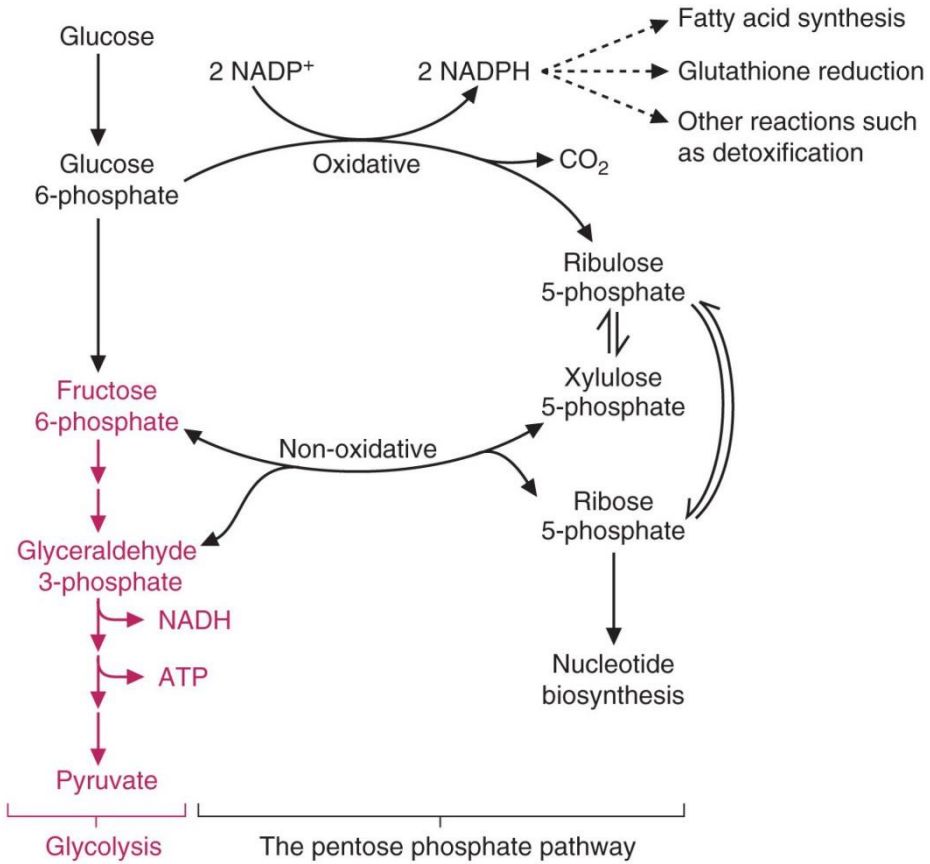
Chapter 27

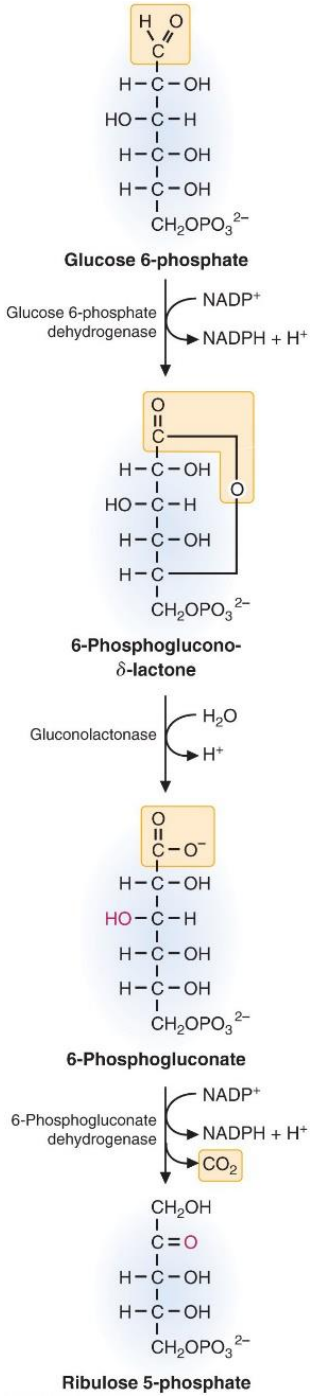
Pentose Phosphate Pathway and the Synthesis of Glycosides, Lactose, Glycoproteins, and Glycolipids

Human Biochemistry



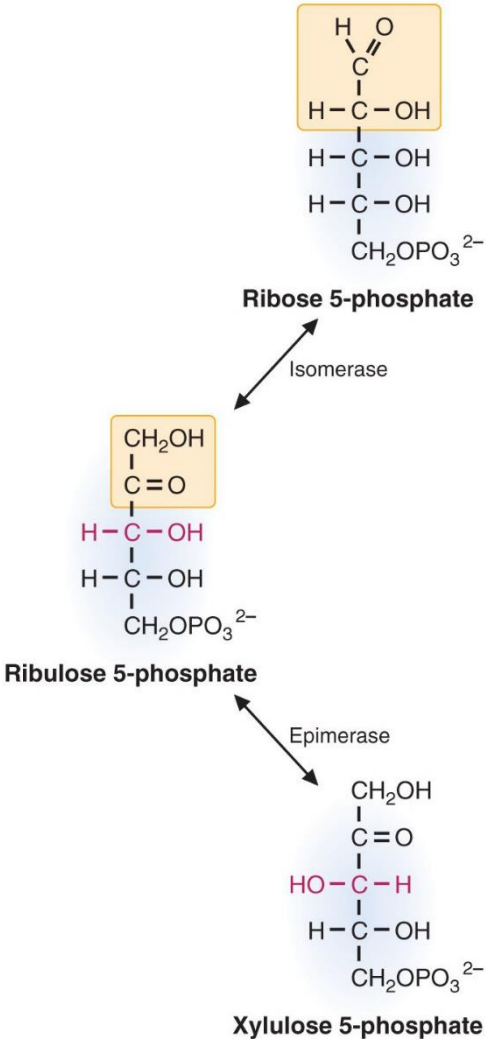
Overview of the pentose phosphate pathway. The pentose phosphate pathway generates NADPH for reactions that require reducing equivalents (electrons) or ribose 5-P for nucleotide biosynthesis. Glucose 6-P is a substrate for both the pentose phosphate pathway and glycolysis. The five-carbon sugar intermediates of the pentose phosphate pathway are reversibly interconverted to intermediates of glycolysis. The portion of glycolysis that is not part of the pentose phosphate pathway is shown in *red*.

- The pentose phosphate pathway (also known as the hexose monophosphate shunt [HMP shunt]) consists of both oxidative and nonoxidative components.
- In the oxidative pathway, glucose 6-phosphate (glucose 6-P) is oxidized to ribulose 5-phosphate (ribulose 5-P), CO_2, and NADPH. Ribulose 5-P, a pentose, can be converted to ribose 5-phosphate (ribose 5-P) for nucleotide biosynthesis.
- The second stage of the pentose phosphate pathway generates ribose 5-P and converts unused intermediates to fructose 6-P and glyceraldehyde 3-P in the glycolytic pathway.



- In the oxidative, first phase of the pentose phosphate pathway, glucose 6-P undergoes and oxidation and decarboxylation to a pentose sugar, ribulose 5-phosphate (ribulose 5-P).
- The first enzyme of this pathway, glucose-6-phosphate dehydrogenase, oxidizes the aldehyde at carbon 1 and reduces NADP to NADPH.
- The gluconolactone that is formed is rapidly hydrolyzed to 6-phosphogluconate, a sugar acid with a carboxylic acid group at carbon 1.
- The next oxidation step releases this carboxyl group as CO_2, with the electrons being transferred to NADP. This reaction is mechanistically very similar to the one catalyzed by isocitrate dehydrogenase in the TCA cycle.
- Thus, 2 moles of NADPH per mole of glucose 6-P are formed from this portion of the pathway.

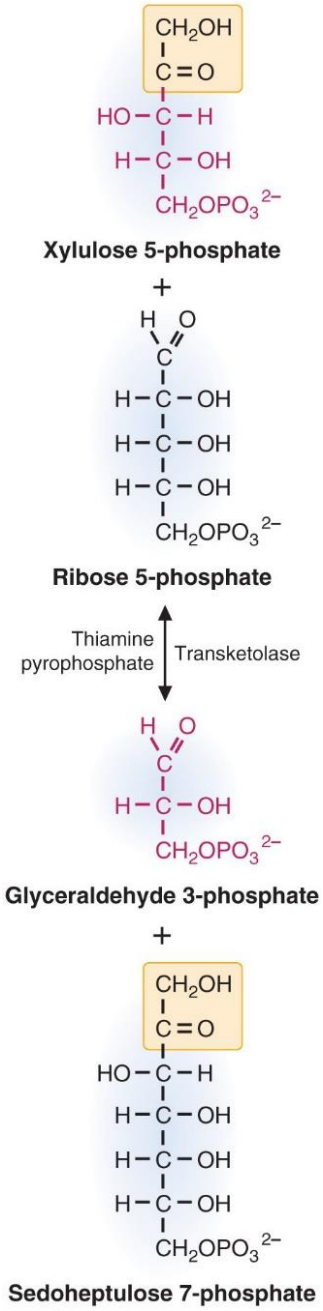
Oxidative portion of the pentose phosphate pathway.
Carbon 1 of glucose 6-P is
oxidized to an acid and then released as CO_2 in an oxidation followed by a decarboxylation reaction. Each of the oxidation steps generates a NADPH.



Ribulose 5-phosphate (ribulose 5-P) is epimerized (to xylulose 5-phosphate [xylulose 5-P], shown in *red*) and isomerized (to ribose 5-phosphate [ribose 5-P], shown in the *yellow* box).

- The nonoxidative portion of the pentose phosphate pathway consists of a series of rearrangement and transfer reactions that first convert **ribulose 5-P** to **ribose 5-P** and **xylulose 5-phosphate** (**xylulose 5-P**), and then the ribose 5-P and xylulose 5-P are **converted to intermediates of the glycolytic pathway**. The enzymes involved are **epimerase**, **isomerase**, **transketolase**, and **transaldolase**.

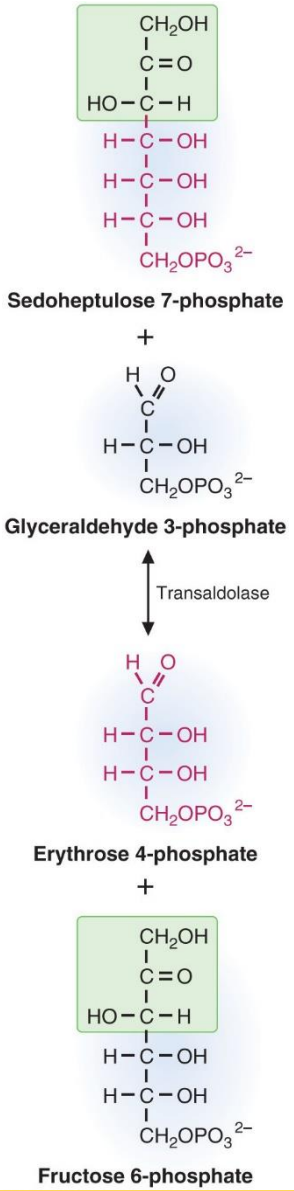
- The epimerase and isomerase convert ribulose 5-P to two other five-carbon sugars. The isomerase converts ribulose 5-P to ribose 5-P. The epimerase changes the stereochemical position of one hydroxyl group (at carbon 3), converting ribulose 5-P to xylulose 5-P.



- Transketolase transfers two-carbon fragments of keto sugars (sugars with a keto group at carbon 2) to other sugars. Transketolase picks up a two-carbon fragment from xylulose 5-P by cleaving the carbon-carbon bond between the keto group and the adjacent carbon, thereby releasing glyceraldehyde 3-P.

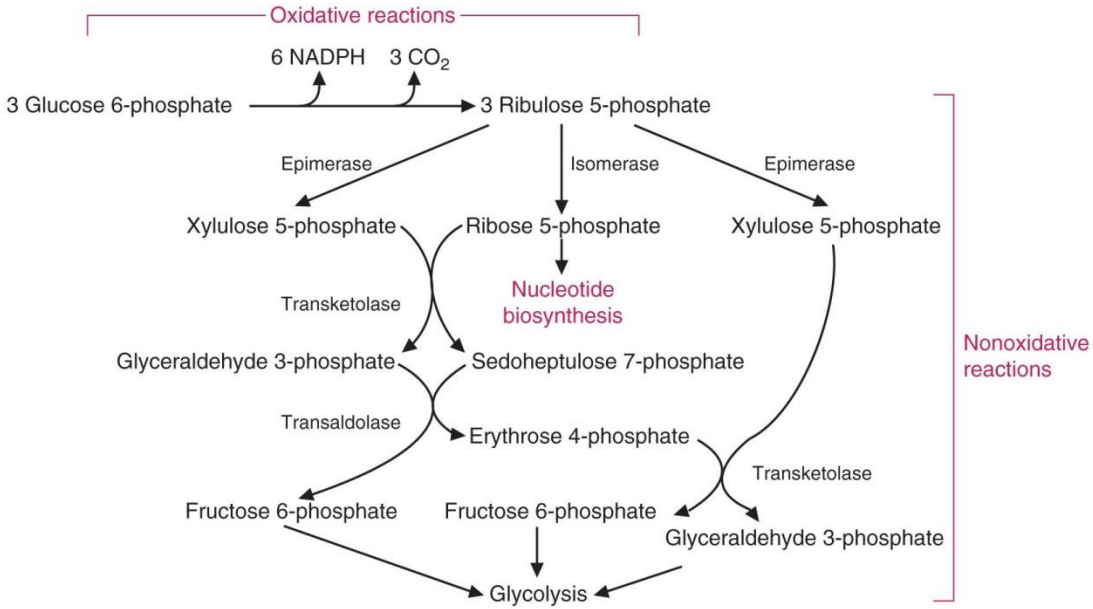
- The two carbon fragment is covalently bound to thiamine pyrophosphate, which transfers it to the aldehyde carbon of another sugar, forming a new ketose. The role of thiamine pyrophosphate here is, thus, very similar to its role in the oxidative decarboxylation of pyruvate and α-ketoglutarate.

Two-carbon unit transferred by transketolase. Transketolase cleaves the bond next to the keto group and transfers the two-carbon keto fragment to an aldehyde. Thiamine pyrophosphate carries the two-carbon fragment, forming a covalent bond with the carbon of the keto group.



Transaldolase transfers a threecarbon fragment that contains an alcohol group next to a keto group.

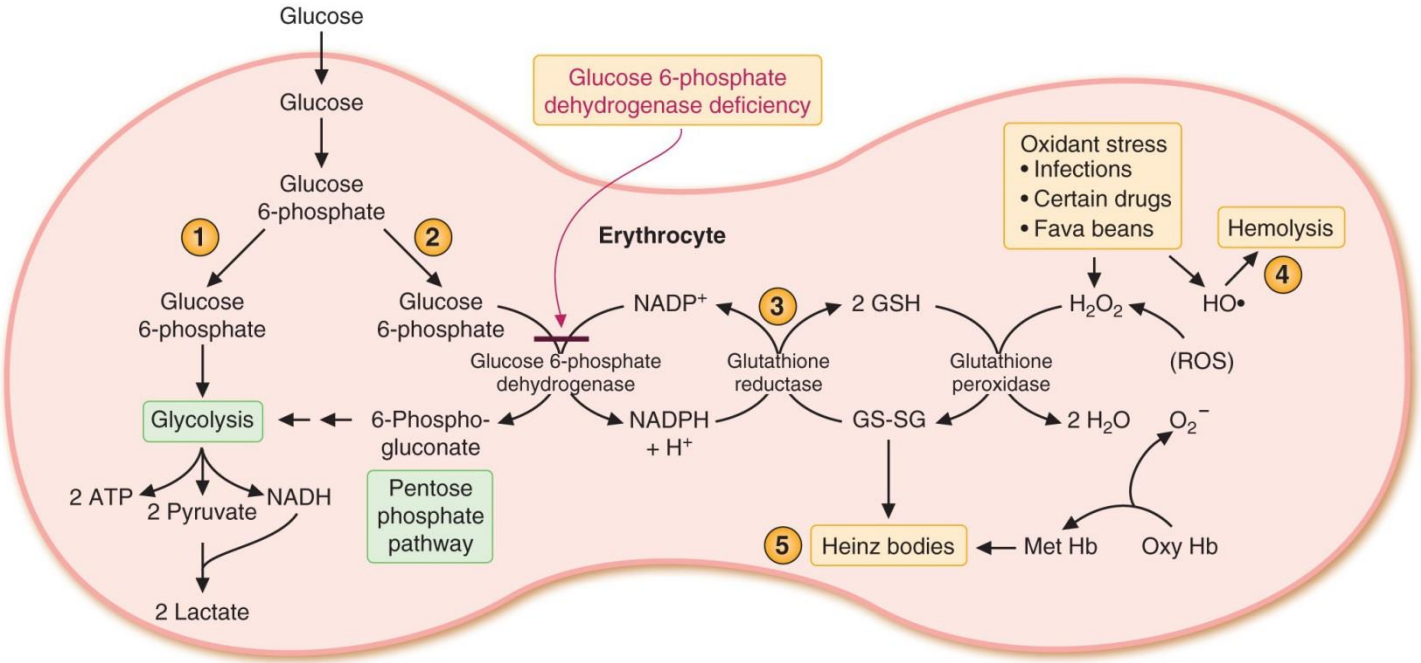
- Transaldolase transfers a three-carbon keto fragment from sedoheptulose 7-P to glyceraldehyde 3-P to form erythrose 4-P and fructose 6-P. The aldol cleavage occurs between the two hydroxyl carbons adjacent to the keto group (on carbons 3 and 4 of the sugar). This reaction is similar to the aldolase reaction in glycolysis, and the enzyme uses an active amino group from the side chain of lysine to catalyze the reaction.



A balanced sequence of reactions in the pentose phosphate pathway.
The interconversion of sugars in the pentose phosphate pathway results in conversion of 3 glucose 6-P to 6 NADPH, 3 CO_2, 2 fructose 6-phosphate (fructose 6-P), and 1 glyceraldehyde 3-P.

- The net result of the metabolism of 3 mol of ribulose 5-P in the pentose phosphate pathway is the formation of 2 mol of fructose 6-P and 1 mol of glyceraldehyde 3-P, which then continue through the glycolytic pathway with the production of NADH, ATP, and pyruvate.

- Because the pentose phosphate pathway begins with glucose 6-P and feeds back into the glycolytic pathway, it is sometimes called the hexose monophosphate (HMP) shunt (a shunt or a pathway for glucose 6-P). The reaction sequence starting from glucose 6-P, involving both the oxidative and nonoxidative phases of the pathway is shown in Figure 27.6.

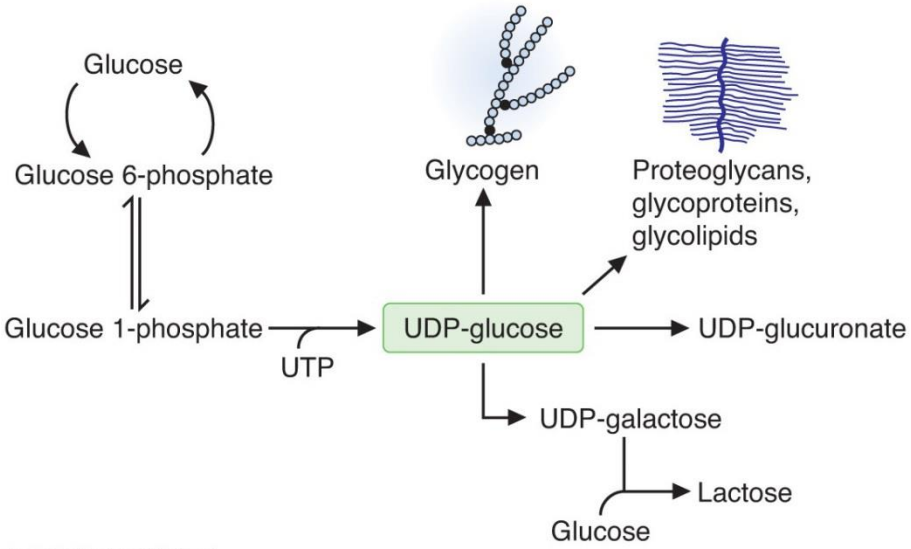


Hemolysis caused by reactive oxygen species (ROS). *(1)* Maintenance of the integrity of the erythrocyte membrane depends on its ability to generate ATP and NADH from glycolysis. *(2)* NADPH is generated by the pentose phosphate pathway. *(3)* NADPH is used for the reduction of oxidized glutathione (GSSG) to reduced glutathione (GSH). Glutathione is necessary for the removal of H_2O_2 and lipid peroxides generated by ROS. *(4)* In the erythrocytes of healthy individuals, the continuous generation of superoxide ion from the nonenzymatic oxidation of hemoglobin provides a source of ROS. The glutathione defense system is compromised by glucose-6-phosphate dehydrogenase deficiency, infections, certain drugs, and the purine glycosides of fava beans. *(5)* As a consequence, Heinz bodies, aggregates of cross-linked hemoglobin, form on the cell membranes and subject the cell to mechanical stress as it tries to go through small capillaries. The action of the ROS on the cell membrane as well as mechanical stress from the lack of deformability result in hemolysis.

- In general, the oxidative phase of the pentose phosphate pathway is the major source of NADPH in cells. NADPH provides the reducing equivalents for biosynthetic reactions and for oxidation-reduction reactions involved in protection against the toxicity of reactive oxygen species (ROS). The glutathione-mediated defense against oxidative stress is common to all cell types (including red blood cells), and the requirement for NADPH to maintain levels of reduced glutathione (GSH) probably accounts for the universal distribution of the pentose phosphate pathway among different types of cells.

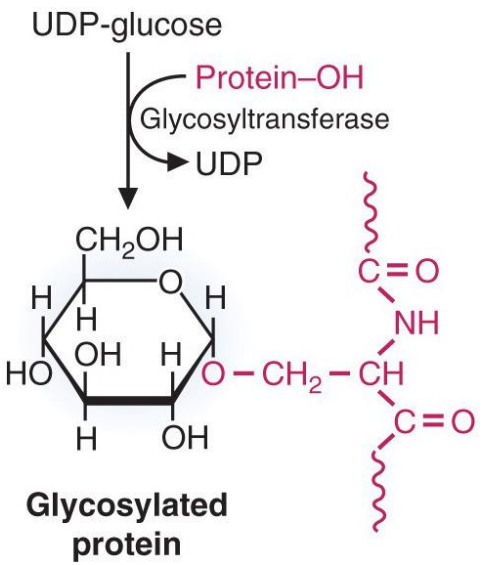
- Figure 27.7 illustrates the importance of this pathway in maintaining the membrane integrity of the red blood cells. NADPH is also used for anabolic pathways, such as fatty acid synthesis, cholesterol synthesis, and fatty acid chain elongation . It is the source of reducing equivalents for cytochrome P450 hydroxylation of aromatic compounds, steroids, alcohols, and drugs. The highest concentrations of glucose-6-phosphate dehydrogenase are found in phagocytic cells where NADPH oxidase uses NADPH to form superoxide from molecular oxygen. The superoxide then generates hydrogen peroxide, which kills the microorganisms taken up by the phagocytic cells.

- The entry of glucose 6-P into the pentose phosphate pathway is controlled by the cellular concentration of NADPH. NADPH is a strong product inhibitor of glucose- 6-phosphate dehydrogenase, the first enzyme of the pathway. As NADPH is oxidized in other pathways, the product inhibition of glucose-6-phosphate dehydrogenase is relieved, and the rate of the enzyme is accelerated to produce more NADPH.



An overview of UDP-glucose metabolism.
The activated glucose moiety of UDP-glucose can be attached by a glycosidic bond to other sugars, as in glycogen or the sugar oligosaccharide and polysaccharide side chains of proteoglycans, glycoproteins, and glycolipids. UDP-glucose also can be oxidized to UDP-glucuronate or epimerized to UDP-galactose, a precursor of lactose.

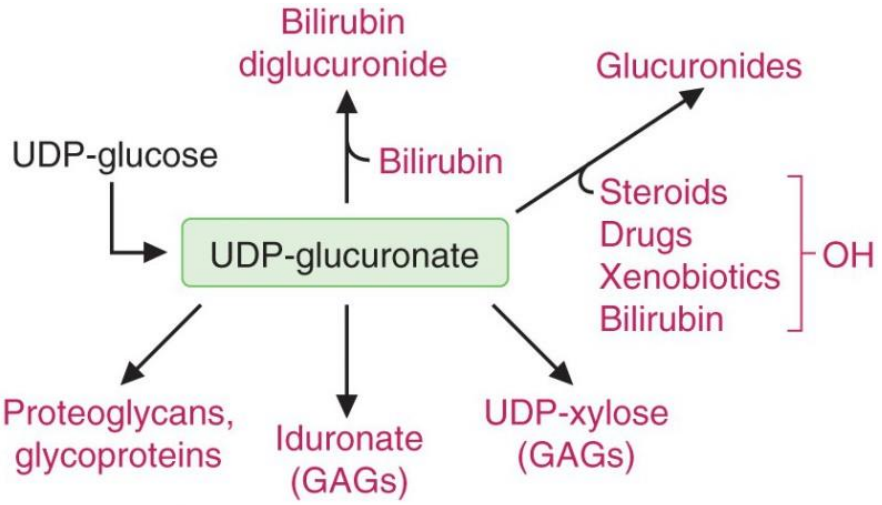
- Uridine diphosphate (UDP)-glucose is an activated sugar nucleotide that is a precursor of glycogen and lactose, UDP-glucuronate and glucuronides, and the carbohydrate chains in proteoglycans, glycoproteins, and glycolipids



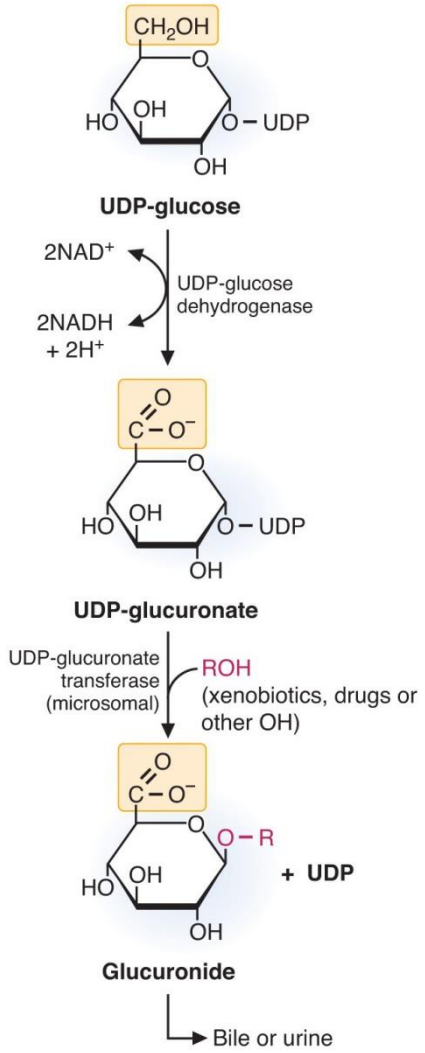
Glycosyltransferases.
These enzymes transfer sugars from nucleotide sugars to nucleophilic amino acid residues on proteins, such as the hydroxyl group of serine or the amide group of asparagine. Other transferases transfer specific sugars from a nucleotide sugar to a hydroxyl group of other sugars. The bond formed between the anomeric carbon of the sugar and the nucleophilic group of another compound is a glycosidic bond.

- In the synthesis of many of the carbohydrate portions of these compounds, a sugar is transferred from the nucleotide sugar to an alcohol or other nucleophilic group to form a glycosidic bond.
- The use of UDP as a leaving group in this reaction provides the energy for formation of the new bond. The enzymes that form glycosidic bonds are sugar transferases (e.g., glycogen synthase is a glucosyltransferase).
- Transferases are also involved in the formation of the glycosidic bonds in bilirubin glucuronides, proteoglycans, and lactose.

- One of the major routes of UDP-glucose metabolism is the formation of UDP-glucuronate, which serves as a precursor of other sugars and of glucuronides (Fig. 27.10).



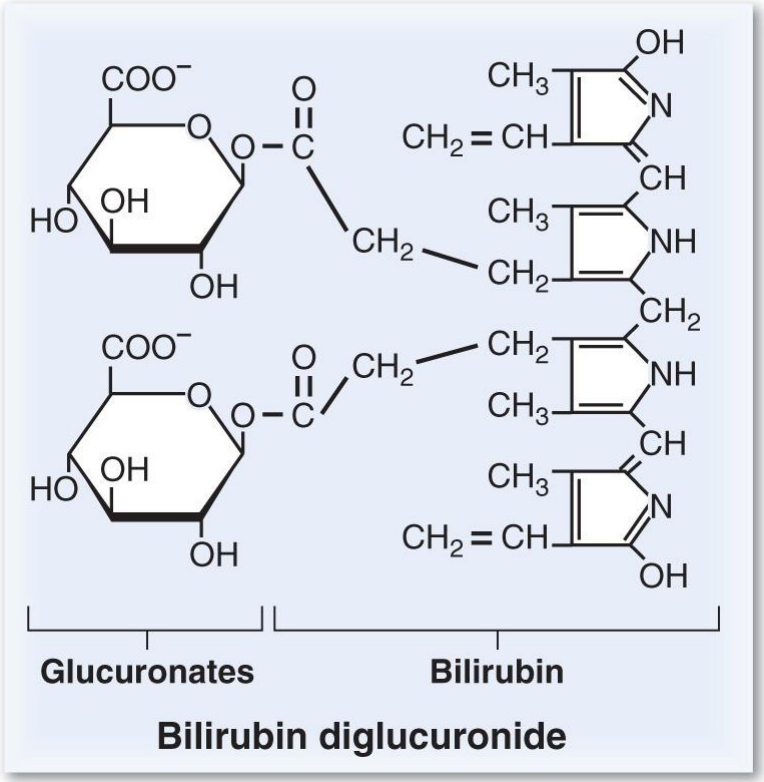
Metabolic routes of UDP-glucuronate.
UDP-glucuronate is formed from UDPglucose (shown in *black*). Glucuronate from UDP-glucuronate is incorporated into glycosaminoglycans (GAGs), where certain of the glucuronate residues are converted to iduronate (see Chapter 49). UDP-glucuronate is a precursor of UDP-xylose, another sugar residue incorporated into glycosaminoglycans. Glucuronate is also transferred to the carboxyl groups of bilirubin or the alcohol groups of steroids, drugs, and xenobiotics to form glucuronides. The "-ide" in the name glucuronide denotes that these compounds are glycosides.
Xenobiotics are pharmacologically, endocrinologically, or toxicologically active substances that are not produced endogenously and, therefore, are foreign to an organism. Drugs are examples of xenobiotics.



- Glucuronate is formed by the oxidation of the alcohol on carbon 6 of glucose to an acid (through two oxidation states) by a NAD^+ dependent dehydrogenase.

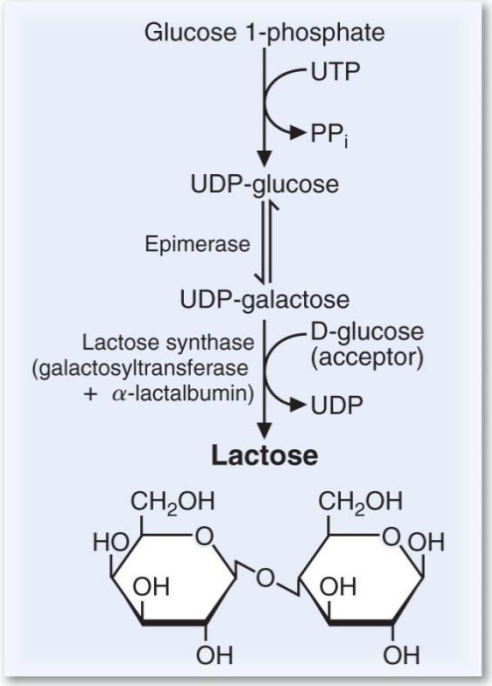
- Glucuronate is also present in the diet and can be formed from the degradation of inositol (the sugar alcohol that forms inositol trisphosphate[IP_3]), an intracellular second messenger for many hormones.

Formation of glucuronate and glucuronides.
A glycosidic bond is formed between the anomeric hydroxyl of glucuronate (at carbon 1) and the hydroxyl group of a nonpolar compound. The negatively charged carboxyl group
of the glucuronate increases the water solubility and allows otherwise nonpolar compounds to be excreted in the urine or bile. The hydrogen atoms have been omitted from the figure for clarity.



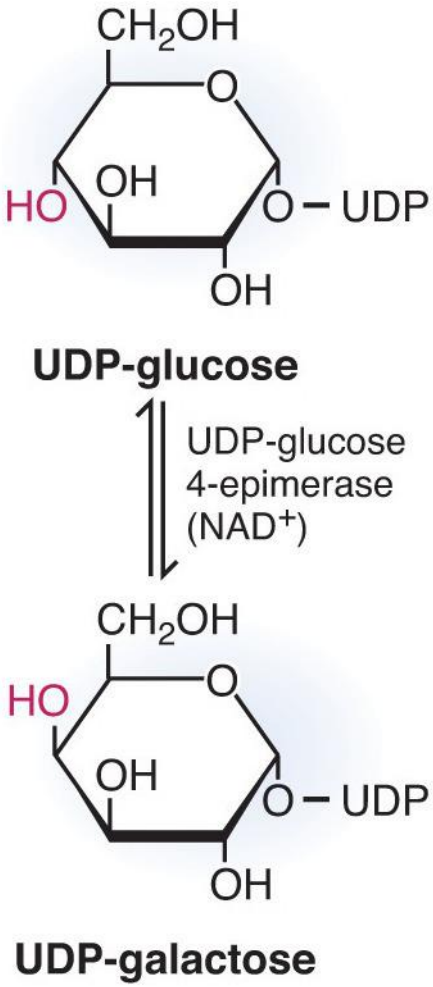
Formation of bilirubin diglucuronide.
A glycosidic bond is formed between the anomeric hydroxyl of glucuronate and the carboxylate groups of bilirubin. The addition of the hydrophilic carbohydrate group and the negatively charged carboxyl group of the glucuronide increases the water solubility of the conjugated bilirubin and allows the otherwise insoluble bilirubin to be excreted in the urine or bile. The hydrogen atoms on the sugars have been omitted from the figure for clarity

- The function of glucuronate in the excretion of bilirubin, drugs, xenobiotics, and other compounds containing a hydroxyl group is to add negative charges and increase their solubility.
- *Bilirubin* is a degradation product of heme that is formed in the reticuloendothelial system and is only slightly soluble in plasma. It is transported to the liver bound to albumin.
- In the liver, glucuronate residues are transferred from UDP-glucuronate to two carboxyl groups on bilirubin, sequentially forming bilirubin monoglucuronide and bilirubin diglucuronide, the “conjugated” forms of bilirubin.
- The more soluble bilirubin diglucuronide (as compared with unconjugated bilirubin) is then actively transported into the bile for excretion.



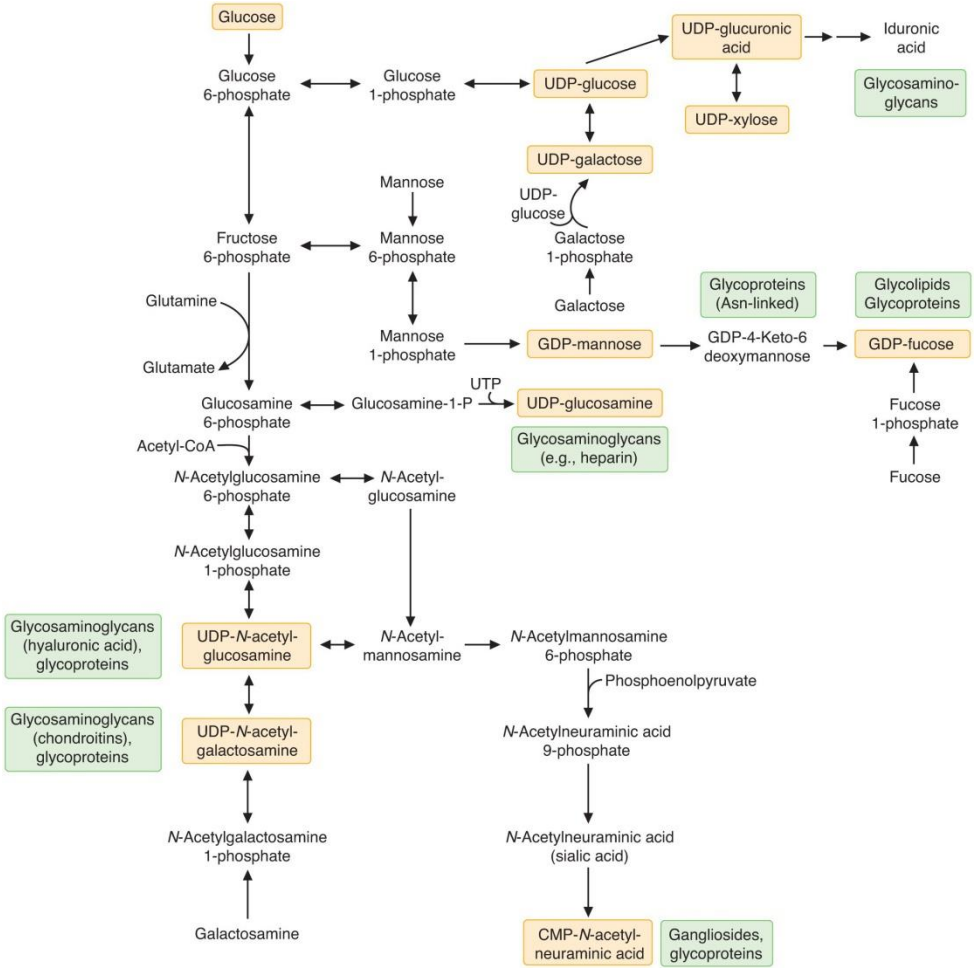
Lactose synthesis.
Lactose is a disaccharide composed of galactose and glucose. UDP-galactose for the synthesis of lactose in the mammary gland is usually formed from the epimerization of UDP-glucose. Lactose synthase catalyzes the attack of the C4 alcohol group of glucose on the anomeric carbon of the galactose, releasing UDP and forming a glycosidic bond. Lactose synthase is composed of a galactosyltransferase and -lactalbumin, which is a regulatory subunit.

- Lactose is synthesized from UDP-galactose and glucose. However, galactose is not required in the diet for lactose synthesis because galactose can be synthesized from glucose.
- Lactose is unique in that it is synthesized only in the mammary gland of the adult female for short periods during lactation.
- *Lactose synthase*, an enzyme present in the ER of the lactating mammary gland, catalyzes the last step in lactose biosynthesis: the transfer of galactose from UDP-galactose to glucose.
- Lactose synthase has two protein subunits: a galactosyltransferase and α-lactalbumin.
  α -Lactalbumin is a modifier protein synthesized after parturition (childbirth) in response to the hormone prolactin.
- This enzyme subunit lowers the *K*m of the galactosyltransferase for glucose from 1,200 to 1 mM, thereby increasing the rate of lactose synthesis. In the absence of -lactalbumin, galactosyltransferase transfers galactosyl units to glycoproteins.



- Galactose and glucose are epimers; they differ only in the stereochemical position of one hydroxyl group, at carbon 4.
- Thus, the formation of UDP-galactose from UDP-glucose is an epimerization (Fig. 27.14).
- The epimerase does not actually transfer the hydroxyl group; it oxidizes the hydroxyl to a ketone by transferring electrons to NAD, and then donates electrons back to re-form the alcohol group on the other side of the carbon.

Epimerization of UDP-glucose to UDP-galactose. The epimerization of glucose to galactose occurs on UDP-sugars. The epimerase uses NAD to oxidize the alcohol to a ketone, and then reduces the ketone back to an alcohol. The reaction is reversible; glucose being converted to galactose forms galactose for lactose synthesis, and galactose being converted to glucose is part of the pathway for the metabolism of dietary galactose. The hydrogen atoms have been omitted for clarity.



- The pathways for use and formation of many of these sugars are summarized in Figure.
- Note that many of the steps are reversible, so that glucose and other dietary sugars enter a common pool from which the diverse sugars can be formed.

Pathways for the interconversion of sugars.
All of the different sugars found in glycosaminoglycans, gangliosides, and other compounds in the body can be synthesized from glucose. Dietary glucose, fructose, galactose, mannose, and other sugars enter a common pool from which other sugars are derived. The activated sugar is transferred from the nucleotide sugar, shown in *orange boxes*, to form a glycosidic bond with another sugar or amino acid residue. The *green box* next to each nucleotide sugar lists some of the compounds that contain the sugar. Iduronic acid, in the upper right corner of the diagram, is formed only after glucuronic acid is incorporated into a glycosaminoglycan (which is discussed in more detail in Chapter 49).

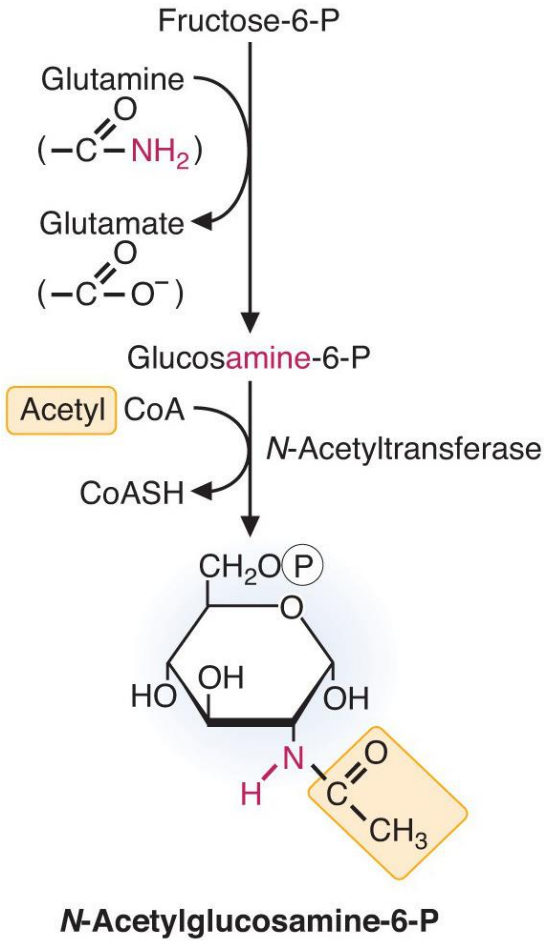
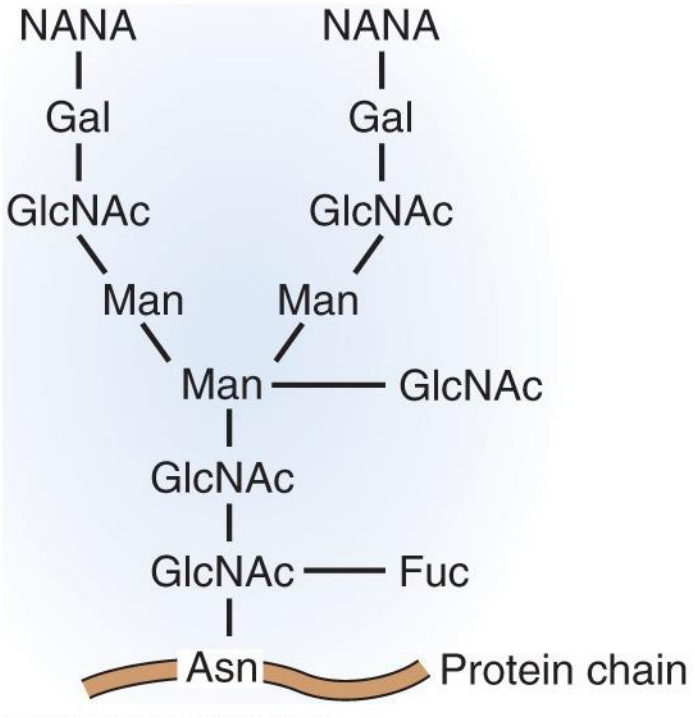


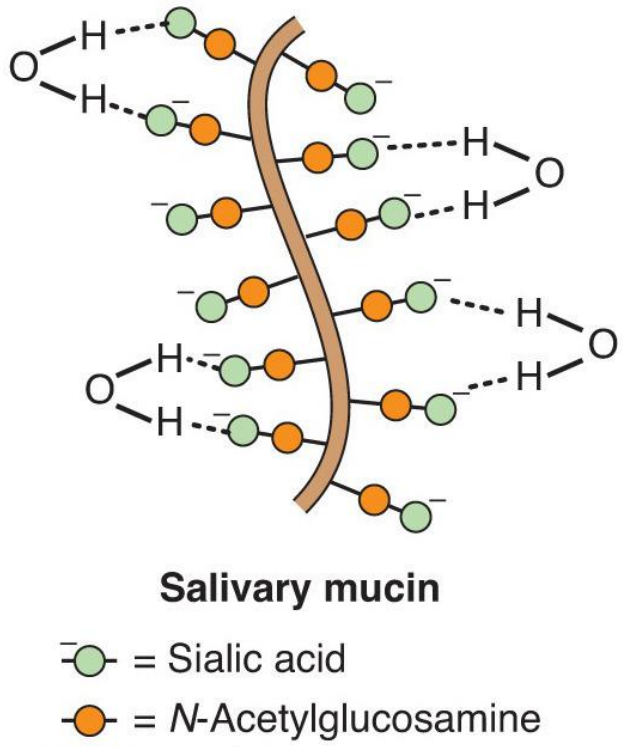
Fig 27.16 The formation of *N*-acetylglucosamine 6-phosphate. The amino sugar is formed by a transfer of the amino group from the amide of glutamine to a carbon of the sugar. The amino group is acetylated by the transfer of an acetyl group from acetyl-CoA. The hydrogen atoms from the sugar have been omitted for clarity.

- The amino sugars are all derived from glucosamine 6-phosphate. To synthesize glucosamine 6-phosphate, an amino group is transferred from the amide of glutamine to fructose 6-phosphate (Fig. 27.16).
- Amino sugars, such as glucosamine, can then be *N*-acetylated by an acetyltransferase.
- *N*-acetyltransferases are present in the ER and cytosol and provide another means of chemically modifying sugars, metabolites, drugs, and xenobiotic compounds.
- Individuals may vary greatly in their capacity for acetylation reactions.



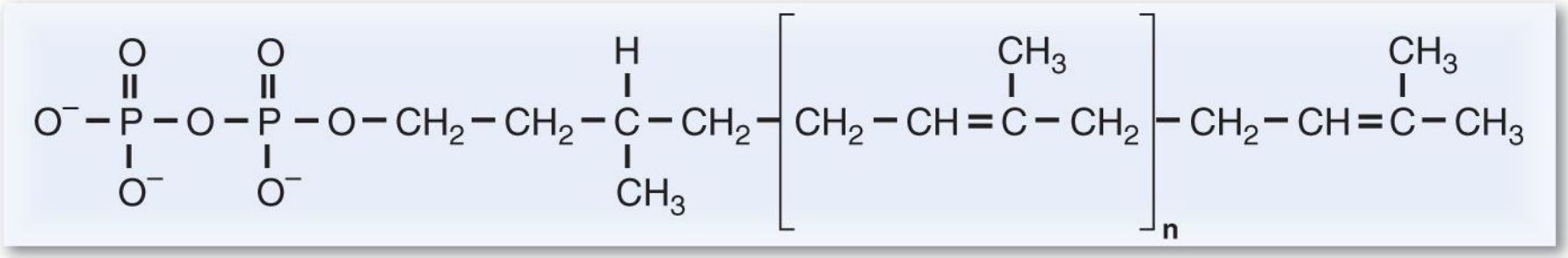
An example of a branched glycoprotein. *NANA*, *N*-acetylneuraminic acid; *Gal*, galactose; *GlcNAc*, *N*-acetylglucosamine; *Man*, mannose; *Fuc*, fucose.

- **Glycoproteins** contain **short carbohydrate chains** covalently linked to either **serine/ threonine** or **asparagine residues** in the protein. These oligosaccharide chains are often branched and they do not contain repeating disaccharides.
- Most proteins in the blood are glycoproteins. They serve as **hormones**, **antibodies**, **enzymes** (including those of the blood clotting cascade), and as structural components of the extracellular matrix. Collagen contains galactosyl units and disaccharides composed of galactosyl-glucose attached to hydroxylysine residues.



Structure of salivary mucin.
The sugars form hydrogen bonds with water. Sialic acid provides a negatively charged carboxylate group. The protein is extremely large, and the negatively charged sialic acids extend the carbohydrate chains (by charge repulsion) so the molecules occupy a large space. All of the salivary glycoproteins contain *O*-linked sugars. NANA is a sialic acid.

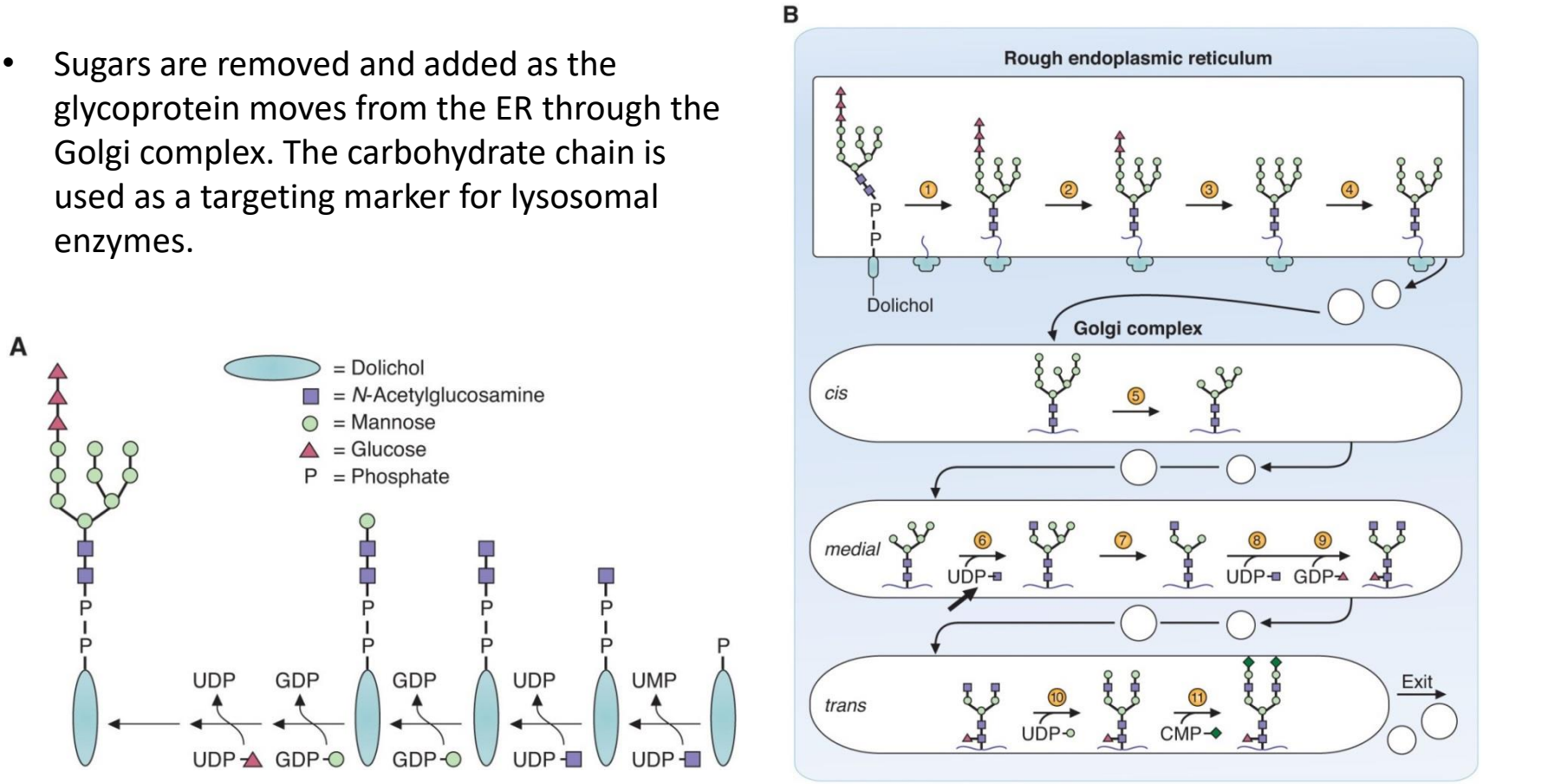
- The secretions of mucus-producing cells, such as **salivary mucin**, are glycoproteins.



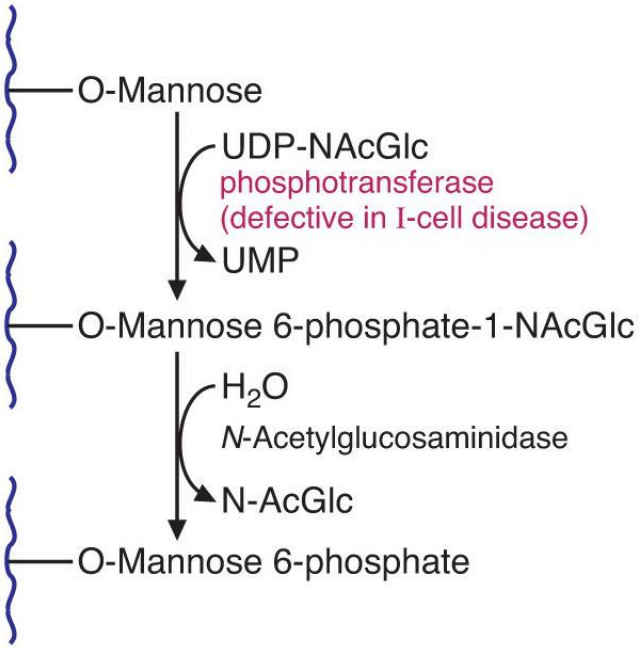
Structure of dolichol phosphate.
In humans, the isoprene unit (*in brackets*) is repeated approximately 17 times (n =~17).

- The protein portion of glycoproteins is synthesized on the endoplasmic reticulum (**ER**). The carbohydrate chains are attached to the protein in the lumen of the ER and the Golgi complex. In some cases, the initial sugar is added to a serine or a threonine residue in the protein, and the carbohydrate chain is extended by the sequential addition of sugar residues to the nonreducing end.
- **UDP-sugars** are the precursors for the addition of four of the seven sugars that are usually found in glycoproteins—glucose, galactose, N-acetylglucosamine, and N-acetylgalactosamine. **GDP-sugars** are the precursors for the addition of mannose and L-fucose, and CMP-NANA is the precursor for NANA.
- **Dolichol phosphate**, which is synthesized from isoprene units is involved in transferring branched sugar chains to the amide nitrogen of asparagine residues.

- Sugars are removed and added as the glycoprotein moves from the ER through the Golgi complex. The carbohydrate chain is used as a targeting marker for lysosomal enzymes.



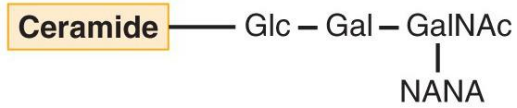
Action of dolichol phosphate in synthesizing the high-mannose form of oligosaccharides (A) and the processing of these carbohydrate groups (B). Transfer of the branched oligosaccharide from dolichol phosphate to a protein in the lumen of the rough endoplasmic reticulum (RER) *(step 1)* and processing of the oligosaccharide *(steps 2–11)*. *Steps 1* through *4* occur in the RER. The glycoprotein is transferred in vesicles to the Golgi complex, where further modifications of the oligosaccharides occur *(steps 5–11)*.
(**B** modified with permission from Kornfeld R, Kornfeld S. Assembly of aspargine-linked oligosaccharides. *Annu Rev Biochem.* 1985;54:631–664. Copyright 1985 by Annual Reviews, Inc.)



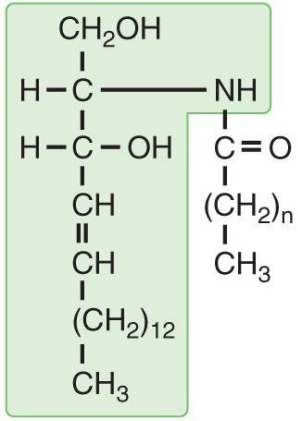
Synthesis of mannose 6-phosphate on the oligosaccharide of lysosomal proteins.
The pathway for phosphorylating a mannose residue within the protein-attached oligosaccharide requires two steps. The first is a transfer of *N*-acetylglucosamine phosphate to the mannose residue, and the second is the release of *N*-acetylglucosamine from the intermediate product, leaving the phosphate behind on the mannose residue.

- I-cell (inclusion cell) disease is a rare condition in which lysosomal enzymes lack the **mannose phosphate marker** that targets them to lysosomes. The enzyme that is deficient in I-cell disease is a phosphotransferase located in the Golgi apparatus.
- The **phosphotransferase** has the unique ability to recognize lysosomal proteins because of their three-dimensional structure, such that they can all be appropriately tagged for transport to the lysosomes.
- Consequently, as a result of the lack of mannose phosphate, **lysosomal enzymes** are secreted from the cells. Because lysosomes lack their normal complement of enzymes, undegraded molecules accumulate within membranes inside these cells, forming **inclusion bodies**.

Galactocerebroside



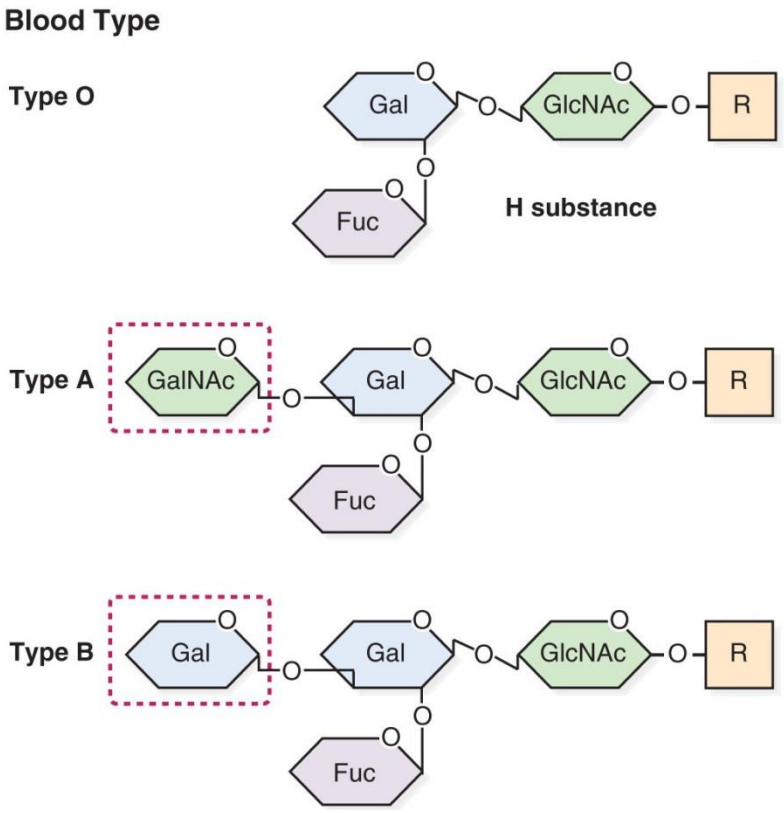
Ganglioside



Ceramide

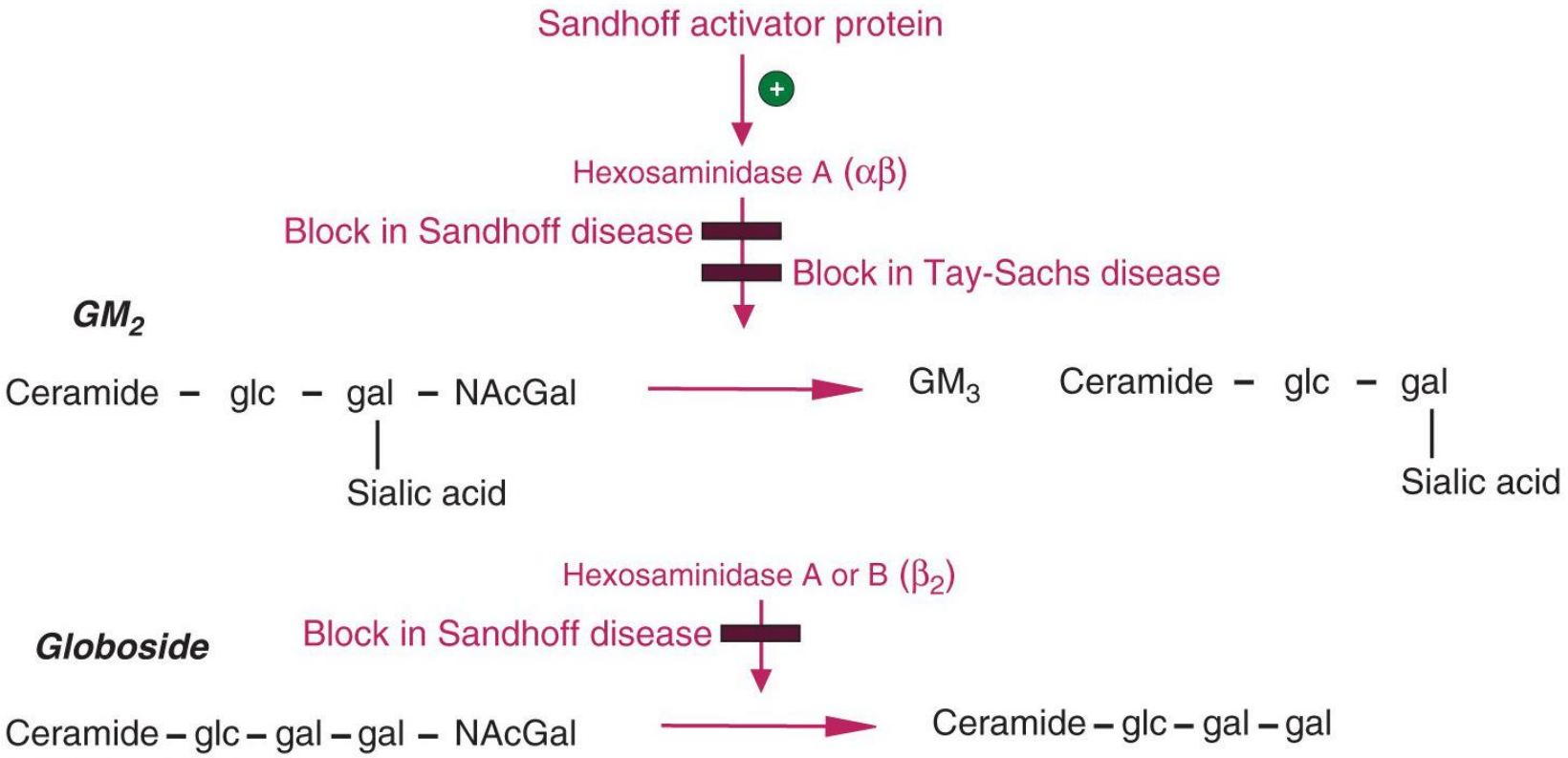
Structures of cerebrosides and gangliosides. n these glycolipids, sugars are attached to ceramide (shown below the glycolipids). The *boxed portion* of ceramide is sphingosine, from which the name “sphingolipids” is derived.

- **Glycolipids** are derivatives of the lipid sphingosine. These sphingolipids include the **cerebrosides** and the **gangliosides**. They contain **ceramide**, with carbohydrate moieties attached to its hydroxymethyl group.



Structures of the blood group substances. Note that these structures are the same except that type A has *N*-acetylgalactosamine (*GalNAc*) at the nonreducing end, type B has galactose (*Gal*), and type O has neither. *R* is either a protein or the lipid ceramide. Each antigenic determinant is boxed. *Fuc*, fucose; *GlcNAc*, *N*-acetylglucosamine; *Gal*, galactose.

- **Glycolipids** are involved in **intercellular communication**. Oligosaccharides of identical composition are present in both the glycolipids and glycoproteins associated with the cell membrane, where they serve as cell recognition factors. For example, carbohydrate residues in these oligosaccharides are the antigens of the **ABO blood group substances**.



Substrate specificities of hexosaminidase A, B, and the function of the activator protein.
Defects in the -subunit inactivate both HexA and HexB activities, leading to G_{M2} and globoside accumulation. A defect in Sandhoff activator protein also leads to G_{M2} accumulation, as HexA activity is reduced. Defects in the -subunit inactivate only HexA activity, such that HexB activity toward globoside is unaffected. *Glc*, glucose; *Gal*, galactose; *NAcGal*, *N*-acetylgalactosamine.

- **Hexosaminidase A**, the defective enzyme in **Tay-Sachs disease**, is actually composed of two subunits, an α- and a β-chain. The stoichiometry is currently considered to be **αβ (a dimer)**. The α-subunit is coded for by the ***HexA* gene**, whereas the β-subunit is coded for by the ***HexB* gene**.
- In Tay-Sachs disease, the α-subunit is defective, and hexosaminidase A activity is lost. However, the β-subunit can form active dimers in the absence of the α-subunit, and this activity, named **hexosaminidase B**, which cleaves the glycolipid globoside, retains activity in children with Tay-Sachs disease. Thus, children with Tay-Sachs disease accumulate the **ganglioside GM_2** but not globoside.
- **Mutation of the *HexB* gene**, and production of a defective β-subunit, leads to inactivation of both hexosaminidase A and B activity. Such a mutation leads to Sandhoff disease. Both activities are lost because both activities require a functional β-subunit. The clinical course of this disease is similar to Tay-Sachs but with an accelerated timetable because of the initial accumulation of both GM_2 and globoside in the lysosomes.
- A third type of mutation also can lead to disease symptoms similar to those of Tay-Sachs disease. Children were identified with Tay-Sachs symptoms, but when both hexosaminidase A and B activities were measured in a test tube, they were normal. This disease, ultimately named **Sandhoff activator disease**, is caused by a mutation in a protein that is needed to activate **hexosaminidase A** activity. In the absence of the activator, hexosaminidase A activity is minimal, and GM2 initially accumulates in lysosomes. This mutation has no effect on hexosaminidase B activity.