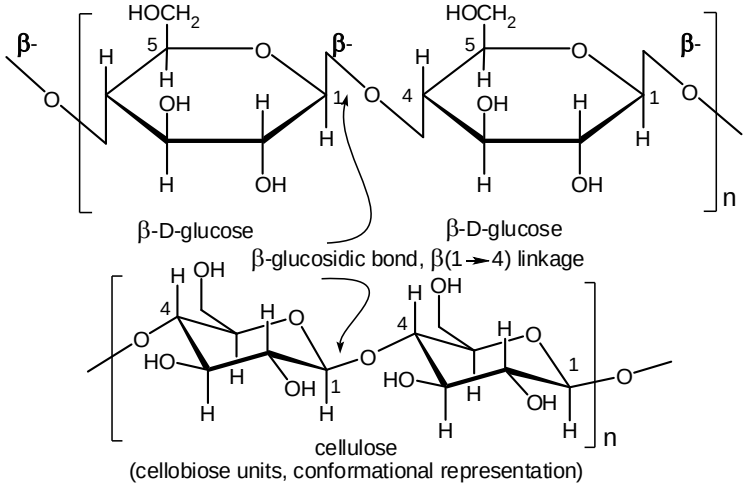
№ 43. POLYSACCHARIDES. TYPES, REPRESENTATIVES AND SOME IMPORTANT PROPERTIES. HETEROPOLYSACCHARIDES.

Polysaccharides are biopolymers that contain many monosaccharide units. The polysaccharide structures are formed of repeating units (either mono- or di-saccharides) joined together by glycosidic bonds. Polysaccharides are often quite heterogeneous, containing slight modifications of the repeating unit. All anomeric carbon atoms, except for the end anomeric carbons, are involved in glycosidic bonds. Therefore polysaccharides are non-reducing; they not respond to Tollens or Fehling reagents; and the do not show mutarotation.

Natural polysaccharides constitute one of the three classes of biopolymers. Many types of polysaccharides are found in Nature serving a wide variety of functions. Such variety is interrelated to the variations in structure, for instance: cellulose – structural component of plants; chitin – structural polysaccharide in cell walls of fungi, exoskeletons of crabs, insects; starch – produced by all green plants as an energy store; starch is the most important carbohydrate in the human diet; glycogen – the glucose store of animals; glycoproteins – contain oligosaccharide chains (glycans) covalently attached to polypeptide; antibodies (immunoglobulins), etc. The latter participate in the immune system response to a foreign body whose polysaccharides on the cell surface are recognized and this triggers the response to destroy the invader cells.

The starches, glycogen, and cellulose are built by one type of building block – D-glucose unit. Such polysaccharides consisting of one type monosaccharide are called collectively **homopolysaccharides**.

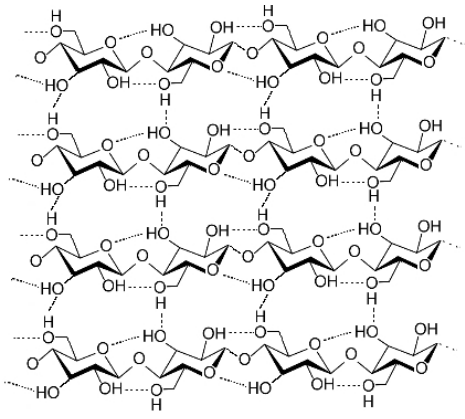
I. Cellulose. Cellulose, $(C_6H_{10}O_5)_n$, is a polysaccharide consisting of a linear chain of several hundred to over ten thousand **β(1,4) linked D-glucose** units. The polymer structure of cellulose was determined in 1920.



Cellulose is both the most common biopolymer and the most common organic compound on Earth. About 33 percent of all plant matter is cellulose (the cellulose content of cotton is 90 percent and that of wood is 50 percent). Plants synthesize cellulose as a structural material to support the weight of the plant.

Cellulose has no taste (anyway humans cannot use it as food); is odorless; is hydrophilic; is insoluble in water and most organic solvents; is chiral, and is biodegradable. It can be broken down chemically into its glucose units by treating it with concentrated acids at high temperature. Complete hydrolysis of all glycosidic bonds of cellulose gives D-glucose. The disaccharide that is obtained from partial hydrolysis is cellobiose.

Cellulose is a straight, unbranched chain polymer. Unlike starch, no coiling or branching is found in cellulose, and the molecule adopts an extended and rather stiff rod-like conformation. The multiple hydroxyl groups on the glucose from one chain form hydrogen bonds with oxygen molecules on the same or on a neighbor chain, holding the chains firmly together side-by-side and forming *microfibrils* with high strength. This strength is important in cell walls, conferring rigidity to plant cells. The hydrogen bonding pattern actually is the reason for rotated to each other in alternating fashion pyran rings, as shown:



With this representation cellulose can give a very good example for structure-function relationship. The formation of many hydrogen bonds between chains in one plane and between molecules in two planes renders cellulose strands with high rigidity and stability.

The dissolution process in some instances is due to chemical

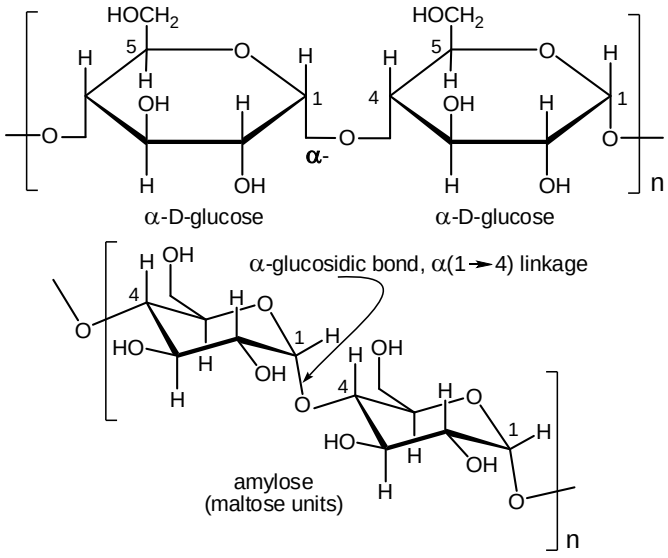
changes. Cellulose dissolves in Schweizer's reagent (ammonia solution of copper(II)hydroxide). This solution is used in the process of preparation of fibers, e.g. artificial silk, viscose process, rayon.

Animals do not possess the enzymes necessary to catalyze the hydrolysis of cellulose and cannot digest it. Some animals can use cellulose as food in an indirect way. In particular, ruminants and termites can digest cellulose with the help of symbiotic micro-organisms that live in their guts. Humans do not have the enzyme necessary to hydrolyse β-glycosidic bond, therefore cellulose is often referred to as "dietary fiber".

Humans use cellulose in various processed forms for basic necessities. Cellulose is the major constituent of paper, paperboard, and card stock and of textiles made from cotton, linen, and other plant fibers. Cellulose as a heat source. The major combustible component of non-food energy crops is cellulose, with lignin second. Cellulose was used to produce the first successful thermoplastic polymer, celluloid (1870). The hydroxyl groups of cellulose can be partially or fully reacted with various reagents to afford derivatives with useful properties. Cellulose esters and cellulose ethers are the most important commercial materials. In principle, cellulosic polymers are renewable resources. Notable among the esters are cellulose acetate and cellulose triacetate, which are film- and fiber-forming materials that find a variety of uses. The inorganic ester nitrocellulose was initially used as an explosive and was an early film forming material.

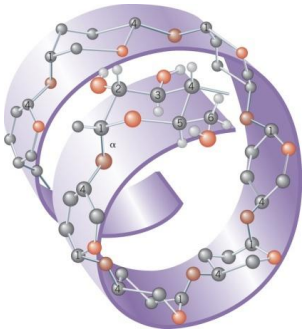
II. Starches: amylose, amylopectin. Starches is a group name for polysaccharides produced by all green plants as an energy store. Starches are the most important carbohydrates in the human diet consumed with potatoes, wheat, maize (corn), rice, etc.. Pure starch is a white, tasteless and odorless powder that is insoluble in cold water or alcohol. Starch consists of two types of molecules: **the water-soluble linear and helical amylose and the water-insoluble branched amylopectin**. Depending on the plant, starch generally contains 20 to 25% amylose and 75 to 80% amylopectin.

Amylose is a linear polymer of glucose linked by **α(1,4) glycosidic bonds**. The polymeric chain can be made of several thousand D-glucose units (n=300-3000).



Amylose molecule is built by linear connection of glucose residues. Amylose is considered true natural polymer of glucose. In both amylose and amylopectin are recognized maltose disaccharide repeating units. Stereochemical difference from cellulose is the α(1,4) linkage which results in striking differences in physical and chemical properties. The α(1,4) glycosidic bonds in amylose promote the formation of a helix structure. The linear representation does not show actual spatial arrangement of the tetrahydropyran rings which form helical conformational structures.

Amylose adopts a very compact helical arrangement with six glucose monomers per helical turn. This folding pattern exposes OH groups to hydrogen bonds with water – amylose is soluble in H_2O whereas cellulose is not.



Cellulose is stiff, rigid; amylose is not.

Iodine molecules fit neatly inside the helical structure of amylose, giving intensely colored deep-blue complex. Iodine gives characteristic deep-blue color reaction with amylose (starch) believed to have iodine molecules inserted in the cavity of this spiral. The color fades away on heating but reappears on cooling. The reaction is very sensitive and can be applied for the detection of starch and iodine. It is the base for "starch-iodine" test for oxidizers. The tested material is added to an aqueous solution of amylose and KI. If the material contains an oxidizer, it oxidizes some of the iodide ions to iodine which forms blue complex with amylose.

Starch does not reduce Fehling solution and ammonia solution of Ag_2O (Tollens reagent).

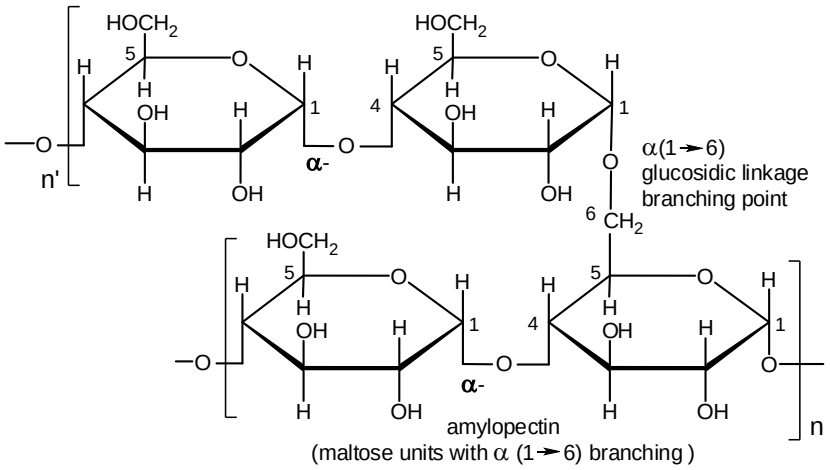
Boiling of starch in the presence of mineral acids or under the action of enzymes gives a product with reducing properties because from hydrolysis more hemiacetal sites are obtained. The final product of starch hydrolysis is glucose.

$$\underset{\text{Starch}}{(C_6H_{10}O_5)_n} \xrightarrow[t^o]{H_2O,\ H^+} \underset{\text{Dextrin}}{(C_6H_{10}O_5)_{n1}} \xrightarrow[t^o]{H_2O,\ H^+} \underset{\text{Maltose}}{C_{12}H_{22}O_{11}} \xrightarrow[t^o]{H_2O,\ H^+} \underset{\text{Glucose}}{n\ C_6H_{12}O_6}$$

Starch is used in the manufacture of various adhesives or glues. Papermaking is the largest non-food application for starches globally.

Unlike cellulose, amylase is excellent food. Starch is a plant's way of storing glucose to meet its energy needs. Animals can use this source by eating starchy foods and, with the aid of their α-glycosidase enzymes, hydrolyze the starch to glucose. The α(1,4) glycosidic bonds are easily hydrolyzed by the enzyme systems, present in all animals. When more glucose is available than is needed as fuel in an organism, animals store the glucose as glycogen.

Amylopectin. Amylopectin is the water-insoluble fraction of starches. Like amylose, **amylopectin** is a polysaccharide of **α(1,4) linked D-glucose** units. Instead of being a continuous length of α(1,4) units, amylopectin **is branched**. The branch points occur every 24-30 glucose units of the main chain. Attached to C-6 at various points on the main chain are short polysaccharide branches joined by α(1,4) glycosidic bonds. Bonds to branches occur between α-OH at C-1 of one glucose unit and C-6 of another (α(1,6) glycosidic bonds). Amylopectin is insoluble in water and hydrolyses slower than amylose.



Plants store starch within specialized organelles. When energy is needed for cell work, the plant hydrolyzes the starch releasing the glucose subunits. Humans and other animals that eat plant foods also use amylase, an enzyme that assists in breaking down amylopectin. Amylopectin serves as food.

III. Glycogen. The amylopectin counterpart in animals is glycogen which has the same composition and structure, but with more extensive **branching via α(1,6) glycosidic bonds** that occurs every 8 to 12 glucose units. The main polysaccharide chain contains many D-glucose units joined by α(1,4) glycosidic bonds. The more extensive branching in glycogen allows for even faster hydrolysis and immediate release of energy. Glycogen is made primarily by the liver and the muscles. The compound is commonly referred to as animal starch. The entire globular granule of glycogen may contain approximately 30,000 glucose units and has a polypeptide (glycogenin) incorporated in the middle.

Glycogen is a very large polysaccharide. Nature utilizes mainly two features of such large structure:

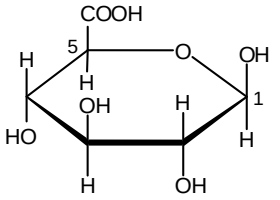
- The large size (M_w>1x10^7) of glycogen prevents it from leaving the storage cell because the molecule has properties of a colloid;
- The storage of tens of thousands of glucose molecules into one molecule greatly relieves the osmotic problem for the storage cell. Such pressure would be caused by the attempted storage of many individual glucose molecules.

Some amount of glycogen is stored in the muscles, ready for immediate hydrolysis and metabolism. More glycogen is stored in the liver. Only the glycogen stored in the liver (up to 8% by mass) can be made accessible to other organs. In the muscles, glycogen is found in a much lower concentration, but the total amount exceeds that in the liver. Muscle cells cannot pass glucose from glycogen into the blood, so the glycogen they store is destined for internal use and is not shared with other cells. This is in contrast to liver cells which, on demand, readily break down their stored glycogen into glucose and send it through the blood stream as fuel for the brain or muscles.

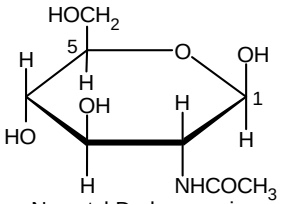
Glycogen functions as the secondary long-term energy storage in animal cells (the other are triglycerides, lipids which are more compact). Glycogen forms an energy reserve that can be quickly mobilized to meet a sudden need for glucose. The highly branched nature of glycogen allows hydrolytic enzymes to have many chain ends available from which glucose molecules can be hydrolyzed.

- Glucose is the source of "ready energy" for the body;
- Long chain fatty acids of triacylglycerols are used for long term energy storage.

IV. Heteropolysaccharides. When all the monosaccharides in a polysaccharide are the same type the polysaccharide is called a **homopolysaccharide** (like cellulose and glycogen), but when more than one type of monosaccharide is present they are called **heteropolysaccharides**. Upon hydrolysis heteropolysacharides give two or more different monosaccharides. Common constituents of heteropolysaccharides are **glucuronic acid**, **iduronic acid** (which is epimer at C-5 of glucuronic acid), **galactose**, **galactosamine**, **glucosamine**.

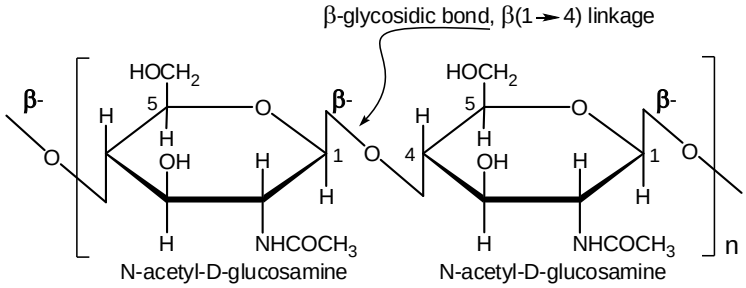


D-glucuronic acid



N-acetyl-D-glucosamine

A clear distinction between homo- and heteropolysaccharides can be made by comparison of the polysaccharides **chitin** and **hyaluronic acid**. **Chitin is a homopolysaccharide** although it contains aminosugar monomeric unit. Chitin is a long-chain polymer of N-acetylglucosamine connected by β(1,4) glycosidic bonds. This homopolysaccharide is a component of the cell walls of fungi, the exoskeletons of crabs, lobsters and insects. When encrusted in calcium carbonate it becomes much harder, as seen in the outer shell of a beetle.



N-acetyl-D-glucosamine N-acetyl-D-glucosamine

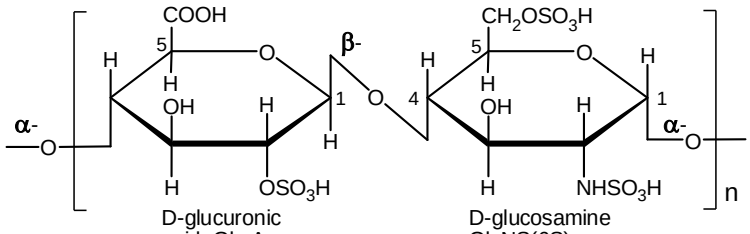
Based on its structure, chitin may be described as cellulose analog with C-2 hydroxyl group on each monomer substituted with an acetyl amino group. This allows for increased hydrogen bonding between adjacent polymers, giving the chitin-polymer matrix increased strength.

In contrast, hyaluronic acid consists of alternating D-glucuronic acid and N-acetylglucosamine residues. Therefore, **hyaluronic acid is a heteropolysaccharide**.

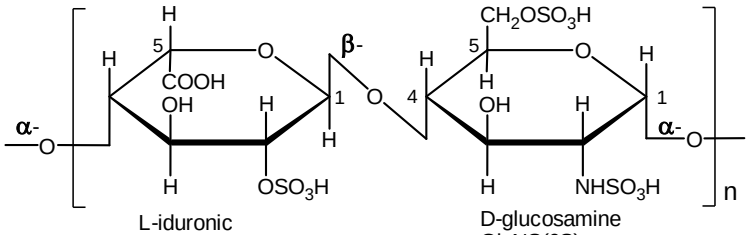
Large and important group of heteropolysaccharides are **glycosaminoglycans** (GAGs), also called **mucopolysaccharides**. They are long unbranched polysaccharides consisting of a repeating disaccharide unit. The repeating unit consists of a hexose or a hexuronic acid, linked to a hexosamine (six-carbon sugar containing nitrogen). GAGs form an important component of connective tissues. GAG chains may be **covalently linked to a protein to form proteoglycans**, also known as **glycoproteins**. The term *Glycan* indicates the carbohydrate portion of a complex molecule.

Examples of glycosaminoglycan found in Nature include **heparin** as an anticoagulant; **hyaluronan** (**hyaluronic acid)** as a component in the synovial fluid, lubricant in body joints; and **chondroitins** which can be found in connective tissues, cartilage and tendons.

Heparin is a highly-sulfated glycosaminoglycan, containing free sulfonic acid residues in ionic form. Therefore, it has the highest negative charge density of any known biological molecule. It is widely used as an injectable anticoagulant; it stops blood from clotting. Heparin is heterogeneous heteropolysaccharide (not a single molecule) due to variably-sulfated repeating disaccharide units.



D-glucuronic acid Glc-A D-glucosamine GlcNS(6S)

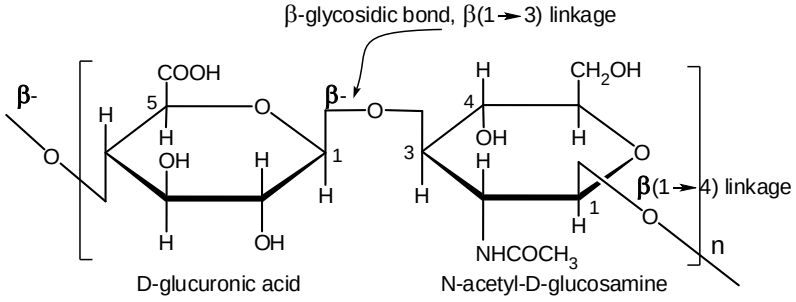


L-iduronic acid (C-5 epimer) IdoA D-glucosamine GlcNS(6S)

Two heparin molecules are shown: one containing sulfonated D-glucuronic acid – D-glucosamine repeating disaccharide, and sulfonated L-iduronic acid – D-glucosamine repeating unit. The connection is described as alternating β(1,4), α(1,4) glycosidic linkages. Iduronic acid is epimer of glucuronic acid at C-5. The sugar abbreviations show also the positions of sulfonic acid residues.

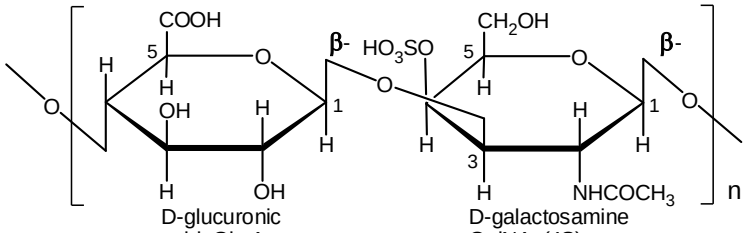
Native heparin is a polymer with a molecular weight ranging 3-30 kDa. Heparin is stored within the mast cells (cells that line the arteries walls), particularly in lungs, liver

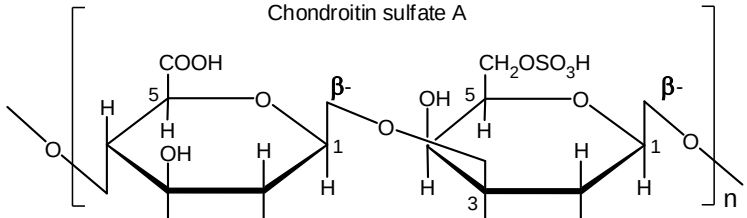
and skin and is released only at sites of tissue injury to prevent extensive clotting. **Heparin acts as an anticoagulant**, preventing the formation of clots and extension of existing clots within the blood. The substance is often administered post surgically. Heparin is given parenterally (IV), as it is degraded when taken by mouth. Is has short biologic half-live of about one hour. Another use of heparin is in the form an inner anticoagulant surface on experimental and medical devices such as test tubes and renal dialysis machines.



Hyaluronan (also called hyaluronic acid or hyaluronate) is an anionic, **non-sulfated glycosaminoglycan** distributed widely throughout connective, epithelial (skin), and neural tissues. Hyaluronic acid contains D-glucuronic acid – N-acetyl-D-glucosamine repeating motive. The bonding between them is β(1,3), β(1,4) glycosidic linkage (notice that the glucosamine ring is turned at 60°, so to present clearer the (1,3) link). Hyaluronic acid is unique among glycosaminoglycans in that it is unsulfated; it forms in the plasma membrane, and can be very large (M_w in millions). Such large molecule gives high viscosity to the liquid which contains the compound, hence – lubricant properties. Hyaluronan is an important component of articular cartilage and in synovial fluid. It is for boundary-layer lubrication, which reduces friction between opposing surfaces of cartilage. The compound is involved in cancer proliferation, metastasis. As one of the chief components of the extracellular matrix (cells glue), hyaluronan contributes significantly to cell proliferation and migration, and may also be involved in the progression of some malignant tumors. Hyaluronan is also a major component of skin, where it is involved in wounds repair. For this reason hyaluronan is a common ingredient in skin care products. Hyaluronan injections temporarily smooth wrinkles by adding volume under the skin, with effects typically lasting for six months.

Chondroitin sulfate is the most prevalent glycoaminoglycan (other are chondroitin – with little or without sulfation, keratosulfate). It is a sulfated GAG composed of a chain of alternating sugars (N-acetylgalactosamine and glucuronic acid). The bonding between them is β(1,3), β(1,4) glycosidic linkage. The polysaccharide is usually found attached to proteins as part of a proteoglycan. A chondroitin chain can have over 100 individual sugars, each of which can be sulfated in variable positions and quantities. Chondroitin sulfate chains are linked to hydroxyl groups on serine residues of certain proteins.



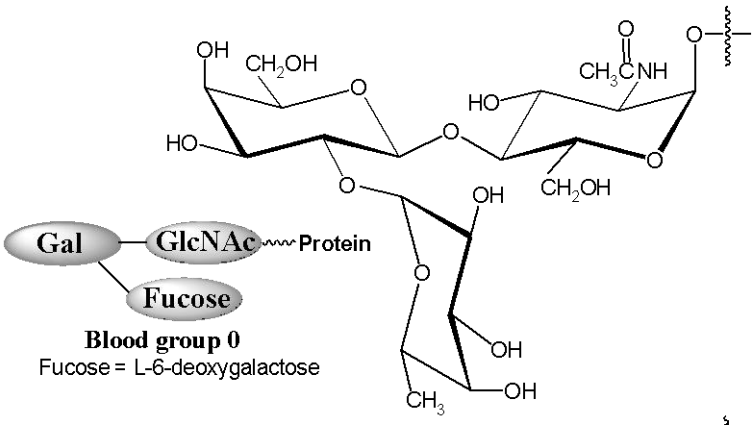


Chondroitin's functions depend largely on the properties of the overall proteoglycan of which it is a part. These functions can be broadly divided into structural and regulatory roles. Chondroitin sulfate is a major component of extracellular matrix, and is important in maintaining the structural integrity of the tissue. This function is typical of the large aggregating proteoglycans. As such it is a major component of cartilage. The tightly packed and highly charged sulfate groups of chondroitin sulfate generate electrostatic repulsion that provides much of the resistance of cartilage to compression (the tissue is flexible yet stiff). Loss of chondroitin sulfate from the cartilage is a major cause of osteoarthritis.

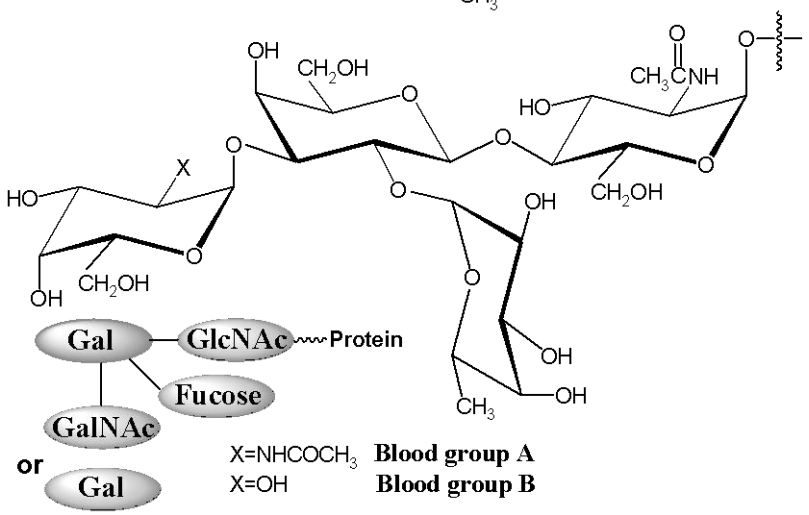
Cell-surface carbobydrates Carbohydrates were once thought to be dull compounds whose only biological purposes were to serve as structural materials and as energy sources. Now it is well established that they perform many other important fuctions. For example, polysaccharides are centrally involved in the critical process by which **one cell recognizes another**. Small polysaccharide chains, covalently bound by

glycosidic links to hydroxyl groups on proteins (glycoproteins), act as biochemical labels on cell surfaces, as illustrated by the **human blood-group** antigens.

It has been known for a long time that human blood can be classified by A, B, AB and 0 types, and that blood from a donor of one type cannot be transfused into a recipient with another type unless the two types are compatible. Otherwise agglutination of incompatible red blood cells (RBC) occurs. It indicates that the body immune system has recognized the presence of foreign cells in the body and has formed antibodies against them. This process is a result from the presence of **polysaccharide characteristic markers** (epitopes, antigenic determinant) **on the surface of the cells**. Their structures are:



Blood group 0
Fucose = L-6-deoxygalactose

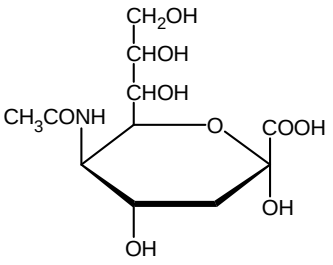


X=NHCOCH$_3$ **Blood group A**
X=OH **Blood group B**

All three blood-group antigenic determinants contain N-acetylamino sugars and the unusual monosaccharide L-fucose. A trisaccharide end-group is characteristic for group 0, and a tetrasaccharide – for groups A and B.

When a cell is attacked by a virus or bacterium or when it interacts with another cell, the drama begins when the foreign particle attaches itself to the surface of the host cell. The invader recognizes the host by the glycoproteins on the cell surface, specifically, it recognizes particular carbohydrate sequence at the end of the glycoprotein. The receptor on the cell surface to which an influenza (flu) virus attaches itself has been identified as a glycoprotein terminating in a disaccharide of N-acetylgalactosamine and N-acetylneuraminic acid (sialic acid derivative, **sialic acid is a generic term for the *N*- or *O*-substituted derivatives of neuraminic acid**, a monosaccharide with a nine-carbon backbone).

One prominent way to disease prevention is to selectively inhibit this "host-guest" interaction. This approach is the foundation of rational drug design.



N-acetylneuraminic acid
sialic acid derivative