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General Biochemistry-2 (BCH 302)

Chapter 3
Carbohydrates_Part2

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Conflicting terms

- **Glycosaminoglycans** (mucopolysaccharides) are linear *polysaccharides* of repeating disaccharides containing amino sugar, either glucosamine or galactosamine
- *Polysaccharides containing aminosugars*
- **Peptidoglycans** are *polysaccharides* consisting of sugars and few amino acids.
- *Polysaccharides + few amino acids*
- **Proteoglycans** are *proteins* that are heavily glycosylated (**95% carbohydrates**). It consists of core protein bound to glycosaminoglycans.
- *Protein + too many sugars*
- **Glycoproteins** are *proteins* that contain covalently bound oligosaccharide chains (1-30% carbohydrates).
- *Protein + few sugars*

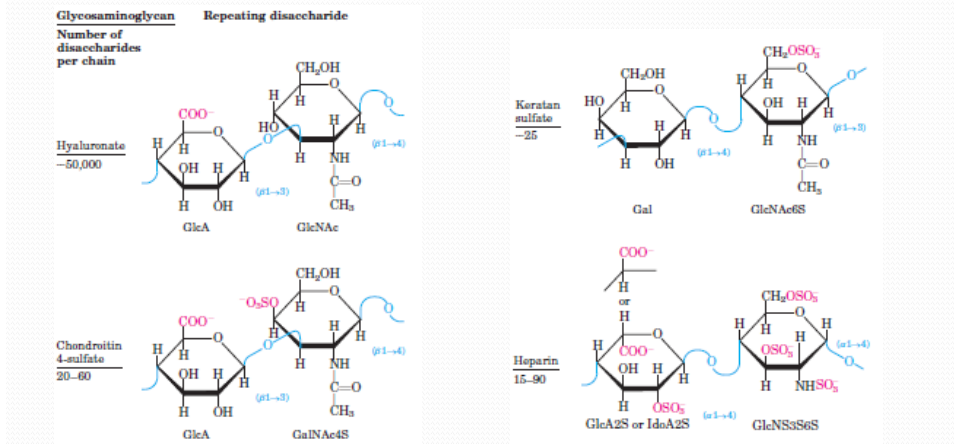
Glycosaminoglycans: Anionic polysaccharide chains made of repeating disaccharide units

- **Glycosaminoglycans** present on the animal cell surface and in the extracellular matrix.
- Glycosaminoglycans (mucopolysaccharides) are **linear** polymers of **repeating disaccharides** units containing a derivative of an amino sugar, either glucosamine or galactosamine
- The constituent monosaccharides tend to be **modified**, with acidic groups, amino groups, and sulfated hydroxyl etc.
- Glycosaminoglycans tend to be **negatively charged**, It is large complexes of negatively charged heteropolysaccharide Chains. This negative charge comes from the prevalence of acidic groups (carboxylate) or due to the presence of sulfate group.
- The Sulfate esters on some of the hydroxyl groups give these polymers a high density of negative charge, forcing them to assume extended conformations.

Glycosaminoglycans: (cont.)

- **So, glycosaminoglycans have the following properties:**
 - Can bind large amounts of water
 - Gel-like matrix
 - Viscous, Lubricating
 - Shock absorbing
 - Negatively charged
- Examples: Chondroitin sulfate, keratan sulfate, heparin, dermatan sulfate, and hyaluronate
- Glycosaminoglycans are usually attached with a small (<5%) amount of protein forming proteoglycans

Repeating units of some common glycosaminoglycans of extracellular matrix



Examples of Glycosaminoglycans

Heparin, is a soluble glycosaminoglycan act as natural anticoagulant and is synthesized in the mast cells in a nonsulfated form, which is then deacetylated and sulfated.

When released into the blood, it inhibits clot formation by interacting with the protein antithrombin.

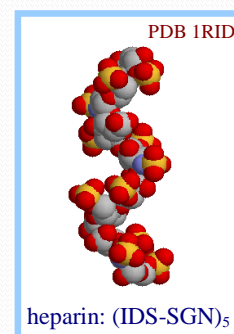
It has a structure similar to that of heparan sulfates, but is more **highly sulfated**.

Heparin has an **extended helical conformation**.

Heparin shown has 10 residues, alternating IDS (iduronate-2-sulfate) & SGN (N-sulfo-glucosamine-6-sulfate).

Heparin has the highest negative charge density of any known biological macromolecule. The charge repulsion by the many negatively charged groups may contribute to its conformation.

Purified heparin is routinely added to blood samples obtained for clinical analysis, and to blood donated for transfusion, to prevent clotting.



Examples of Glycosaminoglycans

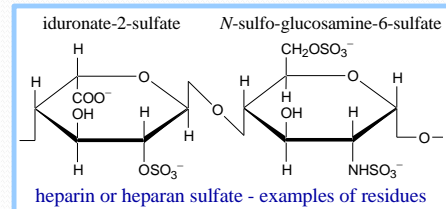
Heparan sulfate

Heparan sulfate is initially synthesized on a membrane-embedded core protein as a polymer of alternating **N-acetylglucosamine** and **glucuronate** residues.

Heparan sulfate is like heparin except that it has :

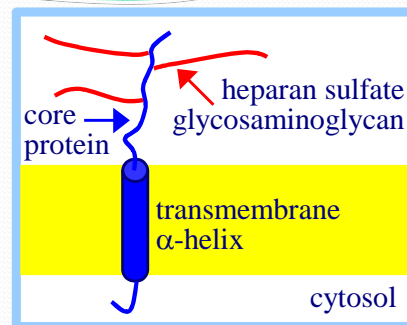
- fewer N- and O-sulfate groups
- more acetyl groups.

Later, in segments of the polymer, glucuronate residues may be converted to the sulfated sugar **iduronic acid**, while *N*-acetylglucosamine residues may be deacetylated and/or sulfated to give heparin.



Some **cell surface heparan sulfate** glycosaminoglycans remain covalently linked to core proteins embedded in the plasma membrane.

The core protein of a **syndecan** heparan sulfate proteoglycan includes a single **transmembrane α -helix**.



Syndecans are single transmembrane domain proteins that act as co-receptors. These core proteins carry three to five heparan sulfate and chondroitin sulfate chains, which allow for interaction with a large variety of ligands

The core protein of a **glypican** heparan sulfate proteoglycan is attached to the outer surface of the plasma membrane via **covalent linkage** to a modified phosphatidylinositol **lipid**.

Glypicans constitute one of the two major families of heparan sulfate proteoglycans. They seem to play a vital role in morphogenesis, and have been suggested as regulators for some cell signaling pathways.

Proteins involved in **signaling** & **adhesion** at the cell surface recognize & bind heparan sulfate chains.

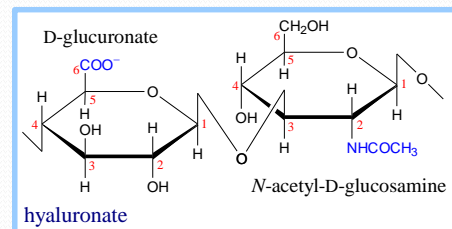
E.g., binding of some **growth factors** (small proteins) to cell surface receptors is enhanced by their binding also to heparan sulfates.

Regulated cell surface **Sulf** enzymes may **remove sulfate** groups at particular locations on heparan sulfate chains to **alter affinity** for signal proteins, e.g., growth factors.

Examples of Glycosaminoglycans

Hyaluronate

Hyaluronate (*hyaluronan*) is an α -glycosaminoglycan that is found in extracellular tissue space, the synovial fluid of joints, and the vitreous humor of the eyes and acts as a binding, lubricating, and protective agent.

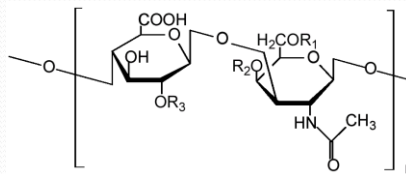


Hyaluronate is a repeating disaccharide consisting of 2 glucose derivatives, glucuronate (glucuronic acid) & N-acetyl-glucosamine.

The glycosidic linkages are $\beta(1\rightarrow3)$ & $\beta(1\rightarrow4)$.

Examples of Glycosaminoglycans

Chondroitin sulfate



- **Chondroitin sulfate** is unbranched polysaccharides.
- It is a sulfated glycosaminoglycan (GAG) composed of a chain of alternating sugars (N-acetylgalactosamine and glucuronic acid). Typically attached to proteins as part of a proteoglycan.
- It helps with tensile strength of cartilage, tendons, and ligaments.

Peptidoglycan

- Peptidoglycan, also known as **murein**, is a polymer consisting of sugars and amino acids that forms a mesh-like layer outside the plasma membrane of most bacteria, forming the cell wall.
- The sugar component consists of alternating residues of β -(1,4) linked *N*-acetylglucosamine and *N*-acetylmuramic acid.
- Attached to the *N*-acetylmuramic acid is a peptide chain of three to *five amino acids*.
- **In case of *Escherichia coli* (a Gram-negative bacterium) the amino acids are:**
 - L-alanine, D-glutamic acid, *meso*-diaminopimelic acid, and D-alanine
- **In case of *Staphylococcus aureus* (a gram-positive bacterium) the amino acids are:**
 - L-alanine, D-glutamine, L-lysine, and D-alanine with a 5-glycine
- The peptidoglycan layer is substantially thicker in gram-positive bacteria (20 to 80 nanometers) than in gram-negative bacteria (7 to 8 nanometers).

Proteoglycans

- **Proteoglycans** are **glycosaminoglycans** that are covalently linked to **serine** residues of specific **core proteins**. The glycosaminoglycan chain is synthesized by sequential addition of sugar residues to the core protein.
- It resembles polysaccharides more than proteins (heavily glycosylated proteins) in as much as the carbohydrate makes up as much as **95%** of the biomolecule by weight.
- Proteoglycans function as lubricants and structural components in connective tissue and mediate adhesion of cells to the extracellular matrix.

Some proteoglycans of the extracellular matrix **bind** non-covalently to **hyaluronate** via protein domains called **link modules**. e.g.:

- Multiple copies of the **aggrecan** proteoglycan associate with hyaluronate in cartilage to form large complexes.
- **Versican**, another proteoglycan, binds hyaluronate in the extracellular matrix of loose connective tissues.

Glycoproteins

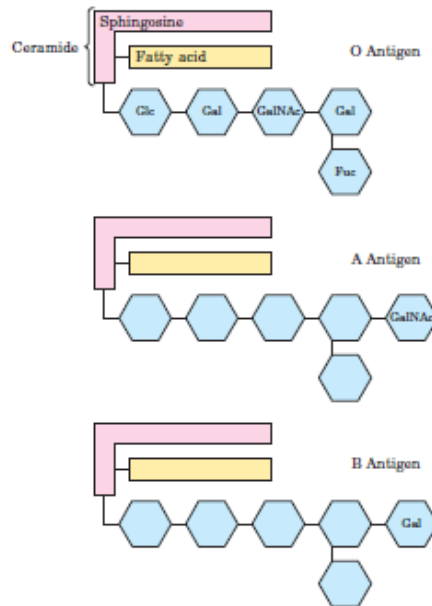
- Glycoproteins contain less carbohydrate than proteoglycans (1-30%).
- **Glycoproteins** have one or several oligosaccharides of varying complexity joined covalently to a protein.
- They are found inside and outside the cells:
 - Inside cells they are found in specific organelles such as Golgi complexes, secretory granules, and lysosomes.
 - Outside the cell on the outer face of the plasma membrane, in the extracellular matrix, and in the blood.
- The oligosaccharide portions of glycoproteins are rich in information, forming highly specific sites for recognition and high-affinity binding by other proteins.
- Cell-surface molecules are contributed to:
 - antigen determinants
 - mediator of cell-cell interaction
 - attachment sites for viruses

Functions of Glycoproteins

- Glycoproteins have many biological functions:
 - 1- Immunological protection
 - 2- Cell-cell recognition
 - 3- Blood clotting
 - 4- Host-pathogen interaction
- **Linkage between sugar and protein part in glycoproteins:**
- Carbohydrates link through the *anomeric* carbon to:
 - The amide nitrogen in the side chain of *asparagine* (N-glycosidic bond) or
 - The hydroxyl oxygen of *serine or threonine* (O-glycosidic bond)

The addition of sugar moiety determines the blood group

- Sugars attached to glycoproteins and glycolipids on the surfaces of red blood cells determine the blood group termed A, B, and O.
- The A and B antigens differ from the O antigen by the addition of one extra monosaccharide *N*-acetylgalactosamine (for A) or galactose (for B) through an α -1,3 linkage to a galactose moiety of the O antigen.,
- The addition of *N*-acetylgalactosamine or galactose is mediated by specific enzyme called glycosyltransferases which add the extra monosaccharide to the O antigen.
- Each person inherits the gene for one glycosyltransferase of this type from each parent.
 - The type A transferase specifically adds *N*-acetylgalactosamine,
 - The type B transferase adds galactose.
 - The O phenotype lack that enzyme due to mutation that leads to premature termination of translation and, hence, it produces inactive glycosyltransferase.



Sugars determining blood groups.

Abbreviations:

Fuc, fucose;

Gal, galactose;

GalNAc, *N*-acetylgalactosamine;

GlcNAc, *N*-acetylglucosamine.

Principles of Biochemistry 4th ed - Lehninger

- **Type A**
 - has lots of sugar bound but there is a different sugar at non-reducing end
 - people have antibodies against B sugars
 - donors (receive blood from): A or O
- **Type B**
 - has lots of sugar bound but there is a different sugar at non-reducing end
 - people have antibodies against A sugars
 - donor (receive blood from): B or O
- **Type AB**
 - mix of both types of sugars as A and B
 - people have no antibodies towards A or B
 - donors (receive blood from): A, B or AB or O
- **Type O**
 - Lack of sugars specific for A and B (lack of terminal *N*-acetylgalactosamine; or terminal galactose)
 - i.e. missing sugar at non-reducing end
 - people have antibodies towards both A or B
 - donors (receive blood from): O only
 - Universal donor : give blood to all groups (A, B, AB and O)

Quiz

- <https://quizlet.com/56413759/test>

Carbohydrates as informational Molecules: The Sugar Code

Many proteins **secreted** by cells have attached N-linked oligosaccharide chains.

Genetic diseases have been attributed to deficiency of particular enzymes involved in synthesizing or modifying oligosaccharide chains of these glycoproteins.

Such diseases, and **gene knockout studies in mice**, have been used to define pathways of modification of oligosaccharide chains of glycoproteins and glycolipids.

Carbohydrate chains of plasma membrane glycoproteins and glycolipids usually face the **outside of the cell**.

They have roles in cell-cell **interaction** and **signaling**, and in forming a protective layer on the surface of some cells.

Lectins are glycoproteins that **recognize** and **bind** to specific **oligosaccharides**.

Concanavalin A & **wheat germ agglutinin** are plant lectins that have been useful research tools.

The **C-type lectin-like domain** is a **Ca⁺⁺-binding** carbohydrate recognition domain in many **animal lectins**.

Recognition/binding of CHO moieties of glycoproteins, glycolipids & proteoglycans **by** animal **lectins** is a factor in:

- cell-cell recognition
- adhesion of cells to the extracellular matrix
- interaction of cells with chemokines and growth factors
- recognition of disease-causing microorganisms
- initiation and control of inflammation.

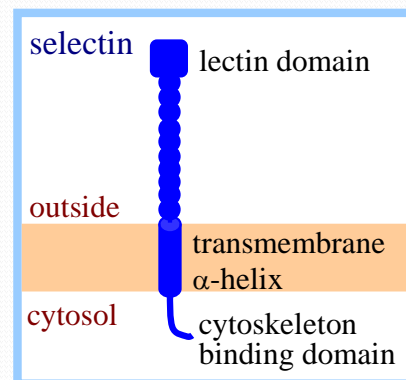
Examples of animal lectins:

Mannan-binding lectin (MBL) is a glycoprotein found in blood plasma.

It binds cell surface carbohydrates of **disease-causing microorganisms** & promotes phagocytosis of these organisms as part of the immune response.

Selectins are integral proteins of mammalian cell plasma membranes with roles in **cell-cell recognition** & binding.

The C-type **lectin-like domain** is at the end of a multi-domain extracellular segment extending out from the cell surface.



A **cleavage site** just outside the transmembrane α -helix provides a mechanism for regulated release of some lectins from the cell surface.

A **cytosolic domain** participates in regulated interaction with the actin cytoskeleton.

Lipopolysaccharides

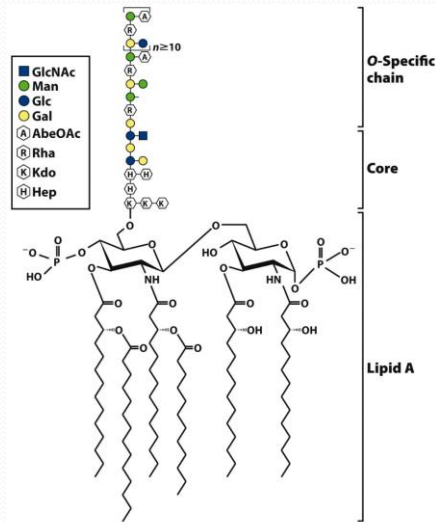


Figure 7-31
Lehninger Principles of Biochemistry, Sixth Edition
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Lipopolysaccharide of
the outer membrane
of the bacterium
Salmonella
typhimurium

Dietary carbohydrates

- Starch
- Sucrose
- Glucose and fructose
- Lactose
- Cellulose
- Other plant polysaccharides

Digestible

Non-digestible
by humans

Only monosaccharides are absorbed into the bloodstream from the gut.

Digestion of carbohydrates involves their hydrolysis into monosaccharides

Digestive Enzymes

Enzymes for carbohydrate digestion

Enzyme	Source	Substrate	Products
α -Amylase	Salivary gland Pancreas	Starch, glycogen	Oligosaccharides
Dextrinase	Small intestine	Oligosaccharides	Glucose
Isomaltase	Small intestine	α -1,6-glucosides	Glucose
Maltase	Small intestine	Maltose	Glucose
Lactase	Small intestine	Lactose	Galactose, glucose
Sucrase	Small intestine	Sucrose	Fructose, glucose

Lactase deficiency produces lactose intolerance

Blood glucose concentrations

Measured in mmol/L = mM or in mg/dL

Conversion factor: 1 mM = 18 mg/dL

Normal plasma glucose concentrations roughly
3.9 – 8.3 mM

Hypoglycemia: < 2.2 mM

Diabetes: > 7.0 mM (fasting)
 > 11.1 mM 2 h after ingestion of 75 g glucose

All cells can use glucose as an energy source
Brain cells and erythrocytes require glucose as an energy source

Quiz

- **Q: Volume of Chondroitin Sulfate in Solution** One critical function of chondroitin sulfate is to act as a lubricant in skeletal joints by creating a gel-like medium that is resilient to friction and shock. This function seems to be related to a distinctive property of chondroitin sulfate: the volume occupied by the molecule is much greater in solution than in the dehydrated solid. Why is the volume so much larger in solution?
- **Ans:** In solution, the negative charges on chondroitin sulfate repel each other and force the molecule into an extended conformation. The polar molecule also attracts many water molecules (water of hydration), further increasing the molecular volume. In the dehydrated solid, each negative charge is counterbalanced by a counterion, such as Na^+ , and the molecule collapses into its condensed form.

Quiz

- **Q: Heparin Interactions** Heparin, a highly negatively charged glycosaminoglycan, is used clinically as an anticoagulant. It acts by binding several plasma proteins, including antithrombin III, an inhibitor of blood clotting. The 1:1 binding of heparin to antithrombin III seems to cause a conformational change in the protein that greatly increases its ability to inhibit clotting. What amino acid residues of antithrombin III are likely to interact with heparin?
- **Ans:** Positively charged amino acid residues would be the best candidates to bind to the highly negatively charged groups on heparin. In fact, Lys residues of antithrombin III interact with heparin.

Quiz

- Q: Glucose Oxidase in Determination of Blood Glucose** The enzyme glucose oxidase isolated from the mold *Penicillium notatum* catalyzes the oxidation of b-D-glucose to D-glucono-d-lactone. This enzyme is highly specific for the b anomer of glucose and does not affect the a anomer. In spite of this specificity, the reaction catalyzed by glucose oxidase is commonly used in a clinical assay for total blood glucose—that is, for solutions consisting of a mixture of b- and a-D-glucose. What are the circumstances required to make this possible? Aside from allowing the detection of smaller quantities of glucose, what advantage does glucose oxidase offer over Fehling's reagent for the determination of blood glucose?
- Ans:** The rate of mutarotation (interconversion of the a and b anomers) is sufficiently high that, as the enzyme consumes b-D-glucose, more a-D-glucose is converted to the b form, and, eventually, all the glucose is oxidized. Glucose oxidase is specific for glucose and does not detect other reducing sugars (such as galactose). Fehling's reagent reacts with any reducing sugar.

Quiz

- Q:** As the table shows, certain pairs of derivatives have the same melting points, although the underivatized monosaccharides do not. Why do glucose and mannose, and similarly galactose and talose, form osazone derivatives with the same melting points?

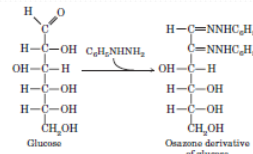
Monosaccharide	MP of anhydrous monosaccharide (°C)	MP of osazone derivative (°C)
Glucose	146	205
Mannose	132	205
Galactose	165–168	201
Talose	128–130	201

- Ans:** The configuration at C-2 of an aldose is lost in its osazone derivative, so aldoses differing only at the C-2 configuration (C-2 epimers) give the same derivative, with the same melting point. Glucose and mannose are C-2 epimers and thus form the same osazone; the same is true for galactose and talose.

Quiz

Q3: Melting Points of Monosaccharide Osazone Derivatives

Many carbohydrates react with phenylhydrazine ($C_6H_5NHNH_2$) to form bright yellow crystalline derivatives known as osazones:



The melting temperatures of these derivatives are easily determined and are characteristic for each osazone. This information was used to help identify monosaccharides before the development of HPLC or gas-liquid chromatography.

Listed below are the melting points (MPs) of some aldoseosazone derivatives:

Monosaccharide	MP of anhydrous monosaccharide (°C)	MP of osazone derivative (°C)
Glucose	146	205
Mannose	132	205
Galactose	165-168	201
Talose	128-130	201

As the table shows, certain pairs of derivatives have the same melting points, although the underivatized monosaccharides do not. Why do glucose and mannose, and galactose and talose, form osazone derivatives with the same melting points?

Ans: The configuration at C-2 of an aldose is lost in its osazone derivative, so aldoses differing only at the C-2 configuration (C-2 epimers) give the same derivative, with the same melting point.

Glucose and mannose are C-2 epimers and thus form the same osazone; the same is true for galactose and talose (see Fig. 7-3).

Quiz

- **A Taste of Honey:** The fructose in honey is mainly in the -D-pyranose form. This is one of the sweetest carbohydrates known, about twice as sweet as glucose. The -D-furanose form of fructose is much less sweet. The sweetness of honey gradually decreases at a high temperature. Also, high-fructose corn syrup (a commercial product in which much of the glucose in corn syrup is converted to fructose) is used for sweetening *cold* but not *hot* drinks.. Draw the two forms and explain why it may not always be wise to cook with honey.
- Heating converts the very sweet pyranose form to the more stable but less sweet furanose form. Consequently, it is difficult to accurately control the sweetness of the preparation, which also accounts for why honey loses sweetness with time.

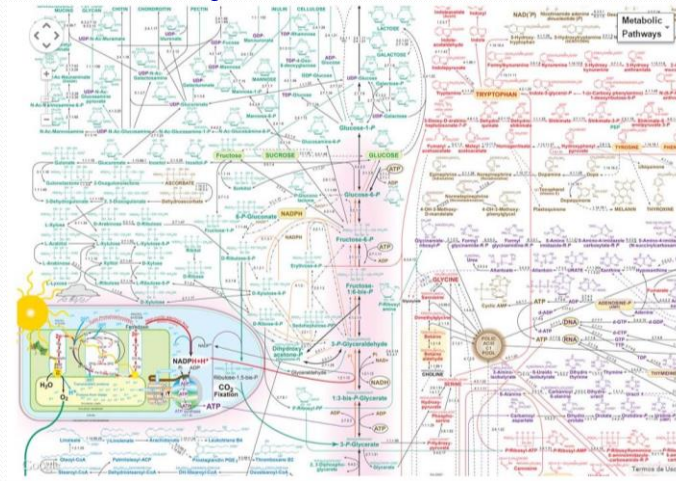
Stryer- Lehninger

Quiz

- **Q: Manufacture of Liquid-Filled Chocolates** The manufacture of chocolates containing a liquid center is an interesting application of enzyme engineering. The flavored liquid center consists largely of an aqueous solution of sugars rich in fructose to provide sweetness. The technical dilemma is the following: the chocolate coating must be prepared by pouring hot melted chocolate over a solid (or almost solid) core, yet the final product must have a liquid, fructose-rich center. Suggest a way to solve this problem. (Hint: Sucrose is much less soluble than a mixture of glucose and fructose.)
- **Ans:** Prepare the core as a semisolid slurry of sucrose and water. Add a small amount of sucrase (invertase), and quickly coat the semisolid mixture with chocolate. After the chocolate coat has cooled and hardened, the sucrase hydrolyzes enough of the sucrose to form a more liquid center: a mixture of fructose, glucose, and sucrose.

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- <https://quizlet.com/128350306/lehninger-biochem-ch-7-flash-cards>
- <https://quizlet.com/161793298/test>
- <https://quizlet.com/161793298/c8-carbohydrates-biochemistry-flash-cards/>

Introduction to Carbohydrate metabolism



Some metabolic pathways of carbohydrates

- 1- Glycolysis
- 2- Krebs cycle
- 3- Glycogenesis
- 4- Glycogenolysis
- 5- Glyconeogenesis

- - Pentose Phosphate Pathway (PPP)
- - Curi cycle
- - Biological oxidation

Metabolism

Metabolism involves :

- *Catabolic reactions* that break down large, complex molecules to provide energy and smaller molecules.
- *Anabolic reactions* that use ATP energy to build larger molecules.

Stages of Carbohydrate Metabolism

Stage 1: Digestion and hydrolysis break down large molecules to smaller ones that enter the bloodstream.

Stage 2: Degradation breaks down molecules to two- and three-carbon compounds.

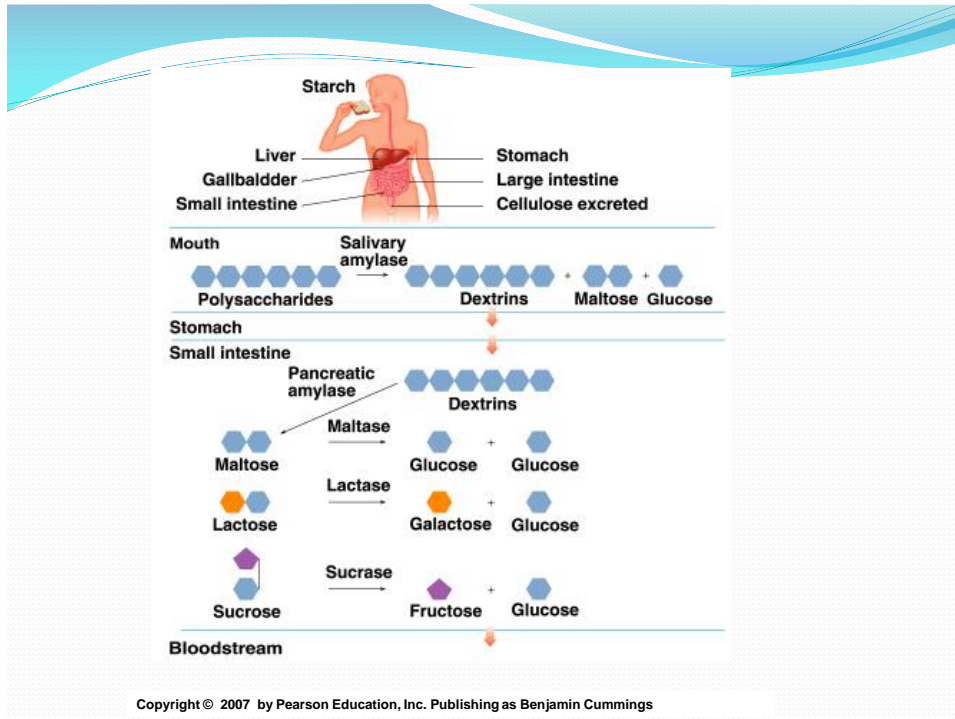
Stage 3: Oxidation of small molecules in the citric acid cycle and electron transport provide ATP energy.

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Stage 1: Digestion of Carbohydrates

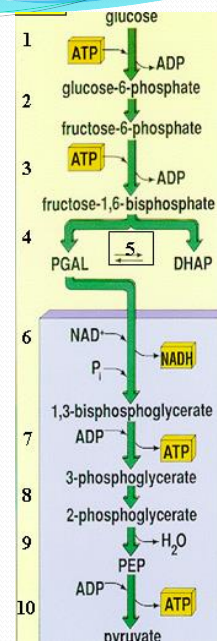
The digestion of carbohydrates:

- Begins in the mouth where salivary amylase breaks down polysaccharides to smaller polysaccharides (dextrins), disaccharide (maltose), and some glucose.
- Continues in the small intestine where pancreatic amylase hydrolyzes dextrins to maltose and glucose.
- Hydrolyzes of disaccharides; maltose, lactose, and sucrose to monosaccharides, mostly glucose, which enter the bloodstream for transport to the cells.



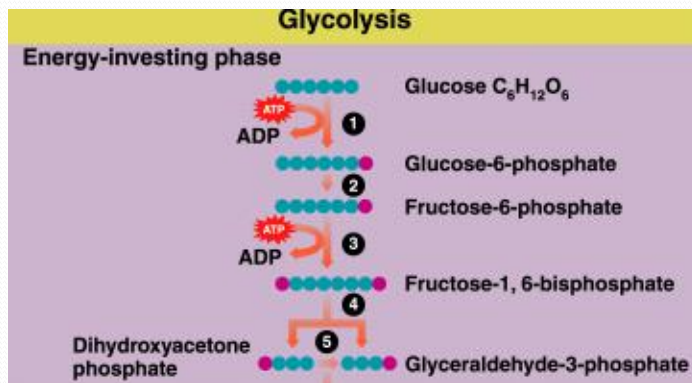
Stage 2: Glycolysis

- Glycolysis is a metabolic pathway that degrades glucose (a six-carbon) to pyruvate (a three-carbon molecules).
- It is an anaerobic process (no oxygen) and occurs in the cytoplasm.
- It is divided into two stages:
 - A- five reactions and consume energy
 - B- five reactions that produce energy



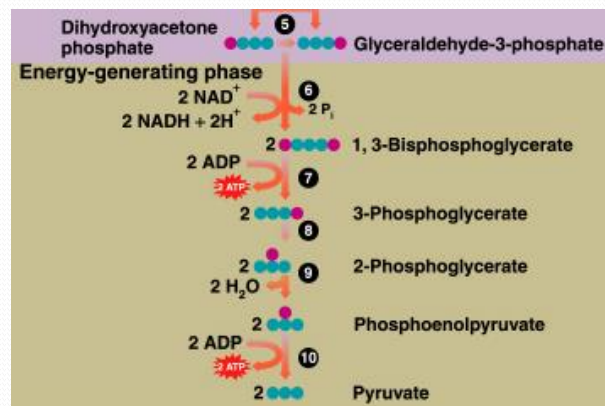
In reactions 1-5 of glycolysis,

- Energy is required to add phosphate groups to glucose.
- Glucose is converted through five enzymatically catalyzed reactions to two three-carbon molecules.



In reactions 6-10 of glycolysis, energy is generated as:

- Sugar phosphates are cleaved to triose phosphates.
- Four ATP molecules are produced.



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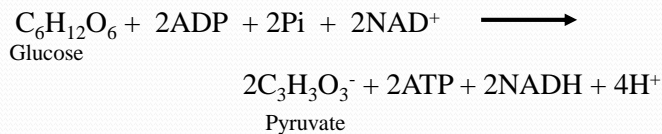
Glycolysis: Overall Reaction

In glycolysis,

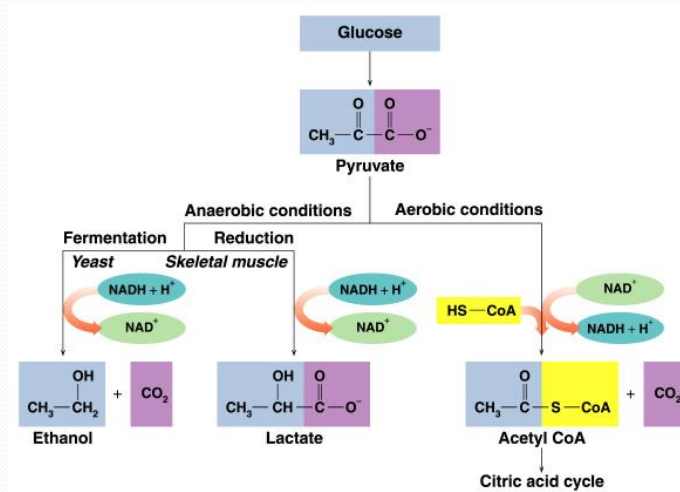
- Two ATP add phosphate to glucose and fructose-6-phosphate.
- Four ATP are formed in energy-generation by direct transfers of phosphate groups to four ADP.
- There is a net gain of 2 ATP and 2 NADH.

In other pathway

- In mitochondria, each of the 2NADH is converted to NAD^+ and 3ATP are produced ($2 \times 3 = 6\text{ATP}$)



The Fate of pyruvate produced from glycolysis

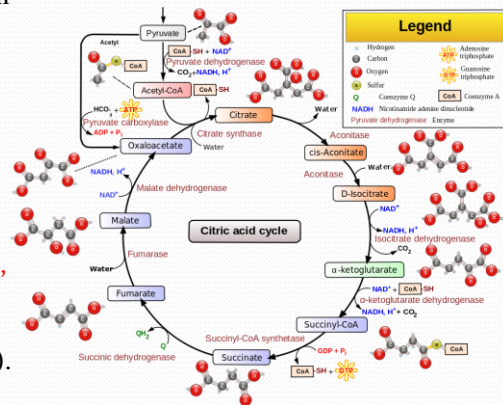


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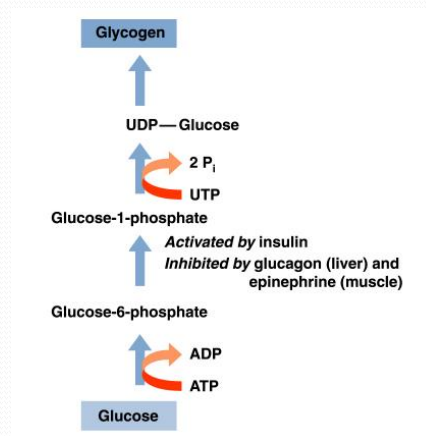
Krebs cycle

- It needs oxygen, so it occurs in all aerobic organisms.
- It is called **citric acid cycle**, **tricarboxylic acid (TCA) cycle** or the **Krebs cycle**.
- It generates energy through the oxidation of acetyl-CoA derived from **carbohydrates, fats and proteins** into CO₂ and chemical energy in the form of adenosine triphosphate (ATP).
- It occurs only in mitochondria which is called the "Power House"



Glycogenesis

- It is the storing of glucose (monosaccharide) by converting to glycogen (polysaccharide) in liver and muscles.
- It operates when high levels of glucose-6-phosphate are formed in the first reaction of glycolysis.
- It does not operate when energy stores (glycogen) are full, which means that additional glucose is converted to body fat.

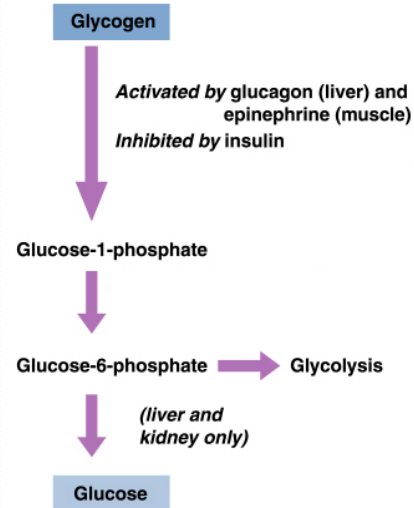


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Glycogenolysis

In **glycogenolysis**

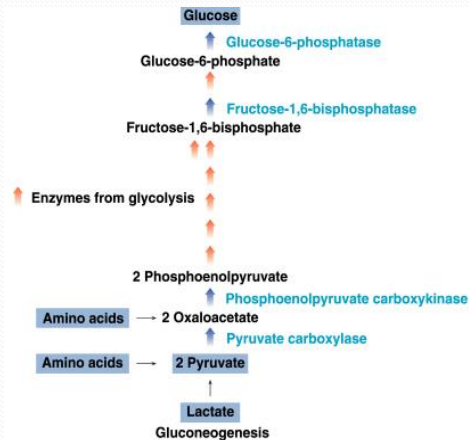
- Glycogen stores in liver and muscles is broken down to glucose.
- Glucose molecules are removed one by one from the end of the glycogen chain to yield glucose-1-phosphate.
- It occurs when the blood glucose level is decreasing to less than the lower limit (70 mg%) to compensate this decrease.



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Gluconeogenesis

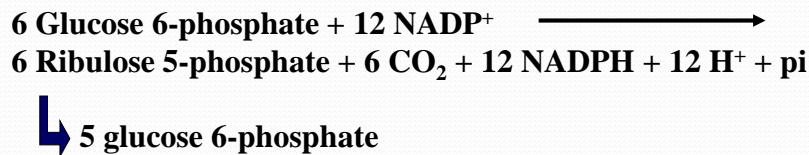
- It is the generation of glucose from certain non-carbohydrate carbon substrates like the **metabolic products of carbohydrates, amino acids and lipid**.
- It occurs when glycogen stores are depleted as a result of starvation or if the body can not utilize glucose as in the case of diabetes.



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Pentose Phosphate Pathway (PPP)

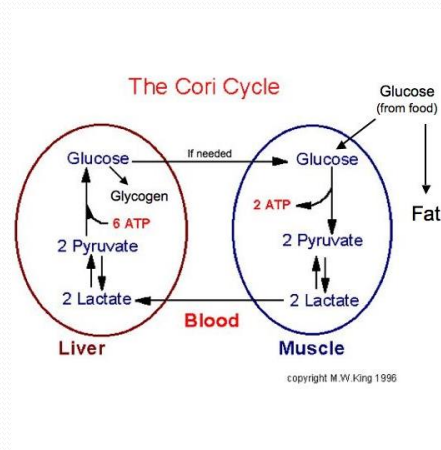
- The **pentose phosphate pathway** is a metabolic pathway parallel to glycolysis.
- It generates NADPH and pentoses as well as Ribose 5-phosphate, the last one a precursor for the synthesis of nucleotides.



Cori Cycle

The Cori cycle

- It is the flow of lactate and glucose between the muscles and the liver.
- It occurs when anaerobic conditions occur in active muscle and glycolysis produces lactate.
- It operates when lactate moves through the blood stream to the liver, where it is oxidized back to pyruvate.
- It converts pyruvate to glucose, which is carried back to the muscles.



Pathways for Glucose

