tissue: by the absorption of this latter tissue the perilymphatic spaces are

developed. The modiolus and osseous spiral lamina of the cochlea are not preformed in cartilage but are ossified directly from connective tissue.

FIG. 903– Transverse section of the cochlear duct of a fetal cat. (After Boettcher and Ayres.)

The middle ear and auditory tube are developed from the first pharyngeal pouch. The entodermal lining of the dorsal end of this pouch is in contact with the ectoderm of the corresponding pharyngeal groove; by the extension of the mesoderm between these two layers the tympanic membrane is formed. During the sixth or seventh month the tympanic antrum appears as an upward and backward expansion of the tympanic cavity. With regard to the exact mode of development of the ossicles of the middle ear there is some difference of opinion. The view generally held is that the malleus is developed from the proximal end of the mandibular (Meckel's) cartilage (Fig. 43), the incus in the proximal end of the mandibular arch, and that thestapes is formed from the proximal end of the hyoid arch. The malleus, with the exception of its anterior process is ossified from a

single center which appears near the neck of the bone; the anterior process is ossified separately in membrane and joins the main part of the bone about the sixth month of fetal life. The incus is ossified from one center which appears in the upper part of its long crus and ultimately extends into its lenticular process. The stapes first appears as a ring (annulus stapedius) encircling a small vessel, the stapedial artery, which subsequently undergoes atrophy; it is ossified from a single center which appears in its base.

The external acoustic meatus is developed from the first branchial groove. The lower part of this groove extends inward as a funnel-shaped tube (primary meatus) from which the cartilaginous portion and a small part of the roof of the osseous portion of the meatus are developed. From the lower part of the funnelshaped

tube an epithelial lamina extends downward and inward along the inferior wall of the primitive tympanic cavity; by the splitting of this lamina the inner part

of the meatus (secondary meatus) is produced, while the inner portion of the lamina forms the cutaneous stratum of the tympanic membrane. The auricula or pinna is developed by the gradual differentiation of tubercles which appear around the margin of the first branchial groove. The rudiment of the acoustic nerve appears about the end of the third week as a group of ganglion cells closely applied to the cephalic edge of the auditory vesicle. Whether these cells are derived from the ectoderm adjoining the auditory vesicle, or have migrated from the wall of the neural tube, is as yet uncertain. The ganglion gradually splits into two parts, thevestibular ganglion and the spiral ganglion. The peripheral branches of the vestibular ganglion pass in two divisions, the pars superior giving rami to the superior ampulla of the superior semicircular duct, to the lateral ampulla and to the utricle; and the pars inferior giving rami to the saccule and the posterior ampulla.

The proximal fibers of the vestibular ganglion form the vestibular nerve; the proximal fibers of the spiral ganglion form the cochlear nerve.

DEVELOPMENT OF THE EYE (2 hours)

The bulb of the eye (bulbus oculi; eyeball), or organ of sight, is contained in the cavity of the orbit, where it is protected from injury and moved by the ocular muscles. Associated with it are certain accessory structures, viz., the muscles, fasciæ, eyebrows, eyelids, conjunctiva, and lacrimal apparatus.

The bulb of the eye is imbedded in the fat of the orbit, but is separated from it by a thin membranous sac, the fascia bulbi (page 1024). It is composed of segments of two spheres of different sizes. The anterior segment is one of a small sphere; it is transparent, and forms about one-sixth of the bulb. It is more prominent than the posterior segment, which is one of a larger sphere, and is opaque, and forms about five-sixths of the bulb. The term anterior pole is applied to the central point of the anterior curvature of the bulb, and that of posterior pole to the central point of its posterior curvature; a line joining the two poles forms the optic axis. The axes of the two bulbs are nearly parallel, and therefore do not correspond to the axes of the orbits, which are directed forward and lateralward.

The optic nerves follow the direction of the axes of the orbits, and are therefore not parallel; each enters its eyeball 3 mm. to the nasal side and a little below the level of the posterior pole. The bulb measures rather more in its transverse and anteroposterior
diameters than in its vertical diameter, the former amounting to about 24
mm., the latter to about 23.5 mm.; in the female all three diameters are rather less than in the male; its antero-posterior diameter at birth is about 17.5 mm., and at puberty from 20 to 21 mm.

FIG. 863– Transverse section of head of chick embryo of forty-eight hours' incubation. (Duval.)

FIG. 864– Transverse section of head of chick embryo of fifty-two hours' incubation. (Duval.))

Development.—The eyes begin to develop as a pair of diverticula from the lateral aspects of the forebrain. These diverticula make their appearance before the closure of the anterior end of the neural tube; after the closure of the tube they are known as the optic vesicles. They project toward the sides of the head, and the

peripheral part of each expands to form a hollow bulb, while the proximal part remains narrow and constitutes the optic stalk (Figs. 863, 864). The ectoderm overlying the bulb becomes thickened, invaginated, and finally severed from the ectodermal covering of the head as a vesicle of cells, the lens vesicle, which constitutes the rudiment of the crystalline lens. The outer wall of the bulb becomes thickened and invaginated, and the bulb is thus converted into a cup, the optic cup, consisting of two strata of cells (Fig. 864). These two strata are continuous with each other at the cup margin, which ultimately overlaps the front of the lens and reaches as far forward as the future aperture of the pupil. The invagination is not limited to the outer wall of the bulb, but involves also its postero-inferior surface and extends in the form of a groove for some distance along the optic stalk, so that, for a time, a gap or fissure, the choroidal fissure, exists in the lower part of the cup (Fig. 865). Through the groove and fissure the mesoderm extends into the

optic stalk and cup, and in this mesoderm a bloodvessel is developed; during the seventh week the groove and fissure are closed and the vessel forms the central

artery of the retina. Sometimes the choroidal fissure persists, and when this occurs the choroid and iris in the region of the fissure remain undeveloped, giving rise to the condition known as coloboma of the choroid or iris.

FIG. 865– Optic cup and choroidal fissure seen from below, from a human embryo of about four weeks. (Kollmann.)

The retina is developed from the optic cup. The outer stratum of the cup persists as a single layer of cells which assume a columnar shape, acquire pigment, and form the pigmented layer of the retina; the pigment first appears in the cells near the edge of the cup. The cells of the inner stratum proliferate and form a layer of considerable thickness from which the nervous elements and the sustentacular fibers of the retina, together with a portion of the vitreous body, are developed. In that portion of the cup which overlaps the lens the inner stratum is not differentiated into nervous elements, but forms a layer of columnar cells which is applied to the pigmented layer, and these two strata form the pars ciliaris and pars iridica retinæ.

The cells of the inner or retinal layer of the optic cup become differentiated into spongioblasts and germinal cells, and the latter by their subdivisions give rise to neuroblasts. From the spongioblasts the sustentacular fibers of Müller, the outer and inner limiting membranes, together with the groundwork of the molecular layers of the retina are formed. The neuroblasts become arranged to form the ganglionic and nuclear layers. The layer of rods and cones is first developed in the

central part of the optic cup, and from there gradually extends toward the cup margin. All the layers of the retina are completed by the eighth month of fetal life.

The optic stalk is converted into the optic nerve by the obliteration of its cavity and the growth of nerve fibers into it. Most of these fibers are centripetal, and grow backward into the optic stalk from the nerve cells of the retina, but a few

extend in the opposite direction and are derived from nerve cells in the brain. The fibers of the optic nerve receive their medullary sheaths about the tenth week after birth. The optic chiasma is formed by the meeting and partial decussation of the fibers of the two optic nerves. Behind the chiasma the fibers grow backward as the optic tracts to the thalami and mid-brain.

The crystalline lens is developed from the lens vesicle, which recedes within the margin of the cup, and becomes separated from the overlying ectoderm by mesoderm. The cells forming the posterior wall of the vesicle lengthen and are converted into the lens fibers, which grow forward and fill up the cavity of the vesicle (Fig. 866). The cells forming the anterior wall retain their cellular character, and form the epithelium on the anterior surface of the adult lens. By the second month the lens is invested by a vascular mesodermal capsule, the capsula vasculosa lentis; the bloodvessels supplying the posterior part of this capsule are derived from the hyaloid artery; those for the anterior part from the anterior ciliary arteries; the portion of the capsule which covers the front of the lens is named the pupillary membrane. By the sixth month all the vessels of the capsule are atrophied except the hyaloid artery, which disappears during the ninth month; the position of this artery is indicated in the adult by the hyaloid canal, which reaches from the optic disk to the posterior surface of the lens. With the loss of its bloodvessels the capsula vasculosa lentis disappears, but sometimes the pupillary membrane persists at birth, giving rise to the condition termed congenital atresia of the pupil.

FIG. 866– Horizontal section through the eye of an eighteen days' embryo rabbit. X 30. (Kölliker.)

The vitreous body is developed between the lens and the optic cup. The lens rudiment and the optic vesicle are at first in contact with each other, but after the closure of the lens vesicle and the formation of the optic cup the former withdraws itself from the retinal layer of the cup; the two, however, remain connected by a network of delicate protoplasmic processes. This network, derived partly from the

cells of the lens and partly from those of the retinal layer of the cup, constitutes the primitive vitreous body (Figs. 867, 868). At first these protoplasmic processes spring from the whole of the retinal layer of the cup, but later are limited to the ciliary region, where by a process of condensation they appear to form the zonula ciliaris. The mesoderm which enters the cup through the choroidal fissure and around the equator of the lens becomes intimately united with this reticular tissue, and contributes to form the vitreous body, which is therefore derived partly from the ectoderm and partly from the mesoderm.

FIG. 867– Sagittal section of eye of human embryo of six weeks. (Kollmann.)

FIG. 868– Section of developing eye of trout. (Szily.)

The anterior chamber of the eye appears as a cleft in the mesoderm separating the lens from the overlying ectoderm. The layer of mesoderm in front of the cleft forms the substantia propria of the cornea, that behind the cleft the stroma of the iris and the pupillary membrane. The fibers of the ciliary muscle are derived from the mesoderm, but those of the Sphincter and Dilatator pupillæ are of ectodermal origin, being developed from the cells of the pupillary part of the optic cup.

The sclera and choroid are derived from the mesoderm surrounding the optic cup.

The eyelids are formed as small cutaneous folds (Figs. 866, 867), which about the middle of the third month come together and unite in front of the cornea. They remain united until about the end of the sixth month.

The lacrimal sac and nasolacrimal duct result from a thickening of the ectoderm in the groove, nasoöptic furrow, between the lateral nasal and maxillary processes. This thickening forms a solid cord of cells which sinks into the mesoderm; during the third month the central cells of the cord break down, and a lumen, the nasolacrimal duct, is established. The lacrimal ducts arise as buds from

the upper part of the cord of cells and secondarily establish openings (puncta lacrimalia) on the margins of the lids. The epithelium of the cornea and conjunctiva, and that which lines the ducts and alveoli of the lacrimal gland, are of ectodermal origin, as are also the eyelashes and the lining cells of the glands which open on the lid-margins.

DIFFUSE ENDOCRINE SYSTEM (2 hours)

Human endocrine system, group of ductless glands that regulate body processes by secreting chemical substances called hormones. Hormones act on nearby tissues or are carried in the bloodstream to act on specific target organs and distant tissues. Diseases of the endocrine system can result from the oversecretion or undersecretion of hormones or from the inability of target organs or tissues to respond to hormones effectively.

It is important to distinguish between an endocrine gland, which discharges hormones into the bloodstream, and an exocrine gland, which secretes substances through a duct opening in a gland onto an external or internal body surface. Salivary glands and sweat glands are examples of exocrine glands. Both saliva, secreted by the salivary glands, and sweat, secreted by the sweat glands, act on local tissues near the duct openings. In contrast, the hormones secreted by endocrine glands are carried by the circulation to exert their actions on tissues remote from the site of their secretion.

The body of knowledge of the endocrine system is continually expanding, driven in large part by research that seeks to understand basic cell functions and basic mechanisms of human endocrine diseases and disorders. The traditional core of an endocrine system consists of an endocrine gland, the hormone it secretes, a responding tissue containing a specific receptor to which the hormone binds, and an action that results after the hormone binds to its receptor, termed the postreceptor response.

Each endocrine gland consists of a group of specialized cells that have a common origin in the developing embryo. Some endocrine glands, such as the thyroid gland and the islets of Langerhans in the pancreas, are derived from cells

that arise in the embryonic digestive system. Other endocrine glands, such as the parathyroid glands and the adrenal medulla, are derived from cells that arise in the embryonic nervous system. Certain glands, including the ovary, testis, and adrenal cortex, arise from a region of the embryo known as the urogenital ridge. There are also several glands that are derived from cells that originate in multiple regions of the embryo. For example, the pituitary gland is composed of cells from the nervous system and the digestive tract.

Each endocrine gland also has a rich supply of blood vessels. This is important not only because nutrients are delivered to the gland by the blood vessels but also because the gland cells that line these vessels are able to detect serum levels of specific hormones or other substances that directly effect the synthesis and secretion of the hormone the gland produces. Hormone secretion is sometimes very complex, because many endocrine glands secrete more than one hormone. In addition, some organs function both as exocrine glands and as endocrine glands. The best-known example of such an organ is the pancreas.

In addition to traditional endocrine cells, specially modified nerve cellswithin the nervous system secrete important hormones into the blood. These special nerve cells are called neurosecretory cells, and their secretions are termed neurohormones to distinguish them from the hormones produced by traditional endocrine cells. Neurohormones are stored in the terminals of

neurosecretory cells and are released into the bloodstream upon stimulation of the

cells.

Most hormones are one of two types: protein hormones (includingpeptides and modified amino acids) or steroid hormones. The majority of hormones are protein hormones. They are highly soluble in water and can be transported readily through the blood. When initially synthesized within the cell, protein hormones are contained within large biologically inactive molecules called prohormones. An enzyme splits the inactive portion from the active portion of the prohormone,

thereby forming the active hormone that is then released from the cell into the blood. There are fewer steroid hormones than protein hormones, and all steroid hormones are synthesized from the precursor molecule cholesterol. These hormones (and a few of the protein hormones) circulate in the blood both as hormone that is free and as hormone that is bound to specific proteins. It is the free unbound hormone that has access to tissues to exert hormonal activity.

Hormones act on their target tissues by binding to and activating specific molecules called receptors. Receptors are found on the surface of target cells in the case of protein and peptide hormones, or they are found within the cytoplasm or nuclei of target cells in the case of steroid hormones and thyroid hormones. Each receptor has a strong, highly specific affinity (attraction) for a particular hormone. A hormone can have an effect only on those tissues that contain receptors specific for that hormone. Often, one segment of the hormone molecule has a strong chemical affinity for the receptor while another segment is responsible for initiating the hormone's specific action. Thus, hormonal actions are not general throughout the body but rather are aimed at specific target tissues.

A hormone-receptor complex activates a chain of specific chemical responses within the cells of the target tissue to complete hormonal action. This action may be the result of the activation of enzymes within the target cell, interaction of the hormone-receptor complex with the deoxyribonucleic acid (DNA) in the nucleus of the cell (and consequent stimulation of protein synthesis), or a combination of both. It may even result in the secretion of another hormone.

The nature of endocrine regulation

Endocrine gland secretion is not a haphazard process; it is subject to precise, intricate control so that its effects may be integrated with those of the nervous system and the immune system. The simplest level of control over endocrine gland secretion resides at the endocrine gland itself. The signal for an endocrine gland to secrete more or less of its hormone is related to the concentration of some substance, either a hormone that influences the function of the gland (a tropic hormone), a biochemical product (e.g., glucose), or a biologically important

element (e.g., calcium or potassium). Because each endocrine gland has a rich supply of blood, each gland is able to detect small changes in the concentrations of its regulating substances.

Some endocrine glands are controlled by a simple negative feedbackmechanism. For example, negative feedback signaling mechanisms in the parathyroid glands (located in the neck) rely on the binding activity of calciumsensitive receptors that are located on the surface of parathyroid cells. Decreased

serum calcium concentrations result in decreased calcium receptor binding activity that stimulates the secretion of parathormone from the parathyroid glands. The increased serum concentration of parathormone stimulates bone resorption (breakdown) to release calcium into the blood and reabsorption of calcium in the kidney to retain calcium in the blood, thereby restoring serum calcium concentrations to normal levels. In contrast, increased serum calcium concentrations result in increased calcium receptor-binding activity and inhibition of parathormone secretion by the parathyroid glands. This allows serum calcium concentrations to decrease to normal levels. Therefore, in people with normal parathyroid glands, serum calcium concentrations are maintained within a very narrow range even in the presence of large changes in calcium intake or excessive losses of calcium from the body.

Control of the hormonal secretions of other endocrine glands is more complex, because the glands themselves are target organs of a regulatory system called the hypothalamic-pituitary-target gland axis. The major mechanisms in this regulatory system consist of complex interconnecting negative feedback loops that involve the hypothalamus(a structure located at the base of the brain and above the pituitary gland), the anterior pituitary gland, and the target gland. The hypothalamus produces specific neurohormones that stimulate the pituitary gland to secrete specific pituitary hormones that affect any of a number of target organs,

including the adrenal cortex, the gonads (testes and ovaries), and the thyroid gland. Therefore, the hypothalamic-pituitary-target gland axis allows for both neural and hormonal input into hormone production by the target gland.

When stimulated by the appropriate pituitary hormone, the target gland secretes its hormone (target gland hormone) that then combines with receptors located on its target tissues. These receptors include receptors located on the pituitary cells that make the particular hormone that governs the target gland. Should the amount of target gland hormone in the blood increase, the hormone's actions on its target organs increases. In the pituitary gland, the target gland hormone acts to decrease the secretion of the appropriate pituitary hormone, which results in less stimulation of the target gland and a decrease in the production of hormone by the target gland. Conversely, if hormone production by a target gland should decrease, the decrease in serum concentrations of the target gland hormone leads to an increase in secretion of the pituitary hormone in an attempt to restore target gland hormone production to normal. The effect of the target gland hormone on its target tissues is quantitative; that is, within limits, the greater (or lesser) the amount of target gland hormone bound to receptors in the target tissues, the greater (or lesser) the response of the target tissues.

In the hypothalamic-pituitary-target gland axis, a second negative feedback loop is superimposed on the first negative feedback loop. In this second loop, the target gland hormone binds to nerve cells in the hypothalamus, thereby inhibiting the secretion of specific hypothalamic-releasing hormones (neurohormones) that stimulate the secretion of pituitary hormones (an important element in the first negative feedback loop). The hypothalamic neurohormones are released within a set of veins that connects the hypothalamus to the pituitary gland

(the hypophyseal-portal circulation), and therefore the neurohormones reach the pituitary gland in high concentrations. Target gland hormones effect the secretion

of hypothalamic hormones in the same way that they effect the secretion of

pituitary hormones, thereby reinforcing their effect on the production of the pituitary hormone.

The importance of the second negative feedback loop lies in the fact that the nerve cells of the hypothalamus receive impulses from other regions of the brain, including the cerebral cortex (the centre for higher mental functions, movement, perceptions, emotion, etc.), thus permitting the endocrine system to respond to physical and emotional stresses. This response mechanism involves the interruption of the primary feedback loop to allow the serum concentrations of hormones to be increased or decreased in response to environmental stresses that activate the nervous system. The end result of the two negative feedback loops is that, under ordinary circumstances, hormone production by target glands and the serum concentrations of target gland hormone are maintained within very narrow limits but that, under extraordinary circumstances, this tight control can be overridden by stimuli originating outside of the endocrine system.

There are important supplemental mechanisms that control endocrine function. When more than one cell type is found within a single endocrine gland, the hormones secreted by one cell type may exert a direct modulating effect upon the secretions of the other cell types. This form of control is known as paracrine control. Similarly, the secretions of one endocrine cell may alter the activity of the same cell, an activity known as autocrine control. Thus, endocrine cell activity may be modulated directly from within the endocrine gland itself, without the need for hormones to enter the bloodstream.

If the requirement that a hormone act at a site remote from the endocrine cells in which the hormone is produced is excluded from the defining characteristics of hormones, additional classes of biologically active materials can be considered as hormones. Neurotransmitters, a group of chemical compounds of variable composition, are secreted at all synapses (junctions between nerve cells over which nervous impulses must travel). They facilitate or inhibit the transmission of neural impulses and have given rise to the science of neuroendocrinology (the branch of medicine that studies the interaction of the

nervous system and the endocrine system). A second group of biologically active substances is called prostaglandins. Prostaglandins are a complex group of fatty acidderivatives that are produced and secreted by many tissues. Prostaglandins mediate important biological effects in almost every organ system of the body. Another group of substances, called growth factors, possess hormonelike activity. Growth factors are substances that stimulate the growth of specific tissues. They are distinct from pituitary growth hormone in that they were identified only after it was noted that target cells grown outside the organism in tissue culture could be stimulated to grow and reproduce by extracts of serum or tissue chemically distinct from growth hormone.

Still another area of hormonal activity that has come under intensive investigation is the effect of endocrine hormones on behaviour. While simple

direct hormonal effects on human behaviour are difficult to document because of the complexities of human motivation, there are many convincing demonstrations of hormone-mediated behaviour in other life-forms. A special case is that of the

pheromone, a substance generated by an organism that influences, by its odour, the behaviour of another organism of the same species. An often-quoted example is the musky scent of the females of many species, which provokes sexual excitation in the male. Such mechanisms have adaptive value for species survival.

The Central Division of APUD cells.

Cell Types

1. Pineal gland (pinealocyte) which produces arginine vasotocin, and lutropin release hormone.
2. Hypothalamic magnocellular portion – produces, arginine vasotocin and arginine vasopressin.
3. Hypothalamic parvocellular portion produces release factors and release inhibiting factor
4. Pituitary pars distalis

with gonadotroph, somatrotroph, mammotroph which produce FSH, LH, ISH, GH, MSH, ß-endorphin, calcitonin.

5. Pituitary, pars intermedia with corticotroph, thyrotroph which

produce ACTH, MSH, ß-endorphin, calcitonin

B. Peripheral Division of APUD cells

Cell Types

1. Adrenomedullary A produces pre-met-enkephalin
2. Adrenomedullary NA produces pre-met-enkephalin
3. Carotid body type 1 produces met-enkephalin
4. Melanoblast/melanocyte produces cysteinyl-DOPA
5. Merkel cell of skin produces calcitonin and somatostatin
6. Thyroid/Ultimobranchial body

produces calcitonin and somatostatin from C cells

7. Pulmonary I, II and III produce bombesin, calcitonin and leuenkephalin
8. Urogenital tract EC (type I)
9. Urogenital tract U (type II)
10. Oviduct APUD cells
11. Uterine endometrial APUD cells
12. APUD cells of cervix and vagina and fallopian tube
13. Pancreas B, A, D and PP (F) cells

produce insulin, glucagon, somatostatin, pancreatic polypeptideand met-enkephalin

14. Stomach G cells produces Gastrin-17, enkephalin, ACTH and MSH
15. Stomach AL cell produces glucagon
16. Stomach ECI cell produces substance P
17. ECL cell or enterochromaffin like cell which produces histamine
18. Intestinal D cell which produces serotonin

19. Intestinal EC1 cell which produces substance P and serotonin
20. Intestinal M cell which produces motilin
21. Intestinal L cell which produces glicentin

22. Intestinal K cell and D cell which produces somatostatin and gastric inhibitory peptide
23. Intestinal N cell which produces neurotensin
24. Intestinal H cell which produces vasoactive inhibitory peptide and

porcine heptacosapeptide.
25. Intestinal I cell and P cell which
produces cholecystokinin and bombesin.
26. Intestinal P cell which produces bombesin
27. Intestinal IG cell which produces gastrin-34
28. Intestinal S cell and L cell which produces secretin and glicentin.
29. Intestine TG cell produces C-terminal gastrin and cholecystokinin
30. Gall bladder APUD cells

GASTROINTESTINAL ASSOCIATED LYMPHOID TISSUE (2 hours)

The lumen of the gastrointestinal tract is outside of the body and much of it is heavily populated with potentially pathogenic microorganisms. It is thus important that the immune system establish and maintain a strong presence at this mucosal boundary, and indeed, the digestive tube is heavily laden with lymphocytes, macrophages and other cells that participate in immune responses. Aside from all of its other functions, the gastrointestinal tract is a lymphoid organ, and the lymphoid tissue within it is collectively referred to as the gutassociated
lymphoid tissue or GALT. The number of lymphocytes in the GALT is roughly equivalent to those in the spleen, and, based on location, these cells are distributed in three basic populations:

Peyer's Patches: These are lymphoid follicles similar in many ways to lymph nodes, located in the mucosa and extending into the submucosa of the small intestine, especially the ileum. In adults, B lymphocytes predominate in Peyer's

patches. Smaller lymphoid nodules can be found throughout the intestinal tract.
In the image of canine ileum below, three lymphoid follicles of a Peyer's patch can be seen. The muscularis is at the top left, and mucosal epithelium in the bottom right.
Lamina propria lymphocytes: These are lymphocytes scattered in the lamina propria of the mucosa. A majority of these cells are IgA-secreting B cells.
Intraepithelial lymphocytes: These are lymphocytes that are positioned in the basolateral spaces between lumenal epithelial cells, beneath the tight junctions (they are "inside" the epithelium, but not inside epithelial cells as the name may incorrectly suggest).
Another important component of the GI immune system is the M or microfold cell. M cells are a specific cell type in the intestinal epithelium over lymphoid follicles that endocytose a variety of protein and peptide antigens. Instead of digesting these proteins, M cells transport them into the underlying tissue, where they are taken up by local dendritic cells and macrophages. Dendritic cells and macrophages that receive antigens from M cells present them to T cells in the GALT, leading ultimately to appearance of immunoglobulin A-secreting plasma cells in the mucosa. Dendritic cells below the epithelium can also sample lumenal antigens by pushing pseudopods between epithelial cells. The

secretory IgA is transported through the epithelial cells into the lumen, where, for example, it interferes with adhesion and invasion of bacteria.
T cells exposed to antigen in Peyer's patches also migrate into the lamina propria and the epithelium, where they mature to cytotoxic T cells, providing another mechanism for containing microbial assaults.
In addition to the GALT discussed above, lymph nodes that receive lymph draining from the gut (mesenteric nodes) and Kupffer cells (phagocytic cells in the liver) play important roles in protecting the body against invasion.
NEUROHUMORAL REGULATION OF DIGESTION (2 hours)

The activities of the digestive system are regulated by both hormones and neural reflexes. Four important hormones and their effects on target cells follow:

Gastrin is produced by enteroendocrine cells of the stomach mucosa.

Effects include:

o Stimulation of gastric juice (especially HCl) secretion by gastric glands.

o Stimulation of smooth muscle contraction in the stomach, small intestine, and large intestine, which increases gastric and intestinal motility.

o Relaxation of the pyloric sphincter, which promotes gastric emptying into the small intestine.

Secretin is produced by the enteroendocrine cells of the duodenal mucosa. Effects include:

o Stimulation of bicarbonate secretion by the pancreas, which stabilizes the pH of the chyme when released into the duodenum.

oStimulation of bile production by the liver.

o Inhibition of gastric juice secretions and gastric motility, which in turn slows digestion in the stomach and retards gastric emptying.

Cholecystokinin (CCK) is produced by the enteroendocrine cells of the duodenal mucosa. Effects include:

oStimulation of bile release by the gallbladder.

oStimulation of pancreatic juice secretion.

o Relaxation of the hepatopancreatic ampulla and opening of the hepatopancreatic sphincter, which allows the flow of bile and pancreatic juices into the duodenum.

The second regulatory agent of the digestive system is the nervous system. Stimuli that influence digestive activities may originate in the head, the stomach, or the small intestine. Based on these sites, there are three phases of digestive regulation:

1.The cephalic phase comprises those stimuli that originate from the head: sight, smell, taste, or thoughts of food, as well as emotional states. In response, the following reflexes are initiated:

1. Neural response: Stimuli that arouse digestion are relayed to the hypothalamus, which in turn initiates nerve impulses in the parasympathetic vagus nerve. These impulses innervate nerve networks of the GI tract (enteric nervous system), which promote contraction of smooth muscle (which causes peristalsis) and secretion of gastric juice. Stimuli that repress digestion (emotions of fear or anxiety, for example) innervate sympathetic fibers that suppress muscle contraction and secretion.

2. General effects: The stomach prepares for the digestion of proteins.

2.The gastric phase describes those stimuli that originate from the stomach. These stimuli include distention of the stomach (which activates stretch receptors), low acidity (high pH), and the presence of peptides. In response, the following reflexes are initiated:

1. Neural response: Gastric juice secretion and smooth muscle contraction are promoted.

2. Hormonal response: Gastrin production is promoted.

3. General effects: The stomach and small intestine prepare for the digestion of chyme, and gastric emptying is promoted.

3.The intestinal phase describes stimuli originating in the small intestine. These include distention of the duodenum, high acidity (low pH), and the presence of chyme (especially fatty acids and carbohydrates). In response, the following reflexes are initiated:

1. Neural response: Gastric secretion and gastric motility are inhibited (enterogastric reflex). Intestinal secretions, smooth muscle contraction, and bile and pancreatic juice production are promoted.

2. Hormonal response: Production of secretin, CCK, and GIP is promoted.

3. General effects: Stomach emptying is retarded to allow adequate time for digestion (especially fats) in the small intestine. Intestinal digestion and

motility are promoted.

REGENERATION OF NERVE FIBERS (2 hours)

tunnel syndrome), autoimmune disease (e.g. Guillain-Barré syndrome), infection

Nerves can be easily damaged in a traumatic event, but can regenerate if the soma and a small portion of the neurilemma remain. Neuroregeneration refers to the regrowth or repair of nervous tissues, cells, or cell products. Such mechanisms may include generation of new neurons, glia, axons, myelin, or synapses. Neuroregeneration differs between the peripheral nervous system (PNS) and the central nervous system (CNS) by the functional mechanisms and especially by the extent and speed. When an axon is damaged, the distal segment undergoes Wallerian degeneration, losing its myelin sheath. The proximal segment can either die by apoptosis or undergo the chromatolytic reaction, an attempt at repair. In the

Nerve injury

Micrograph of a nerve with a decrease in myelinated nerve fibres (pink) and an abnormal increase in fibrous tissue (yellow), as may be seen in nerve injuries.

Nervous system injuries affect more than 90,000 people every year, 10,000 of which are spinal cord injuries. As a result, the field of nerve regeneration and repair, a subfield of neural tissue engineering dedicated to the discovery of new ways to recover nerve functionality after injury, is growing rapidly. The nervous system is divided into two parts: the CNS, which consists of the brain and spinal cord, and the PNS, which consists of cranial and spinal nerves along with their associated ganglia. While the PNS has an intrinsic ability for repair and regeneration, the CNS is for the most part incapable of self-repair and regeneration. There is no current treatment to recover human nerve function after injury to the central nervous system. In addition, multiple attempts at nerve regrowth

across the PNS-CNS transition have not been successful. Although the PNS has the capability for regeneration, much research still needs to be done to optimize the environment for maximum regrowth potential. Nerve regeneration is

Nerve damage can be caused by physical injury or swelling (e.g. carpal
(e.g. neuritis), diabetes, or failure of the blood vessels surrounding the nerve.
CNS, synaptic stripping occurs as glia foot processes invade the dead synapse.
part of the pathogenesis of many diseases, including multiple sclerosis.
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form at the proximal stump and grow until they enter the distal stump. The growth of the sprouts are governed by chemotactic factors secreted from Schwann cells (neurolemmocytes). Injury to the PNS immediately elicits the migration of phagocytes, Schwann cells, and macrophages to the lesion site to clear away debris such as damaged tissue. When a nerve axon is severed, the end still attached to the cell body is labeled the proximal segment, while the other end is called the distal segment. After injury, the proximal end swells and experiences some retrograde degeneration, but once the debris is cleared, it begins to sprout axons and the presence of growth cones can be detected. The proximal axons are able to regrow as long as the cell body is intact and they have made contact with the Schwann cells in the endoneurial channel. Human axon growth rates can reach 2 mm/day in small nerves and 5 mm/day in large nerves. The distal segment, however, experiences Wallerian degeneration within hours of the injury; the axons and myelin degenerate, but the endoneurium remains. In the later stages of regeneration, the remaining endoneurial tube directs axon growth back to the

correct targets. During Wallerian degeneration, Schwann cells grow in ordered columns along the endoneurial tube. This creates a band of Büngner (boB) that protects and preserves the endoneurial channel. Also, macrophages and Schwann regeneration. It is limited by the inhibitory influences of the glial and extracellular environment. The hostile, non-permissible growth environment is in part created by the migration of myelin-associated inhibitors, astrocytes, oligodendrocytes, oligodendrocyte precursors, and microglia. The environment within the CNS, especially following trauma, counteracts the repair of myelin and neurons. Glial scars rapidly form and the glia actually produce factors that inhibit remyelination

and axon repair. The axons themselves also lose the potential for growth with age. Slower degeneration of the distal segment than that which occurs in the peripheral nervous system also contributes to the inhibitory environment; inhibitory myelin and axonal debris are not cleared away as quickly. All these factors contribute to the formation of what is known as a glial scar, which axons cannot grow across.

REGULATION OF OVARIAN-MENSTRUAL CYCLE (1 hours)

NEUROENDOCRINALREGULATION OF MENSTRUALCYCLE – is a complex, genetically determined system of inter-regulation of genitals, central nervous system and target organs. Formation of reproductive system starts antenatally and finishes at th eage of 18-21.

LEVELS OF MENSTRUAL CYCLE REGULATION.

Target organs. These include external and internal genitalorgans, mammary glands, bone tissue andskin. Target organs have receptors for steroidhormones. Influence of sex hormones on these organsleads to formation of secondary sexualcharacter, cyclical processes in endometriumand vagina.

Ovaries. Steroid hormones in the ovary are synthesizeddue to the action pituitary hormones. The hormones synthesis process inside theovary is called steroidogenesis. Adrenal glands and adipose tissue synthesizesteroid hormones too. Steroid hormones are synthesized fromcholesterol and have the same nature. Schematically this process can be representedin the following way: cholesterol – pregnenolone– androgens – estrogens.

Ovaries synthesize 3 types of hormones:estrogens, gestogens, androgens. Femaleorganism produces 3 fractions of estrogens. Estradiol - is the most active estrogen and isproduced by ovaries. Estrone – less active, mainly produced byadipose tissue. Estriol – is a result of transformation ofestradiol, estrone and androgens ofepinephroses. Estriol is produced only during pregnancy andhas minimal hormonal activity.

Progesterone – is the hormone ofyellow body of ovary and is a gestogen.

Major androgen of ovary is testosterone ,which is produced by cells of

internaltheca. Testosterone is not very active. Under the influence of enzyme 5-α-reductase, it is transformed into a moreactive hormone – dehydrotestosterone. Most estrogens and androgens mergewith sex steroid-binding globulin (SSBG). Smaller amount of estrogens merge withalbumin and erythrocytes. Only one per cent of estrogens remainsfree and influences the target organs. SSBG is synthesized by liver, its quantityis proportional to estrogen level, anddecreases under the influence ofandrogens. Today have been discovered progesteronebinding proteins.

PHYSIOLOGIC EFFECTSOF ESTROGEN ON FEMALE ORGANISM

Uterus. Estrogens determine the proliferationprocesses in endometrium, growth ofmyometrium and uterine tubes. Mammary glands. Stimulate growth. Bone tissue.

Estrogens are parathormoneantagonists. They hinder development ofosteoporosis and condense growth zones inbones. Cardiovascular system - i ncrease thearterial pressure and vascular tone.

Circulatory system. Increase the amount offibrin. Mineral metabolism. Estrogens influence thenatrium metabolism they attract sodium fromtissues and can cause oedemas. Lipidic metabolism. Increases quantity ofhigh-density β-lipoproteins; this has anti-atherosclerotic effect. Central nervous system. Estrogens

formoptimal neuropsychic condition. They changesynthesis of pituitary and hypothalamushormone.

PHYSIOLOGIC EFFECTS OF PROGESTERONE. Uterus. In case of sufficient concentration ofestrogens, progesterone exerts influence upontissues. Progesterone creates the evident anti-proliferative effect and conditions the secretionprocesses in endometrium. Besides, progesterone furthers myometriumgrowth. Mammary gland. Along with estrogens andprolactin, progesterone conditions tissuedevelopment. Progesterone also furtherslactation. Cardiovascular system. Progesteronedecreases vessels tone and

arterialpressure. Circulatory system. Progesterone doesnot influence the amount of fibrin. Mineral metabolism. Progesterone hasdiuretic effect. Central nervous system.Progesterone may cause depressions. Itshows evident anti-gonadotrophic action.

EFFECTS OF ANDROGENS. - Androgens in normal concentration aresynergists of estrogens. - In high concentrations androgens show evidentantigonadotrophic action and further thedevelopment of secondary male sexualcharacters. - During antenatal and postnatal periodsincrease of their level causes change of centralnervous system.

Hypophysis.

Hypophysis is divided into 2 lobes:anterior – adenohypophysis and posterior– neurohypophysis. Adenohypophysis consists of groups ofcells, these groups of cells are responsiblefor the synthesis of the followinghormones: growth hormone (somatotropic hormone –STH); thyrotropin (thyroid stimulating hormone –TSH); prolactin (PRL); folliclestimulating hormone (FSH); luteinizing hormone (LH); adrenocorticotropic hormone (ACTH); melanotropic hormone (MH).

LH, FSH, PRL are major hormones whichregulate menstrual cycle. But it is only possible under the conditionof optimal concentrations of other pituitaryhormones. Synthesis of pituitary hormones is realizeddue to stimulating impact ofhypothalamus. PRL synthesis depends on dopamineconcentration. PRL concentration increases whendopamine level decreases.

Hormones are not synthesized inthe posterior lobe of hypophysis. Oxytocin and vasopressin aresynthesized in hypothalamus, butare accumulated in the posteriorlobe of hypophysis.

Hypothalamus. Nucleuses of hypothalamus synthesizethe following neurohormones: libertinesand statines. The libertines stimulateadenohypophysis, statines inhibit it. These hormones are called releasinghormones.

Libertines include the following hormones: adrenocorticotropin-releasing hormone(ACTH-RG); thyrotropin-releasing hormone (TRG); gonadotropinreleasing hormone (GN-RG); growth hormone-releasing hormone(somatoliberin GH-RG); melanoliberin (M-RG)/

Statinesinclude the followinghormones: somatostatin; dopamine (major prolactin-inhibiting factor).

Neurohormones are synthesized not onlyin hypothalamus. Somatostatin is synthesized in tissues ofthyroid gland, bowels. Other hypothalamus peptides – gastrin,cholecystokinin, enkephaline aresynthesized by other tissues also. They create a regulation system called“diffused neuroendocrinal system oforganism”.

Extra-hypothalamic structures. Epiphysis, limbic system, celebrum tonsiland hippocampus influence thereproductive function. They are related to extrahypothalamicstructures.

Function of hypothalamus can bestimulated or inhibited by enkephalins,endorphins, neuropeptides.

NEUROENDOCRINALREGULATIONOF MENSTRUAL CYCLE. At the age of 10-12 years the reproductivesystem starts its development.There are severaltheories, which explain activation ofhypothalamo-pituitary-ovarian system.

1. Theory of late-pubescence. According to thistheory sensitivity of hypothalamus to the steroidhormones changes with the age. Besides,sensitivity of ovaries to gonadotropin increasesalso.

2. Theory of resonance. According tothis theory the increase in electricalactivity of hypothalamus nucleusesstimulates an increase of GN-RGlevel.

3. Theory of block release.According to this theory at the startof pubescence epiphysial functiondecreases and hypothalamicfunction increases.

The activation of hypothalamus makes itoversensitive to decrease in estrogenconcentration. Next, it synthesizes GN-RG, whichstimulates production of

FSH and LH. Gonadotropins influence the process ofgrowth and development of follicle(“folliculogenesis”) in ovaries. This descending process is calleddirect relationship.

The ovary of a newborn girl contains from400.000 to 500.000 primary ovarianfollicles. Only 400 follicles ripen and reach theovulation. Under the influence of FSH thedevelopment of several primari ovarianfollicles starts in the ovary. In the beginning they grow independently.

Later on, their growth depends on the FSH leveland the sensitivity of follicle to the FSH. Therefore, only one follicle reaches the size ofpre-ovulatory, others atrophy. The wall of antrum-containing follicle has 3sheaths: interstitial, internal theca andgranulosis. These sheaths have different sensitivity togonadotrophic hormones. Intersticium and theca are more sensitive to LH;granulosis is more sensitive to FSH.

Follicle synthesizes steroid hormones. This process is called steroidogenesis. Theca and intersticium synthesize steroidsup to androgen fraction; granulosisproduces estrogens. Increase in estrogen concentrationdepresses the function of hypophysis. This process is called negativeinverse relationship.

It takes place in the early pubertal period whenmenstrual cycles are monophase. Positive inverse relationship ischaracterized by maximum estrogenconcentration. Consequently honadotropines and libertinesincrease dramatically. Ovulation follows this process. The ovulation process is a

histochemicalprocess. Estrogens, prostaglandins and histamineinfluence the sheath of the follicle and cause itsrupture.

Granulosis cells are transformed into theyellow body under the influence of LHafter ovulation. The yellow body synthesizesprogesterone. Increase of progesterone inhibits thesynthesis of LH. This causes the death of the yellow body.

The ovarian cycle is a consistent process ofgrowth and development of follicle, ovulation,development and death of the yellow body. Ovarian cycle is

divided into 3 phases: Follicular phase is characterized by growthand development of follicle. It lasts 12-14 days. Ovulatory phase – lasts several hours. Lutein phase is characterized by developmentand functioning of the yellow body.
Uterine cycle is a cyclic process in the ovary,which causes cyclic changes in theendometrium. It has 4 phases: Desquamation. Decrease of steroid hormonescauses spasm, ischemia and rejection spiroidartery of decidual sphere of endometrium. First day of desquamation corresponds to thefirst day of menstruation and menstrual cycle. Regeneration. It corresponds to the earlyperiod of follicular phase in the ovary. Regeneration lasts 4-5 days. It starts with epithelization of endometrium.
Proliferation. It lasts 5-7 days and results inovulation. It corresponds to the late period of follicularphase in the ovary. Is characterized by proliferation of epitheliumand development of spiroid arteries. Secretion. It corresponds to lutein phase in theovary. It lasts 10-12 days. The process of proliferation is superseded bysecretion. If pregnancy did not take place, cyclic processesin the system uterusovaries-
hypothalamic-pituitary system repeat.
MENSTRUAL DISORDERSETIOLOGY Nervous diseases. Mental diseases. Malnutrition. Some occupational hazards. Systemic and gynecologic inflammatory diseases. Illness of the haemopoiesis, cardiovascular and othersystems. Gynecologic operations. Puberty disorders. Age-specific reconstruction of the functional state inhypothalamic-pituitary-ovarian axis in the menopause.
AMENORRHOEA Pathological primary amenorrhoea – when thepatient has never menstruated. Pathological secondary amenorrhoea – whenthe periods are absent for more than 6 months. Physiological amenorrhoea – before puberty,during pregnancy and lactation, and after themenopause. False amenorrhoea – when the flow does notescape because of some obstruction. True amenorrhoea – when the endometrialcycle is absent.
TRUE PATHOLOGICAL AMENORRHOEA

1. Uterine disorders. the uterus may be congenitally defective; the endometrium atrophies after irradiation with X-ray or radium, and hysterectomy.
2. Ovarian disorders. failure of ovarian development occurs in cases of gonadal dysgenesis; Stein-Leventhal syndrome is a disorder of unknown cause. After some years of normal menstruation amenorrhoea occurs with hirsuties. Both

ovaries are enlarged and contain multiple small follicular cysts. There is a block in the normal conversion of progesterone to estrogen so thatan intermediate androgen

substance androstendione appears in excess. The urinary excretion of estrogens is normal or low, while that of pregnantriol (a metabolic product of certain androgens) is raised; arrhenoblastoma is a very rare cause of amenorrhoea; ovarian infections or new growths as processes destroying all ovarian tissue.
3. Pituitary disorders. There is of production of gonadotrophichormones. Amenorrhoea is one aspect of generaldisorders and the gynecologist is seldomresponsible for treatment. Pituitary infantilism (Levi-Loraine syndrome).The adult resembles a child. No effectivetreatment is known. Pituitary cachexia (Simmond's disease). Thisis usually due to ischemic necrosis of thepituitary glands (hypophysis) due tothrombosis of pituitary vessels afterpostpartum hemorrhage and collapse. Failure of lactation is followed by genitalatrophy, loss of pubic hair, weakness,anorexia.
Treatment with cortisone, thyroxin and anabolicsteroids may cause some improvement. Adipogenital dystrophy is characterized bydwarfing, adiposity and genital infantilism, and isusually caused by a craniopharyngioma thatinvolves the pituitary gland and hypothalamus.The treatment is surgical. In acromegaly the eosinophilic adenoma of thepituitary gland may destroy the gonadotrophiccells, and the same may happen with otherpituitary tumors. Small pituitary adenoma may secrete prolactinand cause amenorrhoea with galactorrhoea.
Other endocrine disorders. Amenorrhoea occurs: in severe cases of hyperthyreoidism, myxoedema and cretinism; in some cases of diabetes; in

Addison’s disease; with adrenocortical tumors or hyperplasia.
Nervous disorders (stressor hypothalamic amenorrhoea).This is the commonest type ofsecondary amenorrhoea, andmay be the result of emotionaldisturbances.
Disorders of general health andnutrition. Any chronic or severe illness(including nutritional deficiency) willcause amenorrhoea. 7.Oral contraception. A delayed firstperiod is common after stopping oralcontraception. More prolongedamenorrhoea sometimes occurs.
DIAGNOSIS General examination; Special gynecologic examination; Ultrasonic; X-ray; Hormonal tests.
TREATMENT Sedative therapy; Vitamin therapy; Adequate nutrition, a special diet intended todecrease the body weight; Physiotherapy (endonasal electrophoresis with2% solution of vitamin B1, 0.25% solution ofdyphenhydramine hydrochloride). Drugs suppressing prolactin secretion(bromocriptine, parlodel, dostineks). Hormonal therapy of the cyclic hormones(estrogens followed by progesterone).
DISFUNCTIONAL UTERINE BLEEDING Disfunctional,or anovulatory uterine bleeding is associated with anovulation caused by impaired or unestablished functional relationships in the hypothalamic-pituitary-ovarian axis.

Classification In juvenile age; In reproductive age; In the premenopausal period.
Dysfunctionaluterine bleedings may be divided into anovulatory

and ovulatory ones.
Anovulatory bleedings are induced by theabsence of ovulation and luteal phase ofthe cycle. Anovulatory uterine bleeding develops inpatients with: persistence of an ovarian follicle;persistent follicles release a large numberof estrogens; atresia of a few follicles; atresia of somesmall follicles is associated withhypoestrogenism.

Both account for continuous, monotonoussecretion of estrogens. Ovulation does not occur and the corpus luteumfails to form. Excessive proliferation of the endometriumoccurs as a result of prolongedexposure to estrogens in both processes. Persistent and atretic follicles undergoinvolution. The level of hormones (estrogens) is decreasedin the blood, and bleeding develops.

Circulation in the endometrium isimpaired, the capillary permeability isdecreased, and the sites of dystrophy andnecrosis are manifested. The necrotic mucosa is rejected slowly,which causes prolonged bleeding. Dysfunctional uterine bleedings are notattended with pain.

CLINICAL PICTURE Amenorrhoea: in 4-8 weeks in persistent follicle;3-4 months in atretic follicles; Bleedings are more abundant in persistentfollicles, being occasionally profuse; Anemia; Decrease the patient's working capacity; General fatigue; Headache; Poor appetite; Sleep; Pale skin;

Tachycardia; Decreased blood pressure.

DIAGNOSIS Diagnosis is based on general andgynecologic examination. At generalexamination one should pay attention tothe typical sings: bleedings that follow the suppression ofmenses; monophase basal body temperature; high or low karyopycnotic index.

Dysfunctional uterine bleedings should bedifferentiated from many disease formsthat are attended with bleedings: abortions; interrupted fallopian pregnancy; tumors of the uterus.

TREATMENT Thedoctor's tactics largely depend on the patient's age.

Juvenile bleedings. Conservative treatment (use coagulants,hemostatic agents, stimulants of uterinecontractility). Hormonal haemostasis ("medicamentouscurettage" synthetic estrogen-progesterondrugs (logest, yrina, dgaz, ganin) areprescribed in a dose of 5-6 tablets daily isgradually decreased to 1 tablet per day (thetotal couse of drug administration is 21days). Diagnostic curettage of the uterine mucosa. When bleeding has been arrested, cyclichormone therapy is administered for 6 or 9cycles.

In the child-bearing age. Diagnostic curettage of the uterine mucosa.

Conservative treatment. Hormonal haemostasis (“medicamen-touscurettage” synthetic progestins (norcolut,orgametril, utrogestan, dyphaston) areprescribed in a dose of 5-6 tablets daily isgradually decreased to 1 tablet per day (thetotal couse of 155
drug administration is 21 days). When bleeding has been arrested, cyclichormone therapy is administered for 6 or 9cycles.
Premenopausal and menopausal age Diagnostic curettage of the uterine mucosa. Conservative treatment. Hormonal haemostasis (“medicamentouscurettage”

synthetic progestins (norcolut,orgametril, utrogestan, dyphaston) areprescribed in a dose of 5-6 tablets daily isgradually decreased to 1 tablet per day (thetotal couse of drug administration is 21days). The therapy is directed at regulating themenstrual function (in women under 45years) or its suppression (in women over 45years).
DYSMENORRHOEA(ALGOMENORRHOEA) This term is used to painfulmenstruation. Pain may develop before theonset of menstruation andcontinue throughout the period ofmenstrual flow.
Sometimes, pain is severe and attendedby nausea, vomiting and otherdisturbances, which reduce the patient’sworking capacity. In many cases dysmenorrhoea is just amanifestation of systemic diseases. It may be attributed to retroflexion oranteflexion of the uterus, cicatricialchanges, and narrowing of the cervicalcanal.
Primary and secondary forms ofdysmenorrhoea are distinguished. The formes does not appear to be linkedto any organic disease and is congenital. The latter develops in women withpreviously normal menstruations. Secondary dysmenorrhoea may berelated to inflammatory processes,endometriosis, and genital tumors

REGULATION OF SECRETORY ACTIVITY OF DIGESTIVE

GLANDS. REGENERATION OF DIGESTIVE GLANDS (2 hours)

Chemical digestion in the small intestine relies on the activities of three accessory digestive organs: the liver, pancreas, and gallbladder (Figure). The digestive role of the liver is to produce bile and export it to the duodenum. The gallbladder primarily stores, concentrates, and releases bile. The pancreas produces pancreatic juice, which contains digestive enzymes and bicarbonate ions, and delivers it to the duodenum.

Accessory Organs

The liver, pancreas, and gallbladder are considered accessory digestive organs, but their roles in the digestive system are vital.

The Liver

The liver is the largest gland in the body, weighing about three pounds in an adult. It is also one of the most important organs. In addition to being an accessory digestive organ, it plays a number of roles in metabolism and regulation. The liver lies inferior to the diaphragm in the right upper quadrant of the abdominal cavity and receives protection from the surrounding ribs.

The liver is divided into two primary lobes: a large right lobe and a much smaller left lobe. In the right lobe, some anatomists also identify an inferior quadrate lobe and a posterior caudate lobe, which are defined by internal features. The liver is connected to the abdominal wall and diaphragm by five peritoneal folds referred to as ligaments. These are the falciform ligament, the coronary ligament, two lateral ligaments, and the ligamentum teres hepatis. The falciform ligament and ligamentum teres hepatis are actually remnants of the umbilical vein, and separate the right and left lobes anteriorly. The lesser omentum tethers the liver to the lesser curvature of the stomach.

The porta hepatis (“gate to the liver”) is where the hepatic artery and hepatic portal vein enter the liver. These two vessels, along with the common hepatic duct, run behind the lateral border of the lesser omentum on the way to their destinations. As shown in Figure, the hepatic artery delivers oxygenated blood from the heart to the liver. The hepatic portal vein delivers partially

deoxygenated blood containing nutrients absorbed from the small intestine and actually supplies more oxygen to the liver than do the much smaller hepatic arteries. In addition to nutrients, drugs and toxins are also absorbed. After processing the bloodborne nutrients and toxins, the liver releases nutrients needed by other cells back into the blood, which drains into the central vein and then through the hepatic vein to the inferior vena cava. With this hepatic portal circulation, all blood from the alimentary canal passes through the liver. This largely explains why the liver is the most common site for the metastasis of cancers that originate in the alimentary canal.

The liver receives oxygenated blood from the hepatic artery and nutrient-rich deoxygenated blood from the hepatic portal vein.

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Histology

The liver has three main components: hepatocytes, bile canaliculi, and hepatic sinusoids. A hepatocyte is the liver’s main cell type, accounting for around 80 percent of the liver's volume. These cells play a role in a wide variety of

secretory, metabolic, and endocrine functions. Plates of hepatocytes called hepatic laminae radiate outward from the portal vein in each hepatic lobule.

Between adjacent hepatocytes, grooves in the cell membranes provide room for each bile canaliculus (plural = canaliculi). These small ducts accumulate the bile produced by hepatocytes. From here, bile flows first into bile ductules and then into bile ducts. The bile ducts unite to form the larger right and left hepatic ducts, which themselves merge and exit the liver as the common hepatic duct. This duct then joins with the cystic duct from the gallbladder, forming the common bile duct through which bile flows into the small intestine.

A hepatic sinusoid is an open, porous blood space formed by fenestrated capillaries from nutrient-rich hepatic portal veins and oxygen-rich hepatic arteries. Hepatocytes are tightly packed around the fenestrated endothelium of these spaces, giving them easy access to the blood. From their central position, hepatocytes

process the nutrients, toxins, and waste materials carried by the blood. Materials such as bilirubin are processed and excreted into the bile canaliculi. Other materials including proteins, lipids, and carbohydrates are processed and secreted into the sinusoids or just stored in the cells until called upon. The hepatic sinusoids combine and send blood to a central vein. Blood then flows through a hepatic vein into the inferior vena cava. This means that blood and bile flow in opposite directions. The hepatic sinusoids also contain star-shaped reticuloendothelial cells (Kupffer cells), phagocytes that remove dead red and white blood cells, bacteria, and other foreign material that enter the sinusoids. The portal triad is a distinctive arrangement around the perimeter of hepatic lobules, consisting of three basic structures: a bile duct, a hepatic artery branch, and a hepatic portal vein branch.

Bile

Recall that lipids are hydrophobic, that is, they do not dissolve in water.

Thus, before they can be digested in the watery environment of the small intestine, large lipid globules must be broken down into smaller lipid globules, a process called emulsification. Bile is a mixture secreted by the liver to accomplish the emulsification of lipids in the small intestine.

Hepatocytes secrete about one liter of bile each day. A yellow-brown or yellow-green alkaline solution (pH 7.6 to 8.6), bile is a mixture of water, bile salts, bile pigments, phospholipids (such as lecithin), electrolytes, cholesterol, and triglycerides. The components most critical to emulsification are bile salts and phospholipids, which have a nonpolar (hydrophobic) region as well as a polar (hydrophilic) region. The hydrophobic region interacts with the large lipid molecules, whereas the hydrophilic region interacts with the watery chyme in the intestine. This results in the large lipid globules being pulled apart into many tiny lipid fragments of about 1 μm in diameter. This change dramatically increases the

surface area available for lipid-digesting enzyme activity. This is the same way dish soap works on fats mixed with water.

Bile salts act as emulsifying agents, so they are also important for the

absorption of digested lipids. While most constituents of bile are eliminated in feces, bile salts are reclaimed by the enterohepatic circulation. Once bile salts reach the ileum, they are absorbed and returned to the liver in the hepatic portal blood. The hepatocytes then excrete the bile salts into newly formed bile. Thus,

this precious resource is recycled.

Bilirubin, the main bile pigment, is a waste product produced when the spleen removes old or damaged red blood cells from the circulation. These breakdown products, including proteins, iron, and toxic bilirubin, are transported to the liver via the splenic vein of the hepatic portal system. In the liver, proteins and iron are recycled, whereas bilirubin is excreted in the bile. It accounts for the green color of bile. Bilirubin is eventually transformed by intestinal bacteria into stercobilin, a brown pigment that gives your stool its characteristic color! In some disease states, bile does not enter the intestine, resulting in white (‘acholic’) stool with a high fat content, since virtually no fats are broken down or absorbed.

Hepatocytes work non-stop, but bile production increases when fatty chyme enters the duodenum and stimulates the secretion of the gut hormone secretin. Between meals, bile is produced but conserved. The valve-like hepatopancreatic ampulla closes, allowing bile to divert to the gallbladder, where it is concentrated and stored until the next meal.

The Pancreas

The soft, oblong, glandular pancreas lies transversely in the retroperitoneum behind the stomach. Its head is nestled into the “c-shaped” curvature of the duodenum with the body extending to the left about 15.2 cm (6 in) and ending as a tapering tail in the hilum of the spleen. It is a curious mix of exocrine (secreting digestive enzymes) and endocrine (releasing hormones into the blood) functions

Exocrine and Endocrine Pancreas

The pancreas has a head, a body, and a tail. It delivers pancreatic juice to the duodenum through the pancreatic duct.

The exocrine part of the pancreas arises as little grape-like cell clusters, each

called an acinus (plural = acini), located at the terminal ends of pancreatic ducts. These acinar cells secrete enzyme-rich pancreatic juice into tiny merging ducts that form two dominant ducts. The larger duct fuses with the common bile duct (carrying bile from the liver and gallbladder) just before entering the duodenum via a common opening (the hepatopancreatic ampulla). The smooth muscle sphincter of the hepatopancreatic ampulla controls the release of pancreatic juice and bile into the small intestine. The second and smaller pancreatic duct, the accessory duct (duct of Santorini), runs from the pancreas directly into the duodenum, approximately 1 inch above the hepatopancreatic ampulla. When present, it is a persistent remnant of pancreatic development.

Scattered through the sea of exocrine acini are small islands of endocrine cells, the islets of Langerhans. These vital cells produce the hormones pancreatic polypeptide, insulin, glucagon, and somatostatin.

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Pancreatic Juice

The pancreas produces over a liter of pancreatic juice each day. Unlike bile, it is clear and composed mostly of water along with some salts, sodium bicarbonate, and several digestive enzymes. Sodium bicarbonate is responsible for the slight alkalinity of pancreatic juice (pH 7.1 to 8.2), which serves to buffer the acidic gastric juice in chyme, inactivate pepsin from the stomach, and create an optimal environment for the activity of pH-sensitive digestive enzymes in the small intestine. Pancreatic enzymes are active in the digestion of sugars, proteins, and

fats.

The pancreas produces protein-digesting enzymes in their inactive forms. These enzymes are activated in the duodenum. If produced in an active form, they would digest the pancreas (which is exactly what occurs in the disease, pancreatitis). The intestinal brush border enzyme enteropeptidase stimulates the activation of trypsin from trypsinogen of the pancreas, which in turn changes the pancreatic enzymes procarboxypeptidase and chymotrypsinogen into their active

forms, carboxypeptidase and chymotrypsin.

The enzymes that digest starch (amylase), fat (lipase), and nucleic acids (nuclease) are secreted in their active forms, since they do not attack the pancreas as do the protein-digesting enzymes.

Pancreatic Secretion

Regulation of pancreatic secretion is the job of hormones and the parasympathetic nervous system. The entry of acidic chyme into the duodenum stimulates the release of secretin, which in turn causes the duct cells to release bicarbonate-rich pancreatic juice. The presence of proteins and fats in the duodenum stimulates the secretion of CCK, which then stimulates the acini to secrete enzyme-rich pancreatic juice and enhances the activity of secretin. Parasympathetic regulation occurs mainly during the cephalic and gastric phases of gastric secretion, when vagal stimulation prompts the secretion of pancreatic juice. Usually, the pancreas secretes just enough bicarbonate to counterbalance the amount of HCl produced in the stomach. Hydrogen ions enter the blood when bicarbonate is secreted by the pancreas. Thus, the acidic blood draining from the pancreas neutralizes the alkaline blood draining from the stomach, maintaining the pH of the venous blood that flows to the liver.

The Gallbladder

The gallbladder is 8–10 cm (~3–4 in) long and is nested in a shallow area on the posterior aspect of the right lobe of the liver. This muscular sac stores, concentrates, and, when stimulated, propels the bile into the duodenum via the common bile duct. It is divided into three regions. The fundus is the widest portion and tapers medially into the body, which in turn narrows to become the neck. The neck angles slightly superiorly as it approaches the hepatic duct. The cystic duct is 1–2 cm (less than 1 in) long and turns inferiorly as it bridges the neck and hepatic duct.

The simple columnar epithelium of the gallbladder mucosa is organized in rugae, similar to those of the stomach. There is no submucosa in the gallbladder wall. The wall’s middle, muscular coat is made of smooth muscle fibers. When

these fibers contract, the gallbladder’s contents are ejected through the cystic duct and into the bile duct. Visceral peritoneum reflected from the liver capsule holds the gallbladder against the liver and forms the outer coat of the gallbladder. The gallbladder's mucosa absorbs water and ions from bile, concentrating it by up to
10-fold.
The gallbladder stores and concentrates bile, and releases it into the two-way cystic duct when it is needed by the small intestine.
STRUCTURAL BASIS OF DIGESTION (2 hours)
About the Digestive System
Almost all animals have a tube-type digestive system in which food enters the mouth, passes through a long tube, and exits as feces (poop) through the anus. The smooth muscle in the walls of the tube-shaped digestive organs rhythmically and efficiently moves the food through the system, where it is broken down into tiny absorbable atoms and molecules.
During the process of absorption, nutrients that come from the food (including carbohydrates, proteins, fats, vitamins, and minerals) pass through channels in the intestinal wall and into the bloodstream. The blood works to distribute these nutrients to the rest of the body. The waste parts of food that the body can't use are passed out of the body as feces.
Every morsel of food we eat has to be broken down into nutrients that can be absorbed by the body, which is why it takes hours to fully digest food. In humans, protein must be broken down into amino acids, starches into simple sugars, and fats into fatty acids and glycerol. The water in our food and drink is also absorbed into the bloodstream to provide the body with the fluid it needs.
How Digestion Works
The digestive system is made up of the alimentary canal (also called the digestive tract) and the other abdominal organs that play a part in digestion, such as the liver and pancreas. The alimentary canal is the long tube of organs —

including the esophagus, stomach, and intestines — that runs from the mouth to the

anus. An adult's digestive tract is about 30 feet (about 9 meters) long.

Digestion begins in the mouth, well before food reaches the stomach. When we see, smell, taste, or even imagine a tasty meal, our salivary glands, which are located under the tongue and near the lower jaw, begin producing saliva. This flow of saliva is set in motion by a brain reflex that's triggered when we sense food or

think about eating. In response to this sensory stimulation, the brain sends impulses through the nerves that control the salivary glands, telling them to prepare for a meal.

As the teeth tear and chop the food, saliva moistens it for easy swallowing.

A digestive enzyme called amylase, which is found in saliva, starts to break down some of the carbohydrates (starches and sugars) in the food even before it leaves the mouth.

Swallowing, which is accomplished by muscle movements in the tongue and mouth, moves the food into the throat, or pharynx. The pharynx, a passageway for food and air, is about 5 inches (12.7 centimeters) long. A flexible flap of tissue called the epiglottis reflexively closes over the windpipe when we swallow to prevent choking.

From the throat, food travels down a muscular tube in the chest called the esophagus. Waves of muscle contractions called peristalsis force food down through theesophagus to the stomach. A person normally isn't aware of the movements of the esophagus, stomach, and intestine that take place as food passes through the digestive tract.

At the end of the esophagus, a muscular ring or valve called a sphincter

allows food to enter the stomach and then squeezes shut to keep food or fluid from

flowing back up into the esophagus. The stomach muscles churn and mix the food with acids and enzymes, breaking it into much smaller, digestible pieces. An acidic

environment is needed for the digestion that takes place in the stomach. Glands in the stomach lining produce about 3 quarts (2.8 liters) of these digestive juices each day.

Most substances in the food we eat need further digestion and must travel into the intestine before being absorbed. When it's empty, an adult's stomach has a volume of one fifth of a cup (1.6 fluid ounces), but it can expand to hold more than 8 cups (64 fluid ounces) of food after a large meal.

By the time food is ready to leave the stomach, it has been processed into a thick liquid called chyme. A walnut-sized muscular valve at the outlet of the stomach called the pylorus keeps chyme in the stomach until it reaches the right consistency to pass into the small intestine. Chyme is then squirted down into the small intestine, where digestion of food continues so the body can absorb the nutrients into the bloodstream.

The small intestine is made up of three parts:

1. the duodenum, the C-shaped first part
2. the jejunum, the coiled midsection
3. the ileum, the final section that leads into the large intestine

The inner wall of the small intestine is covered with millions of microscopic, finger-like projections called villi. The villi are the vehicles through which nutrients can be absorbed into the body.

The liver (located under the rib cage in the right upper part of the abdomen), the gallbladder (hidden just below the liver), and the pancreas (beneath the stomach) are not part of the alimentary canal, but these organs are essential to digestion.

The liver produces bile, which helps the body absorb fat. Bile is stored in the gallbladder until it is needed. The pancreas produces enzymes that help digest proteins, fats, and carbohydrates. It also makes a substance that neutralizes stomach acid. These enzymes and bile travel through special channels (called ducts) directly into the small intestine, where they help to break down food. The

liver also plays a major role in the handling and processing of nutrients, which are carried to the liver in the blood from the small intestine.

From the small intestine, undigested food (and some water) travels to the large intestine through a muscular ring or valve that prevents food from returning to the small intestine. By the time food reaches the large intestine, the work of absorbing nutrients is nearly finished. The large intestine's main function is to remove water from the undigested matter and form solid waste that can be excreted. The large intestine is made up of these three parts:

1. The cecum is a pouch at the beginning of the large intestine that joins the small intestine to the large intestine. This transition area expands in diameter, allowing food to travel from the small intestine to the large. The appendix, a small, hollow, finger-like pouch, hangs at the end of the cecum. Doctors believe

the appendix is left over from a previous time in human evolution. It no longer appears to be useful to the digestive process.

2. The colon extends from the cecum up the right side of the abdomen, across the upper abdomen, and then down the left side of the abdomen, finally connecting to the rectum. The colon has three parts: the ascending colon; the transverse colon, which absorb fluids and salts; and the descending colon, which holds the resulting waste. Bacteria in the colon help to digest the remaining food products.

3. The rectum is where feces are stored until they leave the digestive system through the anus as a bowel movement.

The digestive system consists of the digestive tract and its associated glands. Its functions are to obtain from ingested food the metabolites necessary for the growth and energy needs of the body. Before stored or used as energy, food is degested and transformed into small molecules that can be easily absorbed through the lining of the digestive tract. However, a barrier between the environment and

the internal milieu of the body must be maintained. The first step in the comlex process known as digestion occurs in the mouth, where food is ground into smaller pieces by mastification and moistened by saliva, which also initiates the digestion of carbohydrates. Digestion continues in the stomach and small intestine, the food – transformed into basic components (aminoacids, monosaccharides, glycerides, etc) – is absorbed. Water absorption occurs in the large intestine, and as a consequence the undisgested contents become semisolid.

The digestive process commences in the oral cavity with the ingestion, fragmentation and moistening of food but, in addition to its digestive role, the oral cavity is involved in speech, facial expression, sensory reception and breathing. The major structures of the oral cavity, the lips, teeth, tongue, oral mucosa and the associated salivary glands, participate in all these functions. Mastication is the process by which ingested food 1% made suitable for swallowing. Chewing not only involves coordinated movements of the mandible and the cutting and granding action of the teeth but also activity of the lips and tongue, which continually redirect food between the occlusal surfaces of the teeth. The watery component of saliva moistens and lubricates the masticatory process whilst salivary mucus helps to bind the food bolus ready for swallowing. The entire oral cavity is lined by a protective mucous membrane, the oral mucosa, which contains many sensory receptors, including the taste receptors of the tongue.

The epithelium of the oral mucosa is of the stratified squamous type which tends to be keratinised in areas subject to considerable friction such as the palate. The oral epithelium is supported by dense collagenous tissue, the lamina propria. The roof of the mouth consists of the hard and soft palates, both covered with the same type of stratified epithelium. In highly mobile areas such as the soft palate and floor of the mouth, the lamina propria is connected to the underlying muscle by loose submucosal supporting tissue. In contrast, in areas where the oral mucosa overlies bone, such as the hard palate and tooth-bearing ridges, the lamina propria is tightly bound to the periosteum by a relatively thin dense fibrous submucosa.

Throughout the oral mucosa numerous small accessory salivary glands of both serous and mucous types are distributed in the submucosa.

The palatine uvula is a small conical process that extends downward from the center of the lower border of the soft palate. It has a core of muscle and areolar connective tissue covered by typical oral mucosa.\

THE DEVELOPMENT OF ORAL CAVITY AND DIGESTIVE SYSTEM (2 hours)

The oral cavity and pharynx are anatomic spaces defined by hard and soft tissue structures. The shape of these two spaces changes with the normal physiologic function of the surrounding structures during speech, swallowing, and respiration. The oral cavity is bounded anteriorly by the lips, laterally by the cheeks, superiorly by the hard palate, and inferiorly by the mucosa covering the superior surface of the tongue and the sheet of muscles attaching to the inner side of the mandible, including geniohyoid, mylohyoid, and digastric. Although primarily a space through which food and air travel, several structures are found in this space, including the upper and lower dentition, the tongue, salivary glands, mucosal glands, and the mucosal tissue covering the hard palate, which bear the rugae. The oral cavity is continuous with the pharyngeal cavity, a more complex and somewhat irregular space.

The boundaries of the pharynx are the mouth and the nasal choanae anteriorly; the soft palate, or velum, and portions of the skull base superiorly; the posterior tongue inferiorly; and the pharyngeal constrictors posteriorly. In its anterior/inferior aspect, the pharynx joins the larynx; the adjacent lower portion is often referred to as the "hypopharynx" or the "laryngopharynx." In its anterior/superior potion the pharynx joins the nasal cavity and this upper portion is called the "nasopharynx." The midportion of the pharynx, where it joins the oral cavity, is called the "oropharynx." Thus the tongue lies in both the oral cavity and

the pharynx. Various tonsils, composed of lymphoid tissue, are found in the pharynx. The pharyngeal tonsil is found in the roof of the nasopharynx. The palatine tonsils are bilateral structures in the oropharynx, and lingual tonsils lie on the posterior portion of the tongue.

There are several ways of describing the specifics and details of these spaces and the structures that bound them. One classic approach is based on embryology and the developmental history of these spaces. Because the embryology follows the evolutionary origin of these spaces, it has the virtue of delineating the evolutionary constraints of the spaces, and explaining some of the design flaws with respect to function. This approach is very useful for those working with the impact of morphologic birth anomalies on function, as well as the repair of those anomalies. A functional approach, starting with the physiology and mechanics of structures, followed by a description of the sensory and motor mapping to relevant structures is useful for the application of anatomy to understanding normal function, as well as chronic and traumatic dysmorphology.

There are many excellent texts of anatomy, replete with extensive detail. Historically, anatomic atlases provide morphologic detail beyond that available in texts. Recent developments in imaging and publishing extend this tradition. Because the substance of anatomy has changed little over the years, some of the older texts are still both relevant and useful. Additionally, there are numerous texts with a more physiologic outlook, that integrate some portion of anatomy with a more specific functional focus. Finally, there are numerous texts describing, in detail, the embryologic history of the ultimate adult structures.

This review does not duplicate those sources, but rather provides

organization and overview so that basic scientists, clinicians, and other interested readers can utilize that detail as they wish. The information in this review is drawn from many of these sources. Occasionally there are conflicts among these sources (for example, in their description of the motor nerve supply to muscles in the

pharynx), and we have tried to indicate such controversy. Thus, we present both an embryologic overview and a functional overview of these spaces and their associated structures.

The bony skull of adults forms from a complex set of embryologic precursors of multiple tissues. In general, bones of the mammalian skeleton have two different histologic-level origins: either preformed in cartilage (endochondral bone), or direct ossification of mesenchyme (membranous bone). Nearly all postcranial bones are endochondral, but many bones of the head and neck region are membranous bones.

Membranous bones, including the parietal, temporal, and frontal bones, as well as portions of the occipital bones, form the vault of the skull, and are derived from the bony armor of early fish. The maxilla and mandible, as well as portions of the palate and zygomatic bone, are also formed through direct ossification.

The endochondral bones of the skull have several different sources for the cartilage templates. The cranial base bones that underlie the brain are evolutionary descendants of the chondrocranium, a cartilaginous structure that surrounded the brain in early vertebrates. In humans, these tissues are initially a set of capsules (the olfactory, the optic, and the otic) that merge with a set of midline embryologic cartilaginous structures (the prechordal cartilage, the hypophyseal cartilage, and the parachordal cartilage). Together these form the ethmoid bone, the body, the lesser and greater wings of the sphenoid bone, the petrous portion of the temporal bone, and the base of the occipital bone.

Other bones formed from cartilage are derivatives of the pharyngeal arches, complex structures evolutionarily derived from structures that supported and contained the gills in early vertebrates. This set of five serial structures, also called the branchial arches, run from anterior to posterior. They begin to form at day 22 of human development (the beginning of the fourth week) in the head and neck region, but are found in all mammalian embryos. Each arch contains an external covering of ectodermal tissue; a middle core of mesodermal tissue, which will form nerve, muscle, and cartilage; and an inner covering of endoderm. The

cartilage from the arches forms numerous oropharyngeal structures: the bones of the middle ear, the styloid process, the hyoid bone, and the cartilages of the larynx. Tissue in each of the arches also becomes muscle, nerve, and blood vessels. In general, arch one becomes the jaws, arch two becomes aspects of the face and ear, arch three becomes structures associated with the hyoid and upper pharynx, and arches four and six (arch five disappears) become structures associated with the larynx and lower pharynx. Numerous and detailed maps of the various tissues, as well as sensory fields, exist in many embryology texts.

The formation of the face, the palate, and the superior structures of the oral cavity are based on the dynamics of first arch development. The first arch splits

early on into two recognizable entities: a mandibular and a maxillary portion. By the end of the fourth week of development, these in turn form five distinct

swellings: two maxillary, two mandibular, and a superior, midline swelling: the frontonasal process. These tissues are invaded by cells called neural crest cells, which are responsible for the growth of these swellings. Eventually the fusion of these swellings forms the external face. A small gap becomes the mouth, and thickenings in the midline ectoderm eventually become pits that will become nares. Outgrowths of these tissues also become the palate.

Tissue from the pharyngeal arches is also responsible for the formation of the mucosa of the tongue. The complex patterns of both general and special sensation of the tongue are a reflection of its complex development. Mesoderm from the first arch will form the anterior two thirds of the tongue, up to the foramen cecum, whereas third and fourth arch mesoderm forms the posterior one third. Additional tissue from the occipital somites contribute to the tongue musculature. This tissue, originally posterior to the pharyngeal arches, is supplied by cranial nerve (CN) XII, the hypoglossal nerve.

The spaces between the arches externally are called clefts, internally pouches. The outer cleft between the first two arches contributes to the formation

of the external acoustic meatus. The other clefts are absorbed during development. The corresponding inner pouch between the first two arches becomes the tympanic cavity and auditory or eustachian tube. The remainder of the pouches contribute to glandular tissue of the head and neck. These include the palatine tonsils from pouch two, the inferior parathyroid glands and thymus from pouch three, the superior parathyroid glands from pouch four, and the ultimobranchial body from the inferiormost portion of pouch four. Subsequent to formation, these tissues migrate to their adult locations during normal development.

These embryologic relationships, not to mention the adult anatomy, seem only marginally patterned. That is, although the cranial nerves are numbered from I to XII, their fields of innervation are overlapping, and not in a strict superior to inferior or anterior to posterior pattern. In organisms that retain a more primitive anatomy, such as early chordates and even some fish, amphibians, and reptiles, the adult structures are serially organized and accurately reflect the embryologic order of the arches, so that structures supplied by CN II are always anterior to those supplied by CN III. Significant evolutionary reorganization of the head and neck regions results in a mammalian pattern that is no longer linear and neatly

organized.

The evolutionary history that generated these modifications is fascinating. Two distinct factors operate here: constraint and adaptation. Evolution seldom generates morphology of optimal design for function. Instead, to meet the challenges of a changing environment and competition from other organisms, selection operates on variation generated through mutation and frequently small genetic changes that produce more significant changes in the developmental program of an organism. Thus the existing forms, such as the branchial or pharyngeal arches, pose a constraint. However, in adapting to new environments, such as land-dwelling and air-breathing, organisms modify the old structures for

new functions. Subsequent adaptations for endothermy ("warm-blooded"), the
requirement for higher energetic consumption, and for separation of deglutition

and respiration modified the existing morphology further.

It is still useful, however, to appreciate the evolutionary linkage that is manifest in the embryologic development of humans. There are numerous clinical conditions that appear to influence diverse structures. Yet, the affected structures are united by embryologic origin. These problems, often referred to as syndromes of the first and second arch include Goldenhar syndrome, mandibulofacial dysostoses such as Treacher-Collins or Hallermann-Streiff, and DiGeorge syndromes.

There is a functional axis (the foodway) that follows the pathway of ingested food, from the mouth at the anterior end, moving posteriorly through the oral cavity, into the oropharynx, and then inferiorly through the hypopharynx, into the esophageal opening.

The bones that surround the first part of the foodway are the maxilla and mandible, the bones that bear the dentition, and the hard palate, which is composed of the palatine process of the maxilla and the maxillary process of the palatine bones. The cranial base, which forms the roof of the pharynx, is the body of the pterygoid bone, an endochondral bone. The latter part of the foodway is defined by muscle, but the hyoid bone and the cartilages of the larynx are pharyngeal arch structures that anchor this muscle.

The other significant hard tissue structures in the oral cavity are the teeth.

The human dentition, rooted in the maxilla and mandible, consists of 32 teeth in four quadrants, left and right, upper and lower. Humans are born edentulous, and the first deciduous teeth erupt approximately 6 to 8 months after birth. There are five deciduous teeth per quadrant: a medial and a lateral incisor, a canine, and the first and second molars. These teeth are replaced by permanent or adult teeth, and additionally, two premolars and a third molar erupt as part of the adult dentition.

All but the third molar are in place by approximately 12 years of age. The third molar, in modern humans, erupts much later, and often fails to erupt at all.
The muscles that form the walls of the oral cavity are simpler than those that form the walls of the pharynx. The orbicularis oris, which circles the opening of the mouth, and functions as a sphincter to close it, is the anterior boundary of the oral cavity. Other muscles that control the lips, and therefore the opening to the oral cavity are the labial muscles: levator labii superioris, depressor anguli oris, and risorius. The sides of the oral cavity can be considered the dentition, when the mouth is closed and jaw elevated, or the buccinator, the muscle that forms the cheeks. These are considered superficial facial muscles, and receive motor supply from branches of CN VII, the facial nerve.
The muscles of mastication, although not forming the boundaries of the oral cavity or pharynx, are critical for moving the jaws, and therefore oral function. Many of these muscles are supplied by V3 (mandibular branch of the trigeminal): temporalis, masseter, and medial pterygoid and lateral pterygoid.
The lower boundary of the oral cavity could be considered to be either the tongue or the muscles of the oral floor, which are responsible for opening the jaw.

The tongue is a muscular hydrostat, a structure with muscles, but no skeletal support, capable of movement. It consists of four extrinsic muscles—genioglossus (XII), hyoglossus (XII), styloglossus (XII), and palatoglossus (X or XI)—and three

groups of intrinsic fibers—vertical, transverse, and longitudinal fibers—all supplied by the hypoglossal nerve (XII). The genioglossus is an extrinsic muscle in that it originates on the mandible; however, its insertion merges into the intrinsic fibers with no clear demarcation. The neural supply to the tongue consists of three parts. The motor supply, detailed above, a general sensory component, which includes the lingual nerve (V3) to the anterior two thirds, branches of the glossopharyngeal nerve (IX) to the posterior one third, and a small area near the base supplied by the internal laryngeal nerve (X). The special sensation of taste is

supplied by the chorda tympani, a branch of the facial nerve (VII) to the anterior portion, and by the glossopharyngeal nerve (IX) and the internal laryngeal (X) to the posterior one third.

The muscles of the oral floor are sheets of parallel fibered tissue, running from the hyoid bone to the mandible, and include digastric (V3 and VII), mylohyoid (V3), and geniohyoid (XII and C1). Lying on the oral side or inside of geniohyoid is one of the muscles of the tongue, the genioglossus (XII). The general sensation of the oral cavity is from branches of the trigeminal nerve (V). The upper parts, including palate and teeth, are innervated from branches of the maxillary nerve (V2), and the lower parts include oral floor mucosa from the mandibular nerve (V3). There is significant autonomic nerve supply to glandular tissue in the oral cavity. The sympathetic portions derive from the T1 level of the spinal cord, synapse in the superior cervical ganglion, and the postganglionic nerves travel with blood vessels into the target tissue. The parasympathetic structures are branches of the facial nerve (VII), but are distributed with branches of V2 or V3 to the upper or lower portions of the oral cavity. The digastric muscle is believed to be the principal muscle of jaw opening, whereas the geniohyoid is the principal muscle for elevation of the hyoid bone. The thyrohyoid muscle approximates the thyroid cartilage to the hyoid bone during swallowing; thus synergistic contraction of these muscles accounts for laryngohyoid elevation in swallowing.

As food is processed, either through trituration of hard food, or transport of liquids, it leaves the oral cavity and enters the pharynx. The boundaries between these two spaces are the hard–soft palate junction, the foramen cecum marking the boundary between the anterior two thirds and the posterior one third of the tongue, and most importantly, the palatopharyngeal fold of the fauces, containing the palatopharyngeus muscles (X or XI). This muscle originates anteriorly and superiorly from both the hard palate and the aponeurosis of the soft palate and then runs in an inferior/posterior direction into the wall of the pharynx. The palatopharyngeus functionally divides the oral cavity from oropharynx.

The soft palate is the superior boundary of the oropharynx and consists of

several muscles joining in an aponeurosis: tensor veli palatini (V3), levator veli palatini (X or XI), palatopharyngeus (X or XI), uvulus (X or XI), and palatoglossus (X or XI). The principal elevator of the soft palate is the levator veli palatini, but all of these muscles play an important role in opening or closing the airway during

swallowing. The posterior and lateral walls of the pharynx are formed by the groups of muscles known collectively as the pharyngeal constrictors. These muscles, uniting in a midline posterior raphe include the superior, middle, and inferior constrictors, all supplied by branches of the vagus nerve (X). Inside of the

tube of muscle formed by the pharyngeal constrictors and in part of the wall are several longitudinal muscles. The stylopharyngeus runs from the styloid process into the pharyngeal wall, and the palatopharyngeus runs from the soft palate into this wall. These longitudinal muscles are also responsible for the maintenance of the pharyngeal portion of the airway, and may have a role in shortening the pharynx in swallowing (by raising the hypopharynx). The distal end of the pharynx is the upper esophageal sphincter (UES, X or XI), which consists of the lowest part of the inferior constrictor and, according to most authors, the surface of the cricoid cartilage. In some sources, this muscle is divided into the cricopharyngeus and the thyropharyngeus muscles. The cricopharyngeus muscle is unique in that it has no median raphe in its transverse portion. This muscle is tonically active between swallows, holding the UES closed. It is unclear whether the lower fibers of the thyropharyngeus and the upper fibers of the esophageal musculature also contribute to this sphincter closure. The UES represents the end of the pathway of food through the pharynx, and the point of entry of the bolus into the esophagus. The sensory nerve supply to the pharynx is primarily from the glossopharyngeal and vagus nerves (IX and X). Branches of these nerves, including the pharyngeal branch of the glossopharyngeus (IX), small branches from the external and superior laryngeal nerves, and the pharyngeal branches, both from the vagus (X), form the pharyngeal plexus that lies on the pharyngeal wall.

The exception is portions of the nasopharynx, which receive branches of the maxillary nerve (V2). The pharyngeal plexus also holds the motor neurons to the pharyngeal constrictor muscles.

The blood supply to the oral cavity and the pharynx is from branches of the external carotid artery. The branches of the external carotid that supply oral and pharyngeal structures are ascending pharyngeal a., lingual a., facial a., posterior auricular a., superficial temporal a., and numerous branches of the maxillary a. The venous drainage is through many tributaries, including facial, lingual, and pharyngeal vv.

Developmental Changes

Both continuous and discontinuous changes occur in the anatomy of the oral cavity and pharynx over the course of postnatal development. The most anatomically significant change to occur is the eruption of the deciduous dentition starting approximately 6 months after birth. However, the process of changing from a liquid diet to solid food is gradual in humans, and weaning often occurs over many months. Numerous other continuous changes are occurring during this period of time. The most functionally significant change is the descent of the larynx, and associated changes in airway protection.

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In the infant, the

superior position of the larynx places its opening in the nasopharynx. This intranarial position means that infants, as is true of nearly all nonhuman mammals,

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are considered to have obligate nasal respiration. There are, however, pathologic

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conditions, such as adenoid hypertrophy, that result in oral respiration. Despite the "locking" of the larynx into the nasopharynx, respiration uniformly pauses

during swallowing. The laryngeal changes are coordinated with an increase in size of the oropharynx relative to the nasopharynx, changes in the angle of the basicranium, and others subtle changes in pharyngeal morphology that continue through adolescence.

Relevance of Anatomy for Clinical Practice

The descent of the hyoid bone and larynx in early postnatal development has important implications for swallowing and respiration, as well as communication. The neonatal position of the larynx definitively separates the airway from the foodway during tidal respiration. Food accumulating in the oral cavity and oropharynx is prevented from entering the hypopharynx or the larynx by the direct contact of the epiglottis and larynx with the enfolding tissues of the fauces and soft palate. This contact is broken briefly during swallowing, when the soft palate elevates and the epiglottis folds downward, but then is rapidly restored after the swallow. The intranarial position of the larynx also prevents airflow through the mouth, and thereby limits vocalization. The larynx is pulled inferiorly during infant cry, mimicking its adult position.

Laryngeal descent is essential for speech, because it enables airflow through the larynx, pharynx, and oral cavity. Speech obviously carries an enormous benefit for human communication. The question then arises, given the advantage of the descended larynx for speech, why this descent does not occur prenatally. Certainly there are issues regarding the cortical development necessary for speech, and evidence that this development occurs postnatally. But the postnatal development of cerebral capacity for spoken language does not require that development of the peripheral structural mechanisms for speech occur postnatally as well. There is no need to separate the airway from the foodway in utero, because both are filled with amniotic fluid. But the neonate must adjust to huge changes in environment at the moment of birth, with a need to rapidly fill both lungs with air. The intranarial larynx provides an upper airway that is structurally isolated from the oral cavity, with a semirigid cartilaginous framework for the upper airway. This simplifies the

problem of establishing and maintaining airway patency, and reduces the likelihood of airway obstruction. Indeed, the relatively weak muscles of the neonatal pharynx and soft palate may need this structural support to maintain airway integrity for breathing, especially during sleep. The inability to maintain airway patency is a cause for obstructive sleep apnea.

The issue of swallow safety is also critical. Coordination of suckling, swallowing, and breathing is not fully developed in the neonate, creating a risk for deglutitive aspiration. The intranarial larynx prevents the bolus from entering the larynx before and after swallowing, and thus reduces the risk for airway obstruction and/or contamination by ingested milk or other material.

THE DEVELOPMENT OF THE RESPIRATORY SYSTEM

(4 hours)

The rudiment of the respiratory organs appears as a median longitudinal groove in the ventral wall of the pharynx. The groove deepens and its lips fuse to form a septum which grows from below upward and converts the groove into a tube, the laryngo-tracheal tube (Fig. 947), the cephalic end of which opens into the pharynx by a slit-like aperture formed by the persistent anterior part of the groove.

FIG. 947– The head and neck of a human embryo thirty-two days old, seen from the ventral surface. The floor of the mouth and pharynx have been removed. (His.)

The tube is lined by entoderm from which the epithelial lining of the respiratory tract is developed. The cephalic part of the tube becomes the larynx, and its next succeeding part the trachea, while from its caudal end two lateral outgrowths, the right and left lung buds, arise, and from them the bronchi and lungs are developed. The first rudiment of the larynx consists of two arytenoid swellings, which appear, one on either side of the cephalic end of the laryngotracheal

groove, and are continuous in front of the groove with a transverse ridge

(furcula of His) which lies between the ventral ends of the third branchial arches and from which the epiglottis is subsequently developed (Figs. 980, 981). After the separation of the trachea from the esophagus the arytenoid swellings come into contact with one another and with the back of the epiglottis, and the entrance to the larynx assumes the form of a T-shaped cleft, the margins of the cleft adhere to one another and the laryngeal entrance is for a time occluded. The mesodermal wall of the tube becomes condensed to form the cartilages of the larynx and trachea. The arytenoid swellings are differentiated into the arytenoid and corniculate cartilages, and the folds joining them to the epiglottis form the aryepiglottic folds in which the cuneiform cartilages are developed as derivatives of the epiglottis. The thyroid cartilage appears as two lateral plates, each chondrified from two centers and united in the mid-ventral line by membrane in which an additional center of chondrification develops. The cricoid cartilage arises from two cartilaginous

centers, which soon unite ventrally and gradually extend and ultimately fuse on the dorsal aspect of the tube.

3

J. Ernest Frazer 156 has made an important investigation on the development of the larynx and the following are his main conclusions:

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The opening of the pulmonary diverticulum lies between the two fifth arch masses and behind a “central mass” in the middle line—the proximal end of the diverticulum is compressed between the fifth arch masses. The fifth arch is joined by the fourth to form a “lateral mass” on each side of the opening, and these “lateral masses” grow forward and overlap the central mass and so form a secondary transverse cavity, which is really a part of the cavity of the pharynx. The two parts of the cavity of the larynx are separated in the adult by a line drawn back along the vocal fold and then upward along the border of the arytenoid eminence to the interarytenoid notch. The arytenoid and cricoid are developed in the fifth arch

mass. The thyroid is primarily a fourth arch derivative, and if it has a fifth arch element this is a later addition. The epiglottis is derived from the “central mass,” and has a third arch element in its oral and upper aspect; the arch value of the “central mass” is doubtful.

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FIG. 948– Lung buds from a human embryo of about four weeks, showing commencing lobulations. (His.) (See enlarged image)

FIG. 949– Lungs of a human embryo more advanced in development. (His.) (See enlarged image)

The right and left lung buds grow out behind the ducts of Cuvier, and are at first symmetrical, but their ends soon become lobulated, three lobules appearing on the right, and two on the left; these subdivisions are the early indications of the corresponding lobes of the lungs (Figs. 948,949). The buds undergo further subdivision and ramification, and ultimately end in minute expanded extremities—the infundibula of the lung. After the sixth month the air-sacs begin to make their

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appearance on the infundibula in the form of minute pouches. The pulmonary arteries are derived from the sixth aortic arches. During the course of their development the lungs migrate in a caudal direction, so that by the time of birth the bifurcation of the trachea is opposite the fourth thoracic vertebra. As the lungs grow they project into that part of the celom which will ultimately form the pleural cavities, and the superficial layer of the mesoderm enveloping the lung rudiment expands on the growing lung and is converted into the pulmonary pleura.

THE DEVELOPMENT OF THE URINARY SYSTEM (2 hours)

Development of the Urinary System Urinary and genital systems are closely associated Both develop from intermediate mesoderm 7th- 28th somite level(3-d week) Nephrogenic mass (cord) Dorsal side of coelom each cord produces a bulge into the coelom called the urogenital ridge Urinogenital Ridge Form the urinary and genital structures Nephrogenic tissue from 7-14th somite breaks up into

segments called nephrotomes

Fig. 1 - Transverse section and dorsal view of an embryo (trilaminar) (ca. 21 days) Fig. 1 - Transverse section and dorsal view of an embryo (trilaminar) (ca. 21 days) Fig. 1 - Transverse section and dorsal view of an embryo (trilaminar) (ca. 21 days) Transverse section of the three-layered embryo towards the end of the 3rd week of development. 1.Paraxial mesoderm 2.Intermediate mesoderm 3.Lateral mesoderm 4.Notochord 5.Amnion 6.Intraembryonic coelom 7.Endoderm 8.Ectoderm 9.Somatopleural (mesoderm and ectoderm) 10Splanchnopleural (mesoderm and endoderm) 11.Neural groove 12.Neural ridge

1.Neural tube 2.Notochord 3.Aorta dorsalis 4.Dorsal mesentery 5.Intestinal tube 6.Ectoderm 7.Somite 8.Inferior cardinal vein 9.Mesonephric duct (Wolffian duct) 10.Mesonephric tubule 11.Urogenital ridge Mesonephros enlargement point A

The urinary tract develops from the 3rd week of the embryonic period from the intermediate mesoderm as well as from the urogenital sinus. The kidneys develop from the 4th week in three steps: As a first one, a cranial anlage, the pronephros, forms that then later atrophies in the 8th week and is never active functionally. It is followed by a further anlage from the intermediate mesoderm, the mesonephros, that is formed between the 6th and 10th weeks, but is only transitory, and the anlage of the definitive kidneys, the metanephros. They develop from a metanephric anlage (mesodermal origin) and the ureter anlage (that has its origin in the caudal part of the wolffian duct). The urine-excreting part of the kidneys, the nephron, mainly arises from the metanephric anlage (glomerulus, proximal, intermediate and distal tubules), while the rest of the upper urinary tract (collecting ducts, calices, renal pelvis and ureter) develop from the ureter anlage. The lower urinary tract differentiates from the cloaca between the 5th and 8th weeks in that it becomes subdivided by the urorectal septum. The ventral part of the cloaca forms the primary urogenital sinus, out of which the urethra forms in the lower part and the bladder in the upper part. The ureter anlage discharges into the upper posterior wall of the urogenital sinus. In males, the wolffian duct remains

present and forms a connection to the genital tract in the lower part of the urogenital sinus. The numerous induction mechanisms between ureter anlage and metanephric mesenchyma during the development of the renal system, as well as the ascent of the kidneys, originating at the level of the sacrum and moving up to the diaphragm at the end of the development, make it possible for a large number of abnormalities to arise. Many remain asymptomatic whereas others are not compatible with survival.

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Intermediate Mesoderm Cervical region Loses contact with the somite Forms nephrotomes which acquire a lumen and open medially into the intraembryonic

coelom Caudal growth unite and form longitudinal duct Branches off dorsal aorta form glomeruli (ext & int) Thoracic, lumbar, sacral regions Loses contact with coelomic cavity Ext glomeruli fail to develop Segmentation disappears (nephrogenic cord) 2 or more excretory tubules per prior segment Urinary Tubules Associated with a vascular tuft = glomerulus Open tubules = external glomerulus One end opens into the coelom Other end opens into the collecting duct Closed tubules = internal glomerulus Open only into the collecting ducts

Formation of 3 kidney systems Pronephros (simplest & most primitive) 7-10 solid or tubular arranged cell groups in the cervical region (head kidney) Gone by the end of the 4th week Mesonephros (intermediate-more advanced) Appear during regression of pronephros 10-26th somite level Metanephros (permanent kidney) Begins to develop early in 5th week, functions by the 11th week

1.Nephrogenic cord 2.Mesonephric duct (Wolff) 1+2.Mesonephros 3.Intestinal tube 4.Cloaca 5.Atrophying nephrotomes 6.Yolk sac (umbilical vesicle) 7.Allantois 8.Outflow of the mesonephric duct into the cloaca Pronephros (forekidney): transitory structure

Mesonephros Tubules develop from nephrogenic cord (NC) Opens into the

excretory/mesonephric duct Gone by week 10 in females, in males some tubules persist & become vas deferens Approximately 38 pairs of closed tubules S shaped
bend Surrounds internal glomerulus Mesonephric duct develops laterally from NC & extends from 8th somite to urinogenital sinus
Mesonephros: transitory kidney 1.Nephrogenic cord 2.Mesonephric duct 1+2.Mesonephros 3.Intestine 4.Cloaca 5.Atrophied nephrotome 6.Yolk sac (umbilical vesicle) 7.Allantois 8.Outflow of the mesonephric duct into the cloaca 9.Ureter bud (anlage)
1.Neural tube 2.Notochord 3.Aorta dorsalis 4.Dorsal mesentery 5.Intestinal tube 6.Ectoderm 7.Somite 8.Inferior cardinal vein 9.Mesonephric duct (Wolffian duct) 10.Mesonephric tubule 11.Urogenital ridge Mesonephros enlargement point A
1a-Pronephros (atrophying) 1b-Mesonephros (atrophying) 2-Mesonephric duct (Wolffian duct) 3-Nephrogenic cord 4-Ureter anlage 5-Metanephric blastema 6-Liver anlage 7-Cloaca The metanephros: definitive kidney 5th week
Metanephric outflow 1.Cloaca 2.Ureter anlage 3.Metanephric blastema 2+3.Metanephros 4.Mesonephric duct (Wolffian duct) 5.Nephrogenic cord 4+5.Mesonephros
Metanephros Nephrons/tubules develop from nephrogenic mass (26th-28th somite level) Located lateral to mesonephric duct Internal dense layer which forms tubules/nephrons Outer loose layer forms connective tissue capsule Duct system derived from ureteric bud Ureter, renal pelvis, calyces, collecting ducts Ureteric bud elongates and makes contact with nephrogenic mass which surrounds bud like a cap Tubules are closed (internal glomerulus) Migrate from pelvis to abdomen as 148
fetus grows Blood supply from aorta changes as ascent occurs Becomes functional in second ½ of pregnancy
1.Ureter 2.Renal pelvis 3.Vena renalis 4.Arteria renalis 5.Major calix 6.Minor calix 7.Cortex 8.Capsula renis 9.Medullary rays 10.Papilla renalis

11.Sinus renalis 12.Columna renalis 13.Medullary pyramid Kidney at the end of its development

1.Ureter 2.Major calix 3.Minor calix 4.Renal pelvis 5.Collecting duct 6.Metanephric vesicle 7.Kidney lobe Schematic cut through the kidney (sagittal section)

8.Distal tubule 9.Proximal tubule 10.Glomerulus 11.Connecting tubule 13.Intermediate tubule Enlargement of the inset Nephron

Cloaca Caudal end of the hindgut (dilated) In 3 week old embryo the hindgut ends blindly at the cloacal membrane Blind end = cloaca Allantois and mesonephric ducts open into cloaca Cloaca is latin for sewer, a system of pipes used to transport human waste

Urinary Bladder During 4th to 7th week cloaca subdivided Posterior portion = anorectal canal Anterior portion = primitive urogenital sinus Bladder is formed from primitive urogenital sinus Bladder is upper and largest part of urogenital sinus Initially bladder is continuous with the allantois Allantois lumen obilterated & urachus formed connecting apex of bladder with umbilicus In adult urachus =

median umbilical ligament Ureter is outgrowth of mesonephric duct Terminal ends of mesonephric ducts become part of bladder wall Ureter obtains separate entrance into bladder with time

Production of urine by fetus Fetal urine mixes with amniotic fluid Amniotic fluid enters fetal intestinal tract where it is absorbed into bloodstream From the bloodstream to the placenta which transfers metabolic waste to the mother Fetal kidneys are not necessary for exchange of waste products

During the fifth and sixth weeks of development, the mature kidneys lie in the pelvis with their hila pointed anteriorly. As the pelvis and abdomen grow, the kidneys slowly move upward. By the seventh week, the hilum points medially and the kidneys are located in the abdomen. As the embryo continues to grow in a caudal direction, the kidneys are left behind and eventually come to lie in a

retroperitoneal position at the level of L1 by the ninth week of development. In the meantime, the kidneys have completed rotation and the hila now face anteromedially. Ascent of Kidneys