MINISTRY OF PUBLIC HEALTH OF UKRAINE

O. O. BOGOMOLETS NATIONAL MEDICAL UNIVERSITY

Department of Bioorganic and Biological Chemistry

Methodical recommendations for consideration of the topic

"Biochemistry of connective tissue"

on "Biological and bioorganic chemistry"

FOR STUDENTS OF THE 2ST YEAR OF STUDY OF MEDICAL and STOMATOLOGICAL FACULTIES

Kyiv-2020

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O.O. BOHOMOLETS

KYIV NATIONAL MEDICAL UNIVERSITY

DEPARTMENT OF BIOLOGICAL CHEMISTRY

BIOCHEMISTRY OF CONNECTIVE TISSUE

Guide for practical work on Biological Chemistry

Methodical instructions are made for students of the 2nd year of medical medical-psychological, dental faculties and FPTAFU

Kyiv 2020

Topic: Connective Tissue: Structure, Composition and Metabolism.

Relevance of the topic. Connective tissue is a multicomponent, polyfunctional complex of cells and extracellular matrix that serves as a framework for all organs, combining to form a unified organism. It make up a large proportion of the total body mass (more 50 %), are highly specialized and have a diversity of roles. It is a structure responsible for morphogenesis, homeostasis maintenance, biomechanical support, and more. The regeneration potential of connective tissue affects healing of damaged tissue and organs. The breadth and depth of information about connective tissues has fundamental scientific significance as well as applied relevance in clinical medicine.

Theoretical questions.

1. Kinds of Connective tissue and their significance. General characteristics of the biocamical composition of Connective tissue.
2. Fibers of connective tissue: Collagen fibers, Elastic fibers. Formation of Type I collagen.
3. Proteoglycans. Structure and role of proteoglycans and glycosaminoglycans.
4. Characterization and biochemical composition of bone tissue.
5. Spesific bone tissue enzymes.
6. Bone turnover markers
7. Pathobiochemistry of connective tissue.

Chapter 1. THEORETICAL REVIEW.

1. Kinds of Connective tissue and their significance. General characteristics of the biocamical composition of Connective tissue.

Connective tissue (CT) is a one of the four main classes of tissues. Although it is the most abundant and widely distributed of the primary tissues, the amount of connective tissue in a particular organ varies. The connective tissue provided structure and support throughout the body.

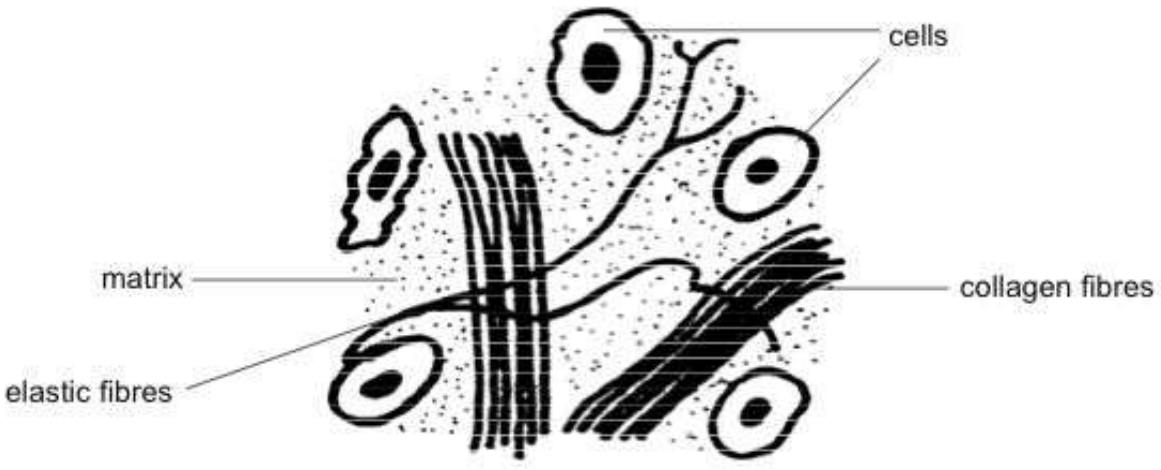
Structure of Connective Tissue

All forms of connective tissue are composed of (1) extracellular fibris, (2) an amorphous matrix called ground substance and (3) stationary and migrating cells. The proportions of these components vary from one part of the body to another depending on the local structural requirements. In some areas, the connective tissue is loosely organized and highly cellular; in others, its fibrous components predominate; and in still others, the ground substance may be its most conspicuous feature. The anatomical classification of the various types of connective tissue is based largely upon the relative abundance and arrangement of these components.

Connective tissue has three main components:

1. Ground substance
2. Fibers
3. Cells

Together the ground substance and fibers make up the extracellular matrix. The composition of these three elements vary tremendously from one organ to the other. This offers great diversity in the types of connective tissue.



Structural elements of connective tissue: Connective tissues consist of three parts: cells suspended in a ground substance or matrix; and most have fibers running through it.

Ground substance is a clear, colorless, viscous fluid that fills the space between the cells and fibers. It is composed of proteoglicans and cell adhesion

proteins that allow the connective tissue to act as glue for the cells to attach to the matrix. The ground substance functions as a molecular sieve for substances to travel between blood capillaries and cells.

Types of Connective Tissue

Connective tissues encompass a diverse array of tissue types that are involved in binding and supporting body structure and tissues.

Connective tissue is divided into four main categories:

1. Connective proper
2. Cartilage
3. Bone
4. Blood

Connective tissue proper has two subclasses: loose and dense. Loose connective tissue is divided into 1) areolar, 2) adipose, 3) reticular. Dense connective tissue is divided into 1) dense regular, 2) dense irregular, 3) elastic.

Areolar Connective Tissue.

These tissues are widely distributed and serve as a universal packing material between other tissues. The functions of areolar connective tissue include the support and binding of other tissues.

It also helps in defending against infection. When a body region is inflamed, the areolar tissue in the area soaks up the excess fluid as a sponge and the affected area swells and becomes puffy, a condition called edema.

Adipose Tissue or Body Fat

This is loose connective tissue composed of adipocytes. It is technically composed of roughly only 80% fat. Its main role is to store energy in the form of lipids, although it also cushions and insulates the body.

The two types of adipose tissue are white adipose tissue (WAT) and brown adipose tissue (BAT). Adipose tissue is found in specific locations, referred to as adipose depots.

Reticular Connective Tissue

This tissue resembles areolar connective tissue, but the only fibers in its matrix are the reticular fibers, which form a delicate network. The reticular tissue is limited to certain sites in the body, such as internal frameworks that can support lymph nodes, spleen, and bone marrow.

Dense Regular Connective Tissue

This consists of closely packed bundles of collagen fibers running in the same direction. These collagen fibers are slightly wavy and can stretch a little bit.

With the tensile strength of collagen, this tissue forms tendons, aponeurosis and ligaments. This tissue forms the fascia, which is a fibrous membrane that wraps around the muscles, blood vessels, and nerves.

Dense Irregular Tissue

This has the same structural elements as dense regular tissue, but the bundles of collagen fibers are much thicker and arranged irregularly. This tissue is found in areas where tension is exerted from many different directions. It is part of the skin dermis area and in the joint capsules of the limbs.

Elastic Connective Tissue

The main fibers that form this tissue are elastic in nature. These fibers allow the tissues to recoil after stretching. This is especially seen in the arterial blood vessels and walls of the bronchial tubes.

Cartilage

This is a flexible connective tissue found in many areas in the bodies of humans and other animals, including the joints between bones, the rib cage, the ear, the nose, the elbow, the knee, the ankle, the bronchial tubes, and the intervertebral discs.

Cartilage is composed of specialized cells called chondroblasts and, unlike other connective tissues, cartilage does not contain blood vessels. Cartilage is classified in three types: 1) elastic cartilage, 2) hyaline cartilage, and 3) fibrocartilage, which differ in the relative amounts of these three main components.

Elastic Cartilage

This is similar to hyaline cartilage but is more elastic in nature. Its function is to maintain the shape of the structure while allowing flexibility. It is found in the external ear (known as an auricle) and in the epiglottis.

Hyaline Cartilage

This is the most abundant of all cartilage in the body. Its matrix appears transparent or glassy when viewed under a microscope. It provides strong support while providing pads for shock absorption. It is a major part of the embryonic skeleton, the costal cartilages of the ribs, and the cartilage of the nose, trachea, and larynx.

Bone tissue is also called the osseous tissue. The osseous tissue is relatively hard and lightweight in nature. It is mostly formed of calcium phosphate in the chemical arrangement termed calcium hydroxyapatite, which gives bones their rigidity. It has relatively high compressive strength, but poor tensile strength, and very low shear stress strength.

The hard outer layer of bones is composed of compact bone tissue, so-called due to its minimal gaps and spaces. Its porosity is 5–30%. This tissue gives bones their smooth, white, and solid appearance, and accounts for 80% of the total bone mass of an adult skeleton.

Filling the interior of the bone is the cancellous or spongy bone tissue, which is composed of a network of rod and plate-like elements that make the overall organ lighter and allow room for blood vessels and marrow.

Function of Connective Tissue

The major functions of connective tissue include:

1. Binding and supporting.
2. Protecting.
3. Insulating.
4. Storing reserve fuel.
5. Transporting substances within the body.

1. **Fibers of connective tissue: Collagen fibers, Elastic fibers**

Three types of fibers are found in connective tissue:

2. Collagen

3. Elastic fibers
4. Reticular fibers

Collagen. The 2 most important fibrous components of the connective tissue are collagen and elastin, both insoluble macromolecular proteins. Collagen is protein molecules made up of amino acids. It provides structural support to the extracellular space of connective tissues. Due to its rigidity and resistance to stretching, it is the perfect matrix for skin, tendons, bones, and ligaments. Generally, they show minimal elongation (less than 10%) under tension

Collagen can be further divided into several groups depending on the type of structures they form. There are 28 various types of collagen that have been discovered, but by far, the most common are types I through IV, with type I comprising over 90% of collagen in the human body.

Type	Polypeptide Composition	Distribution
I	$[\text{alpha 1(I)}]_2$, alpha 2(I)	Skin,bone,tendon,cornea,blood vessels
II	$[\text{alpha 1(II)}]_3$	Cartilage, intervertebral disk
III	$[\text{alpha 1(III)}]_3$	Fetal skin,blood vessels
IV	$[\text{alpha 1(IV)}]_2$, alpha 2(IV)	Basement membrane
V	$[\text{alpha 1(V)}]_2$, alpha 2(V)	Placenta,skin

Amino acids are the building blocks of proteins; therefore, it is no surprise that collagen is comprised of amino acids. The primary amino acid sequence of collagen is glycine-proline-X or glycine-X-hydroxyproline. X can be any of the

other 17 amino acids, and every third amino acid is glycine. Proline or hydroxyproline constitute about 1/6 of the total sequence. Glycine (Gly) is found at almost every third residue. Glycine accounts for 1/3 of the sequence. Proline (Pro) makes up about 17% of collagen. Collagen also has two uncommon derivative amino acids that are not directly inserted during translation. These amino acids are found at specific locations relative to glycine and are modified post-translationally by different enzymes, both of which require vitamin C as a cofactor. Hydroxyproline is derived from proline and Hydroxylysine derived from lysine. Depending on the type of collagen, varying numbers of hydroxylysines are glycosylated (mostly having disaccharides attached).

Collagen is composed of 3 chains. The chains are wound together to form a triple helix. Since glycine is the smallest of all the amino acids, it allows the chain to form a tight configuration, and and it can withstand stress.

The process of collagen synthesis occurs mainly in the cells of fibroblasts which are specialized cells with the main function of synthesizing collagen and stroma. Collagen synthesis occurs both intracellularly and extracellularly. Although different types of collagen may undergo different post-translational modifications, the basic outline for collagen synthesis is listed below.

Formation of Type I collagen. Type I collagen is the most abundant collagen in the body.

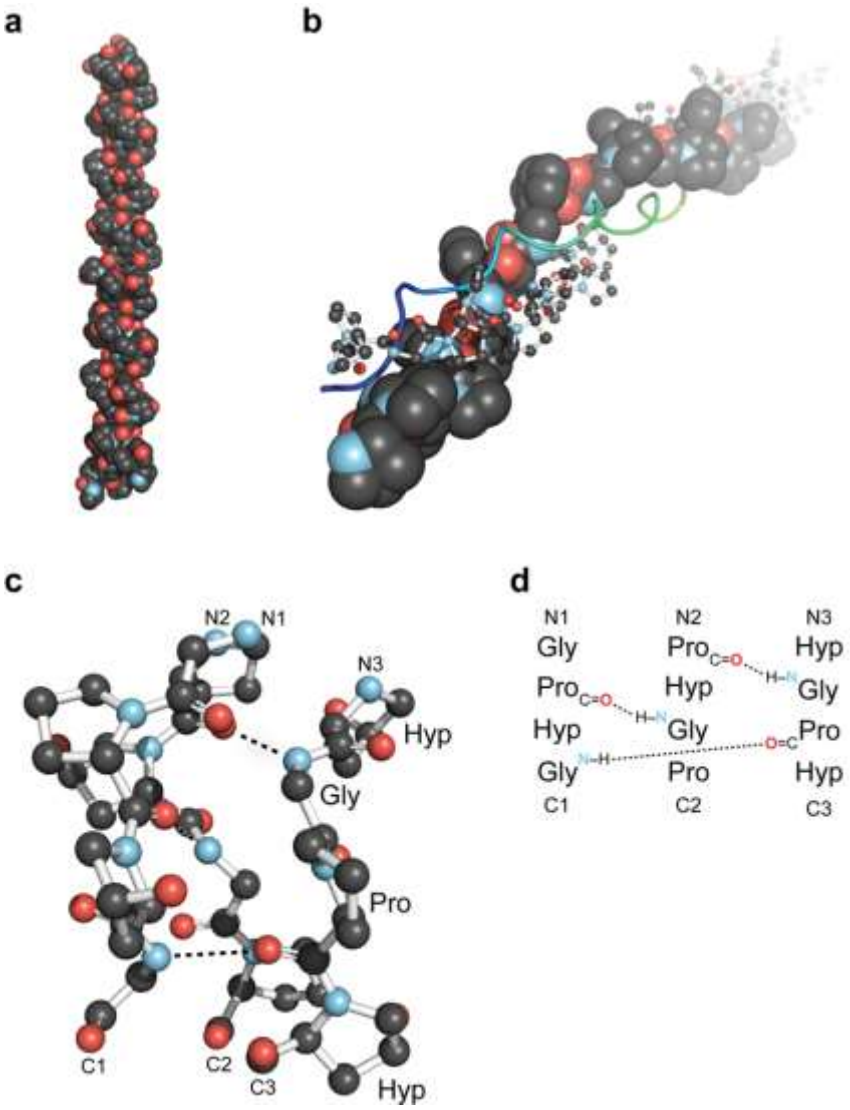
Within the cell

1. During translation, two types of peptide chains are formed on ribosomes along the rough endoplasmic reticulum (RER). These are called the alpha-1 and alpha-2 chains. These peptide chains (known as preprocollagen) have registration peptides on each end and a signal peptide.
2. The preprocollagen is then released into the lumen of the RER. Thereafter the signal peptides are cleaved inside the RER and the peptide chains are now called pro-alpha chains.

3. Hydroxylation of lysine and proline amino acids occurs inside the lumen. This process is dependent on ascorbic acid (Vitamin C) as a cofactor. Further glycosylation of the selected hydroxyl groups on lysine with galactose and glucose.
4. Three of the hydroxylated and glycosylated pro-a-chains assemble by twisting into a triple helix by zipper-like folding. The triple helix configuration is 3 left-handed helices twisted into a right-handed coil. Triple helical structure is formed inside the endoplasmic reticulum from each two alpha-1 chains and one alpha-2 chain. This is called procollagen.
5. Procollagen molecule is ready to move to the Golgi apparatus for final modifications and assembled into secretory vesicles to enter the extracellular space.

Outside the cell

1. Once outside the cell, the registration peptides are cleaved and tropocollagen is formed by procollagen peptidase.
2. These tropocollagen molecules gather to form collagen fibrils, via covalent cross-linking by lysyl oxidase which links hydroxylysine and lysine residues. Multiple collagen fibrils form into collagen fibers.
3. Collagen may be attached to cell membranes via several types of protein, including fibronectin and integrin.

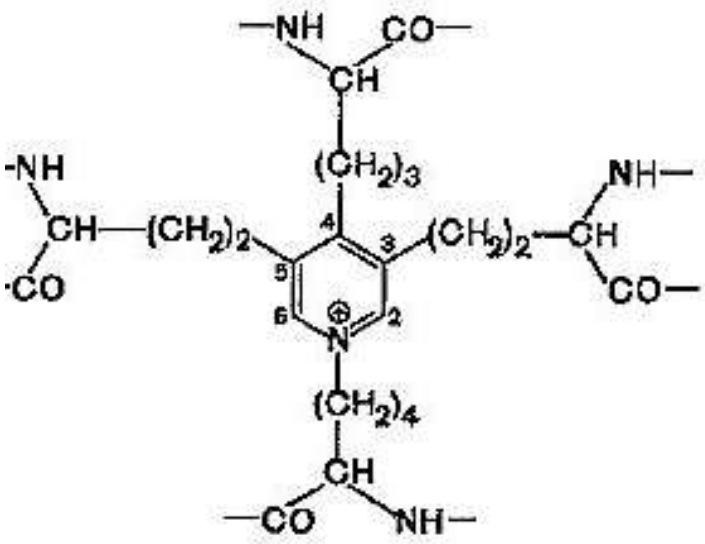


Overview of the collagen triple helix. (*a*) High-resolution crystal structure of a collagen triple helix. (*b*) View down the axis of a triple helix. (*c*) Image of a segment of collagen triple helix.(*d*) Stagger of the three strands in the segment in panel *c*.

Elastic fibers. Elastin is a key extracellular matrix (ECM) protein that provides resilience and elasticity to tissues and organs. Elastin is roughly 1000 times more flexible than collagens; thus, the main function of elastin is the elasticity of tissues. It is the dominant protein in extensible tissues and is primarily present in the lungs, aorta, and skin. Elastin rich in *proline, lysine, glycine*, unlike collagen, can exist in nonglycosylated forms. Elastic fibers are long, thin fibers that form branching network in the extracellular matrix. They help the connective tissue to stretch and recoil. Elastic fibers may increase their length by 150%, yet still return to their previous configuration.

Elastic fibers: extensible elements of the extracellular matrix. Elastic fibers in the ECM allow tissues such as skin, the lungs, and blood vessels to withstand repeated

stretching and considerable deformation and to return to a relaxed state. The arrangement of elastin varies and depends largely on the strength and direction of forces on the tissue. The fibers may be organized into concentric fenestrated sheets (eg, aorta), as small individual fibers (eg, skin, lung), or as a 3-dimensional honeycomb-like network of fine fibers (eg, elastic cartilage). Elastic fibers are composed of an elastin core and microfibrils located mostly around the periphery. The microfibrils, which are chiefly made up of *fibrillin*, initially act as a scaffold on which elastin is deposited, but once the core elastin is generated, the majority of microfibrils are displaced to the outer aspect of the fiber. Elastin contains 2 amino acids (ie, *desmosine and isodesmosine)* that form cross-linkages between adjacent tropoelastin chains and are important in imparting the elastic properties to elastin. The exact mechanism of extensibility is not clearly understood, but the quantity of elastin found within the tissue usually reflects the amount of mechanical strain imposed on it and the requirement for reversible deformation.



Desmosine

3.Proteoglycans. Structure and role of proteoglycans and glycosaminoglycans.

Proteoglycans consist of a core protein domain covalently linked to glycosaminoglycans (GAGs). These GAGs form long, negatively charged, linear repeats of disaccharide units, and provide proteoglycans with their unique ability to bind water, which is critical for imparting compressive resistance to tissues. Proteoglycans are found in all connective tissues, extracellular matrix (ECM) and on the surfaces of many cell types. Proteoglycans are remarkable for their *diversity* (different cores, different numbers of GAGs with various lenghts and compositions).

They perform numerous vital functions within the body. GAG dependent functions can be divided into two classes: the biophysical and the biochemical. The biophysical functions depend on the unique properties of GAGs: the ability to fill the space, bind and organize water molecules and repel negatively charged molecules. Because of high viscosity and low compressibility they are ideal for a lubricating fluid in the joints. On the other hand their rigidity provides structural integrity to the cells and allows the cell migration due to providing the passageways between cells.

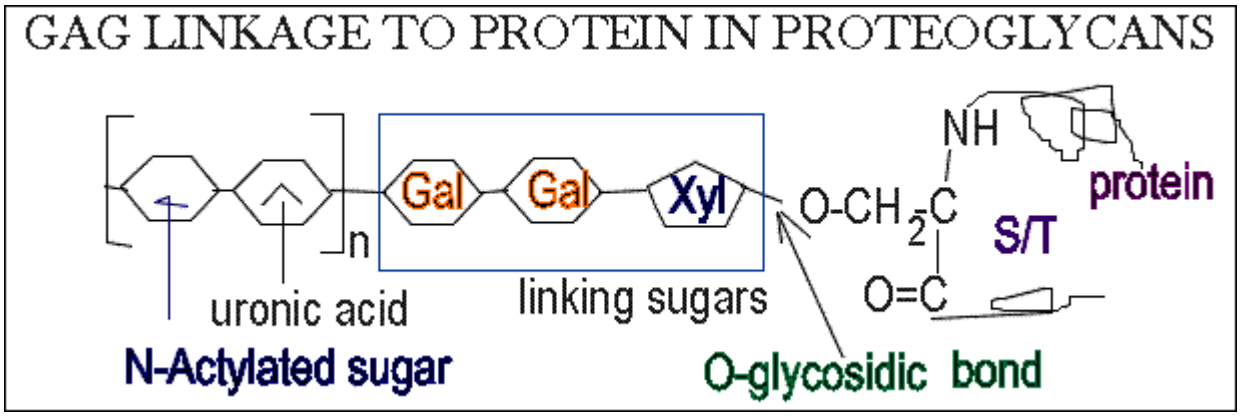
For example the large quantities of chondroitin sulfate and keratan sulfate found on *aggrecan* play an important role in the hydration of cartilage. They give the cartilage its gel-like properties and resistance to deformation.

Aggrecan is one of the most important extracellular proteoglycans. It forms very large aggregates (a single aggregate is one of the largest macromolecules known ; it can be more than 4 microns long). Aggrecan molecules are non-covalently bound to the long molecule of hyaluronan (like bristles to the backbone in a bottlebrush). It is faciliated by the linking proteins. To each aggrecan core protein multiple chains of chondroitin sulfate and keratan sulfate are covalently attached through the trisacharide linker. The other, more biochemical functions of GAGs are mediated by specific binding of GAGs to other macromolecules, mostly proteins. Proteoglycans participate in cell and tissue development and physiology.

Structure of proteoglycans

The GAGs extend perpendicular from the core protein in a bottlebrush- like structure. The linkage of GAGs such as (*heparan sulfates and chondroitin sulfates*) to the protein core involves a specific trisaccharide linker.

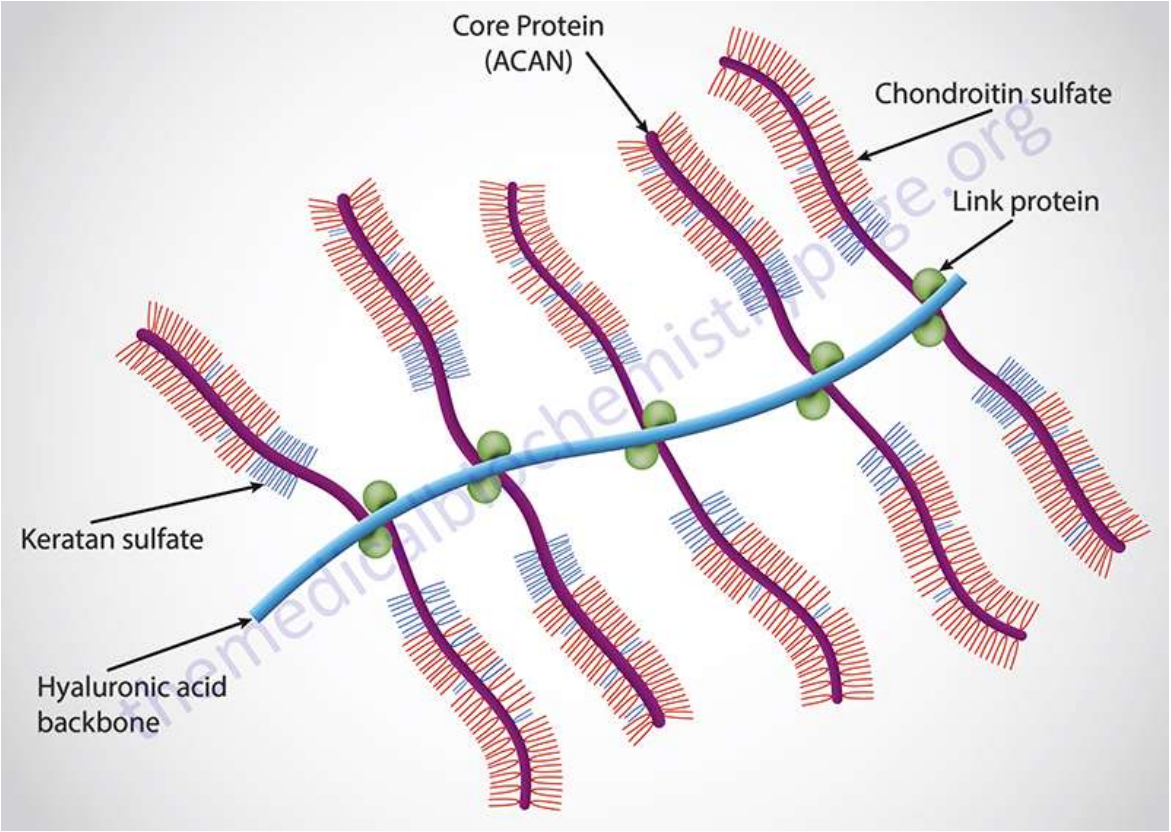
The linkage of GAGs to the protein core, in most but not all proteoglycans, involves a specific tetrasaccharide linker composed of a glucuronic acid (GlcA) residue, two galactose (Gal) residues, and a xylose (Xyl) residue forming a structure such as: $GAG_{(n)}$–GlcA–Gal–Gal–Xyl–Ser–protein. The tetrasaccharide linker is coupled to the protein core through an *O*-glycosidic bond to a Ser residue in the protein.



Some forms of *keratan sulfates* are linked to the protein core through an **N**-asparaginyl bond.

The protein cores of proteoglycans are rich in Ser and Thr residues which allows multiple GAG attachment.

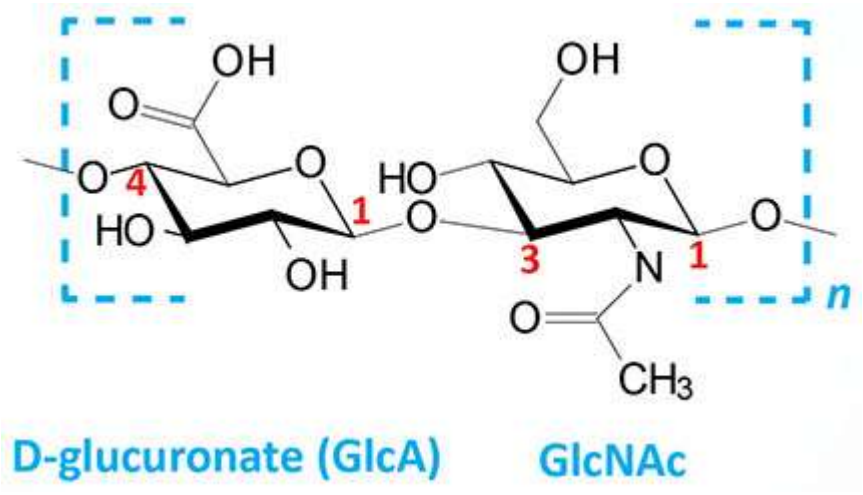
The GAG molecules are long unbranched polysaccharides containing a repeating disaccharide unit. The disaccharide units contain either of two modified sugars, *N*-acetylgalactosamine (GalNAc) or *N*-acetylglucosamine (GlcNAc), and a uronic acid such as glucuronate (GlcA) or iduronate (IdoA). GAGs are highly negatively charged molecules, with extended conformation that imparts high viscosity to the solution in which they reside.



Structure of an aggrecan-type complex proteoglycan

Along with the high viscosity of GAGs comes low compressibility, which makes these molecules ideal for a lubricating fluid in the joints. At the same time, their rigidity provides structural integrity to cells and provides passageways between cells, allowing for cell migration. The specific GAGs of physiological significance are hyaluronic acid, dermatan sulfate, chondroitin sulfate, heparin, heparan sulfate, and keratan sulfate.

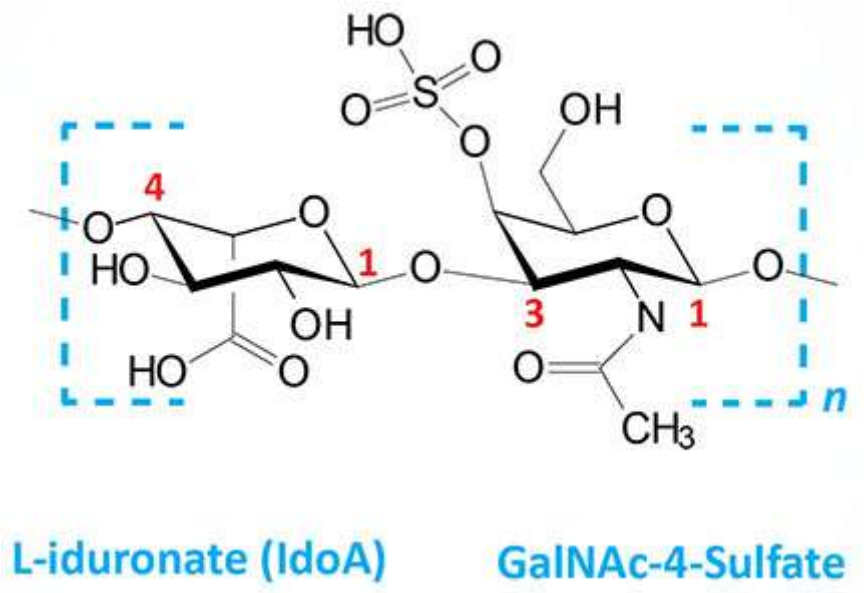
Hyaluronic acid composed of D-glucuronate (GlcA) plus GlcNAc; linkage is β(1,3)



Occurence: synovial fluid, ECM of loose connective tissue

Hyaluronic acid (HA) is a high molecular weight polysaccharide that is distributed in all bodily tissues and fluids. Hyaluronic acid is unique among the GAGs because it does not contain any sulfate and is not found covalently attached to proteins. It *forms non-covalently linked complexes with proteoglycans* in the ECM. The liver is the most important organ involved in the synthesis and degradation of HA. Hyaluronic acid polymers are very large (100 - 10,000 kD) and can displace a large volume of water.

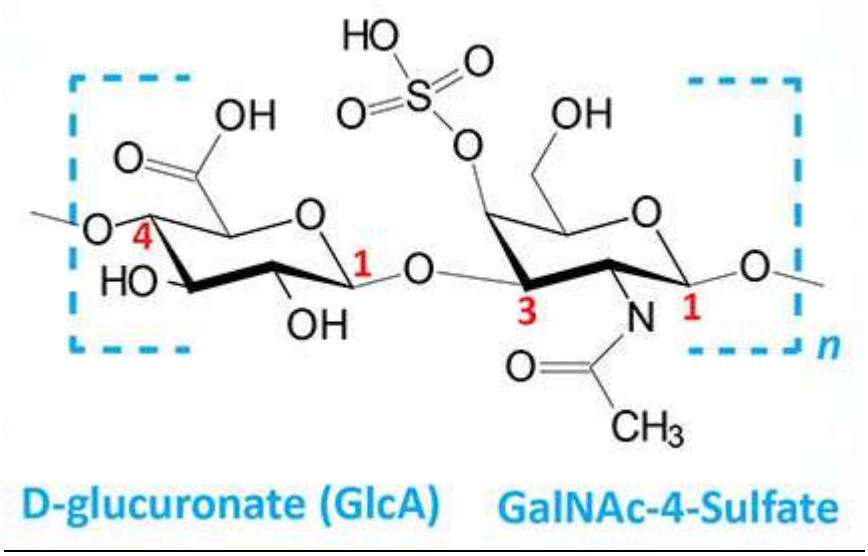
Dermatan sulfate composed of L-iduronate (IdoA) or D-glucuronate (GlcA) plus GalNAc-4-sulfate; GlcA and IdoA sulfated; linkages is β(1,3) if GlcA, α(1,3) if IdoA



Occurence : skin, blood vessels, heart valves.

Dermatan sulfates in humans are composed of repeating disaccharide units of iduronic acid and GalNAc. The name of this class of GAG is derived from the fact that they represent the predominant GAG in the skin (dermis). Although the presence of GalNAc technically identifies dermatan sulfate as a chondroitin sulfate, the presence of the iduronic acid establishes the dermatan sulfates as a distinct class of GAG. Dermatan sulfates are generated from chondroitin sulfates via the epimerization of the glucuronate (GlcA) residues of chondroitins to iduronate (IdoA). The epimerization reaction is catalyzed by an enzyme which is often referred to as uronyl C5-epimerase. Dermatan sulfates regulate specific functions of the *hemostasis cascade*. Dermatan sulfates also interact with numerous growth factors, thereby playing a role in the regulation of cell proliferation. The proteoglycan, decorin, is a DSPG that is important for the binding of collagen and fibronectin. It serves to function as a regulator of wound repair and skin strength.

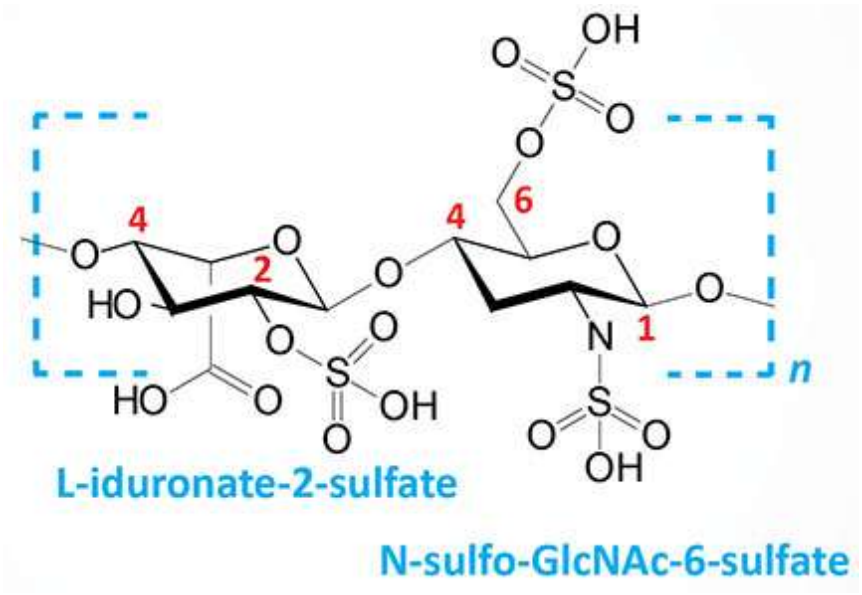
Chondroitin 4- and 6-sulfates composed of D-glucuronate (GlcA) and GalNAc-4- or 6-sulfate; linkage is β(1,3) (the figure contains GalNAc 4-sulfate).



Occurence : cartilage, bone, heart valves ; It is the most abundant GAG.

The typical chondroitin sulfate disaccharide unit in humans is composed of GalNAc and GlcA, both of which can be highly sulfate modified. Chondroitin sulfate is an important structural component of cartilage and provides much of its resistance to compression. Along with glucosamine, chondroitin sulfate has become a widely used dietary supplement for treatment of osteoarthritis.

Heparin and heparan sulfate composed of L-iduronate(IdoA: many with 2-sulfate) or D-glucuronate (GlcA: many with 2-sulfate) and *N*-sulfo-D-glucosamine-6-sulfate; linkage is α(1,4) if IdoA, β(1,4) if GlcA: heparans have less overall sulfate than heparins



Heparans have less sulfate groups than heparins

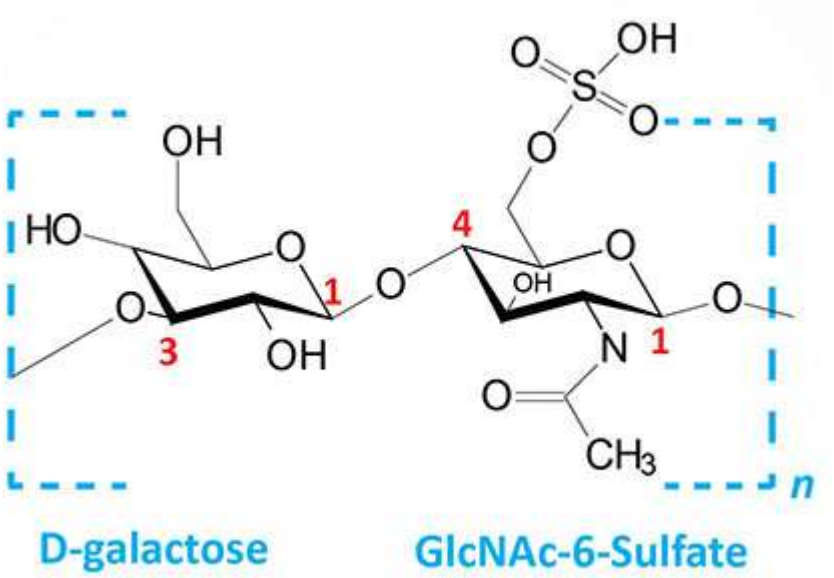
Occurence :

Heparin: component of intracellular granules of mast cells lining the arteries of the lungs, liver and skin

Heparan sulfate: basement membranes, component of cell surfaces

Heparin acts as an anticoagulant, preventing the formation of clots and extension of existing clots within the blood. While heparin does not break down clots that have already formed

Keratan sulfate composed of galactose plus GlcNAc-6-sulfate; linkage is β(1,4).



Occurence : cornea, bone, cartilage ;

Keratan sulfates are often aggregated with chondroitin sulfates.

The term keratan was originally coined in reference to the fact that this GAG structure was originally identified in the cornea. Keratan sulfates are large, highly hydrated molecules which in joints can act as a cushion to absorb mechanical shock.

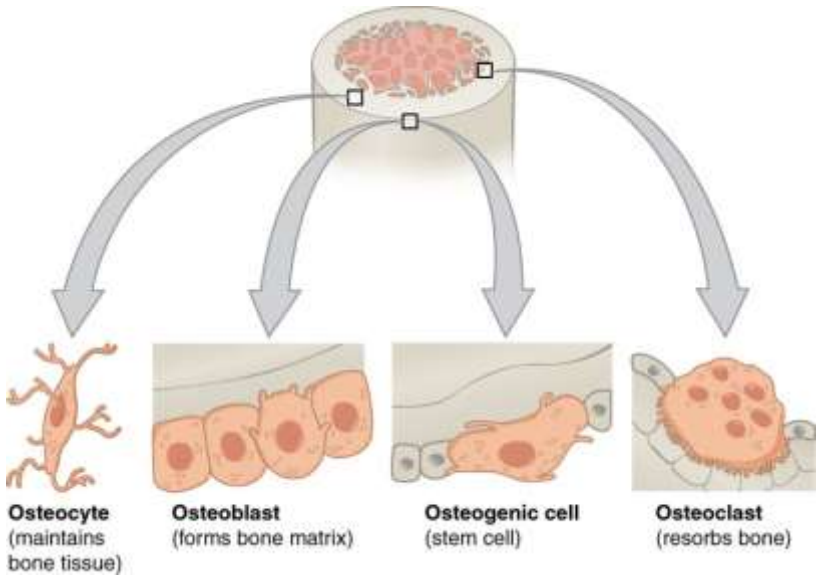
4. Characterization and biochemical composition of bone tissue

Bone tissue (osseous tissue) differs greatly from other tissues in the body. Bone is hard and many of its functions depend on that characteristic hardness. Bone is also dynamic in that its shape adjusts to accommodate stresses.

Bone consists of four types of cells: osteoblasts, osteoclasts, osteocytes, and osteoprogenitor (or osteogenic) cells. Each cell type has a unique function and is found in different locations in bones. *The osteoblast*, the bone cell responsible for forming new bone, is found in the growing portions of bone, including the periosteum and endosteum. Osteoblasts, which do not divide, synthesize and secrete the collagen matrix and calcium salts. As the secreted matrix surrounding the osteoblast calcifies, the osteoblast becomes trapped within it. As a result, it changes in structure, becoming an *osteocyte,* the primary cell of mature bone and the most common type of bone cell. Each osteocyte is located in a space (lacuna) surrounded by bone tissue. Osteocytes maintain the mineral concentration of the matrix via the secretion of enzymes. As is the case with osteoblasts, osteocytes lack mitotic activity. They are able to communicate with each other and receive nutrients via long cytoplasmic processes that extend through canaliculi (singular = canaliculus), channels within the bone matrix.

Bone Cells		
Cell type	**Function**	**Location**
Osteogenic cells	Develop into osteoblasts	Deep layers of the periosteum and the marrow
Osteoblasts	Bone formation	Growing portions of bone, including periosteum and endosteum
Osteocytes	Maintain mineral concentration of matrix	Entrapped in matrix
Osteoclasts	Bone resorption	Bone surfaces and at sites of old, injured, or unneeded bone

Bone cell types: Table listing the function and location of the four types of bone cells.



Four types of bone cells: Four types of cells are found within bone tissue. Osteogenic cells are undifferentiated and develop into osteoblasts. When osteoblasts get trapped within the calcified matrix, their structure and function changes; they become osteocytes. Osteoclasts develop from monocytes and macrophages and differ in appearance from other bone cells.

If osteoblasts and osteocytes are incapable of mitosis, then how are they replenished when old ones die? The answer lies in the properties of a third category of bone cells: *the osteogenic cell*. These osteogenic cells are undifferentiated with high mitotic activity; they are the only bone cells that divide. Immature osteogenic cells are found in the deep layers of the periosteum and the marrow. When they differentiate, they develop into osteoblasts. The dynamic nature of bone means that new tissue is constantly formed, while old, injured, or unnecessary bone is dissolved for repair or for calcium release. The cell responsible for bone resorption, or breakdown, is *the osteoclast*, which is found on bone surfaces, is multinucleated, and originates from monocytes and macrophages (two types of white blood cells) rather than from osteogenic cells. Osteoclasts continually break down old bone while osteoblasts continually form new bone.

Bone tissue is continuously remodeled. Bone remodeling is a highly complex process by which old bone is replaced by new bone, in a cycle comprised of three phases: initiation of bone resorption by osteoclasts, the transition (or reversal period) from resorption to new bone formation, and the bone formation by

osteoblasts. The formation, proliferation, differentiation, and activity bone cells are controlled by local and systemic factors.

The local factors include autocrine and paracrine molecules such as growth factors, cytokines, and prostaglandins produced by the bone cells.

The systemic factors which are important to the maintenance of bone homeostasis include parathyroid hormone (PTH), calcitonin, 1,25-dihydroxyvitamin D_3 (calcitriol), glucocorticoids, androgens, and estrogens.

Normal bone remodeling is necessary for fracture healing and skeleton adaptation to mechanical use, as well as for calcium homeostasis. On the other hand, an imbalance of bone resorption and formation results in several bone diseases. For example, excessive resorption by osteoclasts without the corresponding amount of nerformed bone by osteoblasts contributes to bone loss and osteoporosis, whereas the contrary may result in osteopetrosis. Recent studies have shown that bone influences the activity of other organs and the bone is also influenced by other organs and systems of the body.

Bone organic matrix. Bone is composed by inorganic salts and organic matrix. The organic matrix contains collagenous proteins (90%), predominantly type I collagen, and noncollagenous proteins including osteocalcin, osteonectin, fibronectin and growth factors. There are also small leucine-rich proteoglycans. The inorganic material of bone consists predominantly of phosphate and calcium ions; however, significant amounts of bicarbonate, sodium, potassium, citrate, magnesium, carbonate, zinc, barium, and strontium are also present. Calcium and phosphate ions nucleate to form the hydroxyapatite crystals, which are represented by the chemical formula $Ca_{10}(PO_4)_6(OH)_2$. Together with collagen, the noncollagenous matrix proteins form a scaffold for hydroxyapatite deposition and such association is responsible for the typical stiffness and resistance of bone tissue. Bone matrix constitutes a complex and organized framework that provides mechanical support and exerts essential role in the bone homeostasis. The bone matrix also has a participation in the bone remodeling.

5. Spesific bone tissue enzymes.

Alkaline phosphatase is an ubiquitous membrane-bound glycoprotein that catalyzes the hydrolysis of phosphate monoesters at basic pH values. Alkaline phosphatase is divided into four isozymes depending upon the site of tissue localization that are Intestinal ALP, Placental ALP, Germ cell ALP and tissue nonspecific alkaline phosphatase or liver/bone/kidney (L/B/K) ALP.

Mammalian alkaline phosphatases (ALPs) are zinc-containing metalloenzymes. They has four metal binding sites -two for zinc, one for magnesium, and one for calcium ion. Three metal ions including two Zn^{2+} and one Mg^{2+} in the active site are essential for enzymatic activity. However, these metal ions also contribute substantially to the conformation of the ALP monomer. Alkaline phosphatase activity is important for the mineralization of bone and represents a useful biochemical marker of bone formation. Its elevation in serum is correlated with the presence of bone, liver, and other diseases. High ALP levels can show that the bile ducts are obstructed. Also, elevated ALP indicates that there could be active bone formation occurring as ALP is a byproduct of osteoblast activity or a disease that affects blood calcium level (hyperparathyroidism), vitamin D deficiency. In violation of bone formation, there is a decrease in the content and activity of alkaline phosphatase in bone, plasma and other tissues.

Tartrate resistant acid phosphatase is concentrated in osteoclasts. It is a lysosomal enzyme. It has often been used to assess bone absorption. Tartrate resistant acid phosphatase might serve as a biochemical marker for osteoclast function.

6. Bone turnover markers

Bone markers can be divided into formation and resorption markers, although some markers may reflect both activities. Bone formation markers are produced by osteoblastic cells or derived from procollagen metabolism, whereas resorption markers are the degradation products of osteoclasts or collagen degradation.

Bone formation markers.

- *Procollagen type 1 extension peptides*

Type 1 collagen is derived from its precursor, procollagen. The procollagen molecule contains amino- and carboxy-terminal extensions, which are removed enzymatically during extracellular processing, resulting in the production of type 1 collagen. This cleavage results in the release of procollagen type 1 N-terminal propeptide and procollagen type 1 C-terminal propeptide.

- *Bone-specific alkaline phosphatase*

Bone-specific alkaline phosphatase (BALP) is produced by osteoblasts and its production is correlated positively with bone formation rate.

Bone resorption markers

- *Tartrate-resistant acid phosphatase*
- *Hydroxyproline*

Hydroxyproline (OHP) is an amino acid that is derived from the post-translational hydroxylation of proline. It contributes about 13–14% of the amino acid content of collagen, and hence, it is found in collagen-containing tissues other than bone such as cartilage and skin. Although it is used principally as a resorption marker, OHP is released into the circulation during bone resorption from collagen degradation as well as during bone formation (~10%) from newly synthesized procollagen peptides. Therefore, OHP is neither specific to bone tissue nor to the resorption process.

- *Cathepsin K*

Cathepsins are members of the cysteine protease family, and 11 isoforms have been identified. Osteoclasts secrete cathepsin K into bone resorption lacunae for degradation of bone matrix proteins including type I collagen, osteopontin and osteonectin, and hence, cathepsin K plays an important role in bone resorption. Cathepsin K inhibitors are currently being evaluated as a potential treatment option for osteoporosis.

7. ***Pathobiochemistry of connective tissue. Types of connective tissue disease***

There are several types of connective tissue disease. It's useful to think of two major categories. The first category includes those that are inherited, usually due to a single-gene defect called a mutation. The second category includes those where the connective tissue is the target of antibodies directed against it. This condition causes redness, swelling, and pain (also known as inflammation).

Connective tissue diseases due to single-gene defects. Connective tissue diseases due to single-gene defects cause a problem in the structure and strength of the connective tissue. Examples of these conditions include:

- Ehlers-Danlos syndrome (EDS)
- Marfan syndrome
- Osteogenesis imperfecta

Connective tissue diseases characterized by inflammation of tissues are caused by antibodies (called autoantibodies) that the body incorrectly makes against its own tissues. These conditions are called autoimmune diseases. Included in this category are the following conditions:

- Rheumatoid arthritis (RA)
- Scleroderma
- Vasculitis
- Sjogren's syndrome.

People with diseases of connective tissue may have symptoms of more than one autoimmune disease. In these cases, doctors often refer to the diagnosis as mixed connective tissue disease.

The causes and symptoms of connective tissue disease caused by single-gene defects vary as a result of what protein is abnormally produced by that defective gene.

Ehlers-Danlos syndrome (EDS) is caused by a collagen formation problem. EDS is actually a group of over 10 disorders, all characterized by stretchy skin, abnormal growth of scar tissue, and over-flexible joints. Depending on the particular type of EDS, people may also have weak blood vessels, a curved spine, bleeding gums or problems with the heart valves, lungs, or digestion. Symptoms range from mild to

extremely severe. The most dangerous is type IV due to the tendency to rupture of arteries or intestines due to violations of type III collagen. Type VI is associated with a deficiency of lysyl hydroxylase. It is characterized by increased mobility of the joints and a tendency to eye ruptures.

Marfan syndrome is caused by a defect in the connective tissue protein fibrillin. It affects the ligaments, bones, eyes, blood vessels, and heart. People with Marfan syndrome are often unusually tall and slender, have very long bones and thin fingers and toes. Abraham Lincoln may have had it. Sometimes people with Marfan syndrome have an enlarged segment of their aorta (aortic aneurysm) which can lead to fatal bursting (rupture).

Osteogenesis imperfect. People with different single-gene problems placed under this heading all have collagen abnormalities along with typically low muscle mass, brittle bones, and relaxed ligaments and joints. Other symptoms of osteogenesis imperfecta are dependent upon the specific strain of osteogenesis imperfecta they have. These may include thin skin, a curved spine, hearing loss, breathing problems, teeth that break easily, and a bluish gray tint to the whites of the eyes.

Rheumatoid Arthritis (RA). Rheumatoid arthritis is one of the most common connective tissue diseases and can be inherited. RA is an autoimmune disease, meaning the immune system attacks its own body. In this systemic disorder, immune cells attack and inflame the membrane around joints. It also can affect the heart, lungs and eyes. It affects many more women than men (an estimated 71% of cases).

Scleroderma. An autoimmune condition that causes scar tissue to form in the skin, internal organs (including the GI tract), and small blood vessels. It affects women three times more often than men throughout life, occurring at a rate of 15 times greater for women during childbearing years.

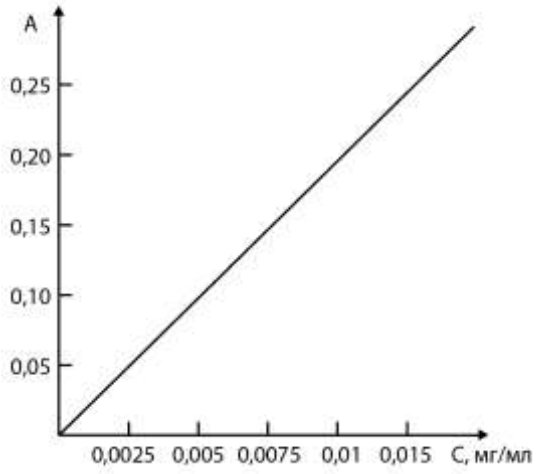
Sjogren's syndrome. The main symptoms of Sjogren's syndrome are dry mouth and eyes. People with this condition can also experience extreme fatigue and pain in the joints. The condition increases the risk of lymphoma and can affect the lungs, kidneys, blood vessels, digestive system, and nervous system.

Chapter 2. PRACTICAL PART.

Task 1. Determination of free oxyproline in urine.

Principle of the method: the method is based on the oxidation of hydroxyproline to a compound close to the structure to the pyrrole, which, when condensed with the Ehrlich reagent (p-dimethylaminobenzaldehyde), gives a pink color. The intensity of the color of the solution is proportional to the concentration of oxyproline.

Progress. Two test tubes (control and test) are applied: in the first test tube - 1 ml of filtered urine, in the second - 1 ml of distilled water (control test). In both test tubes add 1 ml of 0,01 M solution of copper sulphate, 1 ml of 2,5 N of sodium hydroxide solution, 1 ml of 6% solution of hydrogen peroxide. Samples are stirred for 5 minutes, after which they are heated for 3 minutes in a boiling water bath, then the test tubes are cooled with water from the tap. 4 ml of 3 N solution of sulfuric acid and 1 ml of Eric's reagent are added to the test tube, placed for 1 min in a boiling water bath, cooled, and then the optical density is measured at the FEC against the control at a wavelength □ = 500-560 nm (green filter) in a dish 10 mm thick. From the extinction of the experiment, take extinction of the control and determine the content of oxyproline in 1 ml of urine according to the calibration graph. Calculate the amount of oxyproline in 100 ml or daily urine.



Norm. In an adult with urine a day, up to 8 mg of free oxyproline is excreted.

Clinical and diagnostic value. The content of hydroxyproline in blood and urine characterizes the intensity of catabolism of collagen and the rate of exchange of this amino acid. Oxiprolin may be bound in the form of proteins, peptides, and also in the free state of serum of blood and urine. Sharply increases the excretion of hydroxyproline with urine in collagenoses (rheumatism, rheumatoid arthritis, systemic scleroderma, dermatomyositis), with hyperparathyroidism, Paget's disease (up to 1 g per day). Even more oxyproline is excreted in hereditary hyperhydroxyprolinemia, due to the deficiency of the hydroxyprolin oxidase enzyme,as a result of which the exchange of hydroxyproline is violated.

Task 2.. Qualitative reaction to glycosaminoglycans (Berry-Spinarder test).

The principle of the method. In the interaction of glycosaminoglycans with toluidine blue in an acidic medium, a red color (metachromassage) is formed.

Material supply: toluidine blue (0,04% solution in acetate acetate buffer with pH 2,0), 10% solution of acetate acid, filter paper, micro-pepper.

Procedure: On a strip of filter paper, at a distance of 1 cm each, put a micropipette of 0.005, 0.01 and 0.025 ml of urine, dry the paper at room temperature, and then immerse it in a 0.04% solution of toluidine blue for 1 min. Take out a strip of paper and wash the reagent with a 10% solution of acetate acid. If the concentration of glycosaminoglycans in the urine under investigation exceeds 10 mg / 100 ml, then a red color appears on one of the applied stains.

Conclude.

Clinical and diagnostic value. In the urine of a healthy person contains 2.7 - 7.5 mg / day of acidic glycosaminglycans (mainly chondroitin sulfates A and C). Negative reaction is observed in a healthy child already on the 2nd week of life. With gorogolizma and Gunter's syndrome, glycosaminoglycans with urine (mucopolysaccharidurіa) are significantly increased to 30-80 mg / day.

Situational challenges.

Task number 1. The patient revealed the fragility of the walls of the blood vessels, increased bleeding, reduced strength and elasticity of the skin, swinging and falling teeth. Lack of what vitamin can lead to these violations? In the biosynthesis of which component of the connective tissue he is involved? What is the name of this state?

The standard of the answer: These symptoms are due to collagen biosynthesis. In the process of its post-translational modification from procolagen to collagen, hydroxylation reactions of proline and lysine occur in hydroxyproline and hydroxylizine with the participation of vitamin C. Its lack also leads to a violation of the formation of transverse bonds, resulting in deterioration of the density and mechanical properties of collagen fibers. Such signs are characteristic of scurvy.

Task number 2. In a two-year-old child with mucopolysaccharidosis, there is a delay in physical and neuro- mental development, deformation of the skeleton and other disorders of the musculoskeletal system What metabolism of substances of connective tissue is disturbed during mucopolysaccharidosis? Why these diseases are called lysosomal? What excretion of substances with urine significantly increases with mucopolysaccharidosis?

The standard of the answer: Mucopolysaccharide, glycosaminoglycans, proteoglycans. It is in the lysosomes that enzymes-glycosidases are localized that their function is inadequate for this disease. With mucopolysaccharidosis, the excretion of glycosaminoglycans and hydroxyproline in urine is significantly increased.

Task number 3. Pathogenic microorganisms are capable of destroying hyaluronic acid, isolating the enzyme hyaluronidase. What is their advantage over microbes that do not show hyaluronidase activity?

The standard of the answer: Hyaluronidase allows certain pathogenic microorganisms to penetrate the human body through mucous membranes and skin, causing hydrolysis (depolymerization) of hyaluronic acid, and thus

contributes to their spread in the body. The action of such microorganisms can be judged by the presence in the blood and urine of the products of disintegration of connective tissue - amino sugars, glucuronic, neuraminic and sialic acids. Gram-positive microorganisms produce a protective capsule of hyaluronic acid, which increases their pathogenicity.

Task number 4 A patient with a burn disease is threatened with the formation of blood clots in the blood vessels due to increased blood coagulation. What glycosaminoglycan can be used to prevent the formation of blood clots?

The standard of the answer: An anticoagulant from derivatives of glycosaminglycans belongs to heparin. Its anticoagulation effect is due to the fact that it binds to the inhibitor of the factors of coagulation with antithrombin III, changes its conformation and thus increases its resistance to thrombin. Antithrombin retained in this way counteracts intravascular coagulation of blood.

Task number 5 At aging of an organism the skin shrivels, its dryness grows, excretion of hydroxyproline in the urine is weakened. What are the main biochemical changes happening at the same time?

The standard of the answer: The ratio of proteoglycan / collagen is reduced due to a decrease in the content of proteoglycans and an increase in the content of collagen. This is observed when the activity of various enzymes of fibroblasts, where they are synthesized, and lysosomes, where their decay occurs, is observed. Proteoglycans significantly reduce the content of hyaluronic acid, which leads to a decrease in the hydration of proteoglycans in the skin, its elasticity (turgor) is lost, its dryness increases, etc. At the same time, the physical and chemical properties of collagen change (the number of intra-and inter-molecular transverse bonds increases, structural stability of collagen fibers increases, their elasticity decreases, their ability to swell, etc.) and its catabolism weakens.

Tests from Krok – 1 (restractor «A»)

1. With osteolaterizm, the strength of collagen decreases, which is due to a marked decrease in the formation of cross-links in collagen fibrils. The reason for this phenomenon is a decrease in the activity of such an enzyme:

A.* Lysyloxidase.

B. Monoamine oxidase

C. Prolyl hydroxylase

D. Lysyl hydroxylase

E. Collagenase

2. Most of all with age, the skin of a person preperepaet changes associated with a decrease in its elasticity. What elements of connective tissue ensure its elasticity?

A.*Collagen and Elastic fibers.

B. Substance.

C. Cells of the epidermis.

D. Cells of connective tissue.

E. Reticular fibers

3. Human skin is very strong to break. It is known that the skin consists of epithelial tissue and two types of connective tissue. Which of the following fabrics provides strength to the skin?

A.*Dense unformed connecting.

B. Stratified squamous epithelium.

C. Loose connective tissue.

D. Single-layered epithelium.

E. Transitional epithelium

4. When examining the patient, a characteristic clinic of collagenosis was revealed. Urinary indications are typical for this pathology:

A.*Hydroxyproline.

B. Arginine.

C. Glucose.

D. Mineral salts

E. Ammonium salts.

5. A 53-year-old male patient is diagnosed with Paget's disease The concentration of oxyproline in daily urine is sharply increased, which primarily means intensified disintegration of:

A.*Collagen.

B. Albumin.

C. Keratin.

D. Hemoglobin.

E. Fibrinogen

6. A cosmetologist asked the patient to get rid of the tattoo on his shoulder. What substance contained in the connective tissue makes such a "painting" possible?

A.*Hyaluronic acid.

B. Elastin.

C. Gamma-globulin.

D. Fibronectin

E. Heparin.

7. A woman 30 years old is ill for about a year, when for the first time there were pains in the area of the joints, their swelling, redness of the skin over them. Preliminary diagnosis of rheumatoid arthritis. One of the causes of this disease is a change in the structure of connective tissue protein:

A. Troponin

B. Mutsina

C. Myosin

D. Ovoalbumin

E. * Collagen

8. After healing of the wound, a scar was formed. What substance is the main component of this kind of connective tissue?

A.*Collagen

B. Elastin

C. Hyaluronic acid

D. Chondroitin sulfate

E. Keratansulphate

9. As anticoagulants, various substances are used, including natural polysaccharides, namely:

A.*Heparin

B. Hyaluronic acid

C. Dermatan sulfate

D. Chondroitin sulfate

E. Dextran

10. In patients with collagenosis, destruction of connective tissue is observed. What researches of laboratory parameters of blood and urine is advisable to appoint a patient suspected of having collagenosis (chronic form)?

A.*The content of oxyproline and oxilysin in blood and urine.

B. The activity of isoenzymes in blood LDH

C. The content of urates in the blood

D. C-reactive protein in the blood

E. Transaminases of blood activity

Key Terms.

- *extracellular matrix*: Cells of the connective tissue are suspended in a non-cellular matrix that provides structural and biochemical support to the surrounding cells.
- *fibroblast:* A type of cell found in connective tissue that synthesizes the extracellular matrix and collagen.
- *connective tissue*: A type of tissue found in animals whose main function is to bind, support, and anchor the body.

- *cartilage*: A type of dense, non-vascular connective tissue, usually found at the end of joints, the rib cage, the ear, the nose, in the throat, and between intervertebral disks.
- *adipose tissue*: Connective tissue that stores fat and cushions and insulates the body.
- *collagen* is the main structural protein in the extracellular space in the various *connective tissues*.

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