The pentose phosphate pathway. Metabolism of fructose and galactose. The uronic acid pathway. The synthesis of amino sugars and glycosyl donors in glycoprotein synthesis.

Significance of pentose phosphate pathway

- source of NADPH (reductive syntheses, oxygenases with mixed function, reduction of glutathion)
- as a source of ribose-5-P (nucleic acids, nucleotides)
- metabolic use of five carbon sugars obtained from the diet

No ATP is directly consumed or produced

The pentose phosphate pathway

(Hexose monophosphate shunt)

Tissue location:

liver, adipose tissue (up to 50% of glucose metab.), erythrocytes, adrenal gland, mammary gland, testes, ovary etc.

(generally tissues, where the reductive syntheses or hydroxylations catalyzed by monooxygenases occur)

The other tissues use only some reactions of pentose phosphate pathway

Cell location: cytoplasma

Two phases of pentose phosphate pathway

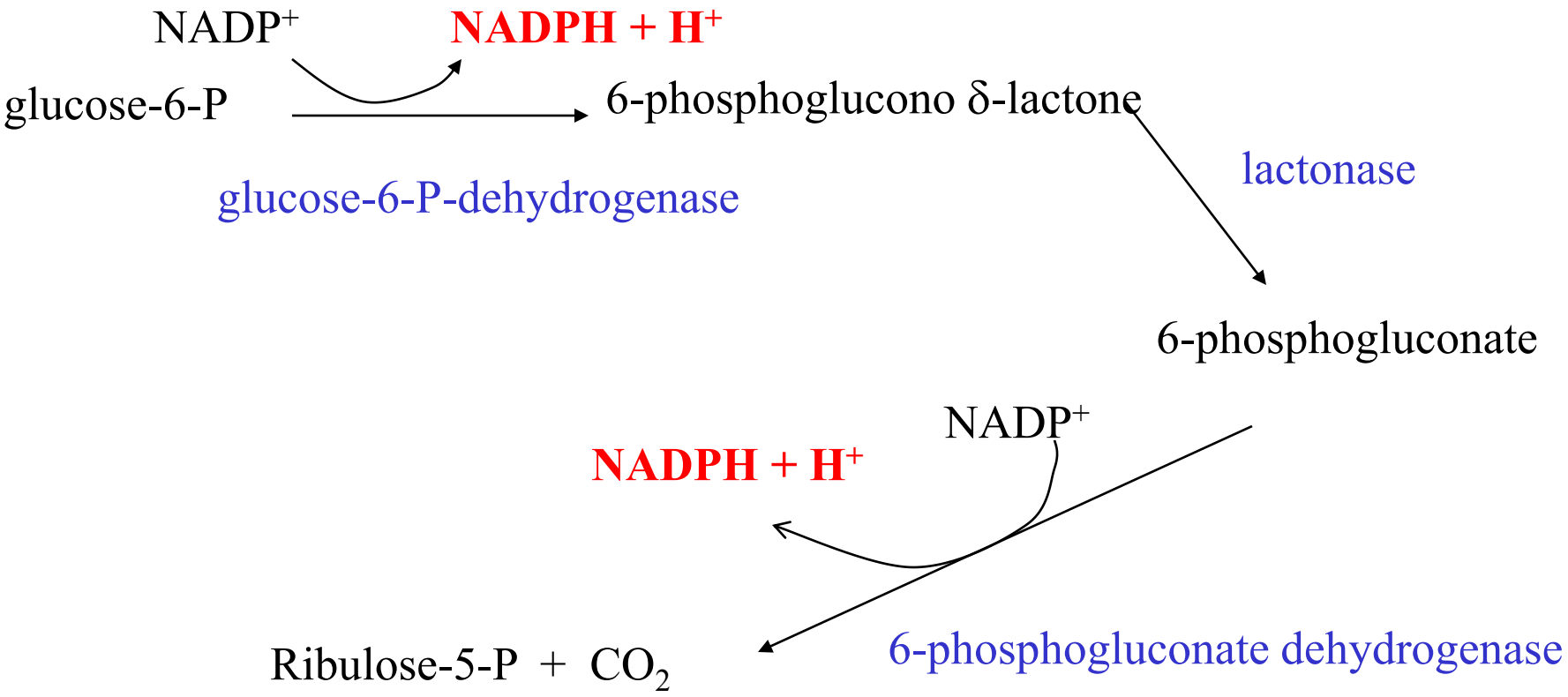
Oxidative phase **irreversible reactions**

synthesis of NADPH and pentoses

Nonoxidative (interconversion) phase reversible reactions

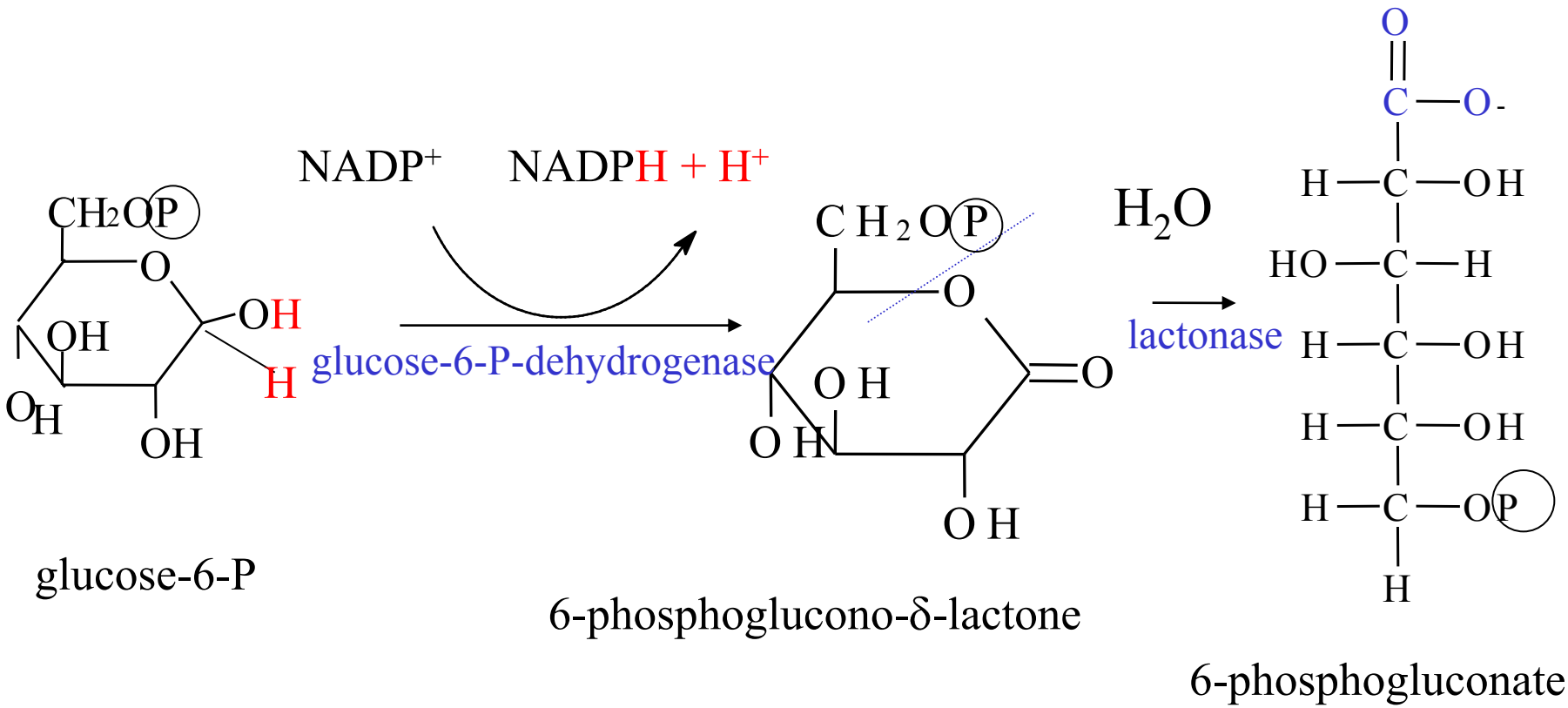
conversion of remaining pentoses to glucose

Oxidative part of pentose phosphate pathway

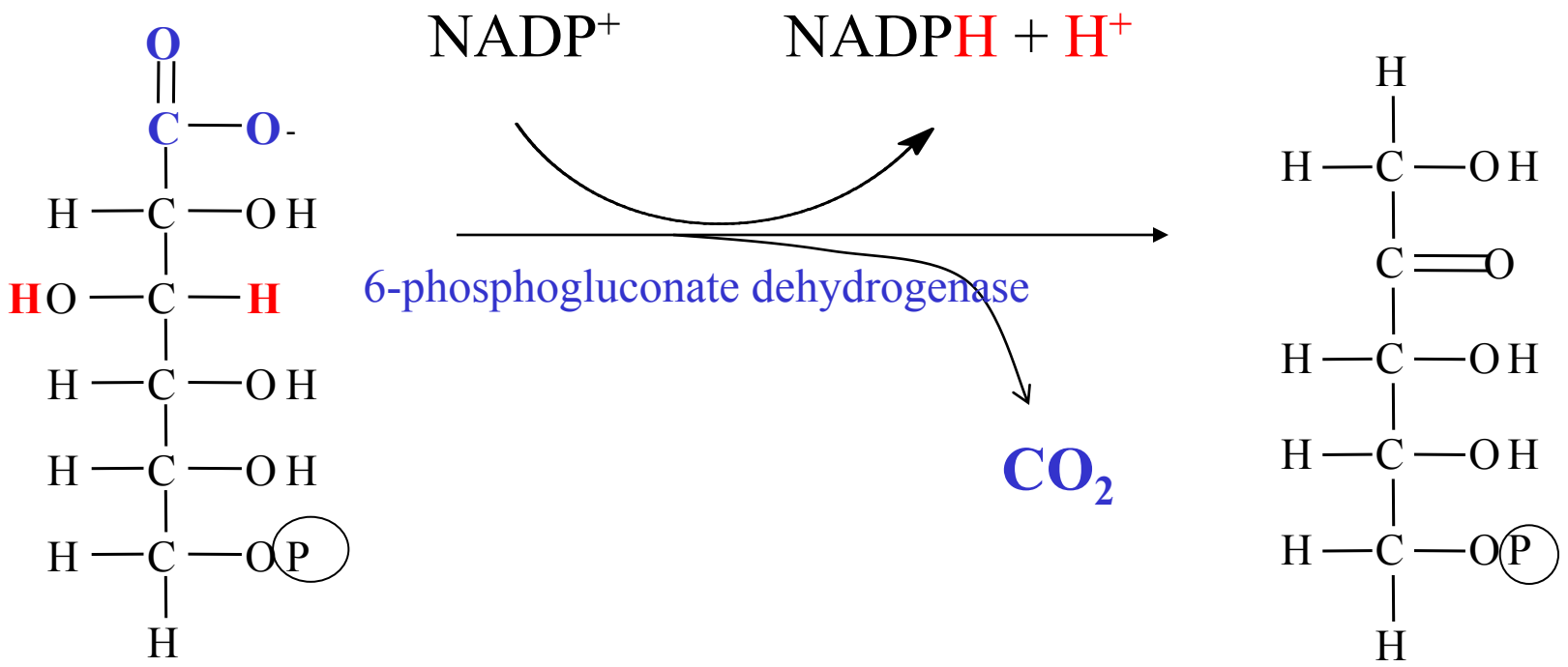


Glucose 6-phosphate dehydrogenase is the regulated key enzyme of the pathway.
Factors affecting the reaction:
inhibition by NADPH
Availability of $NADP^+$
Induction of the enzyme by insuline

Oxidative part of pentose phosphate pathway with structural formulas – formation of 6-phosphogluconate



Oxidative part of pentose phosphate pathway with structural formulas – conversion of 6-phosphogluconate



6-phosphogluconate

ribulose-5-P

The yield of oxidative phase of pentose phosphate pathway:

2 mols of NADPH

1 mol of pentose phosphate

Reversible nonoxidative reactions of pentose phosphate pathwayy

Summary equation:

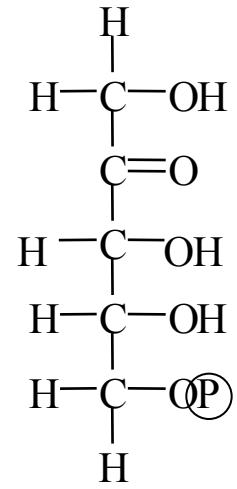
3 Ribulose-5-P $\rightleftharpoons$ 2 fructose-6-P + Glyceraldehyde-3-P

What is the significance of this phase?

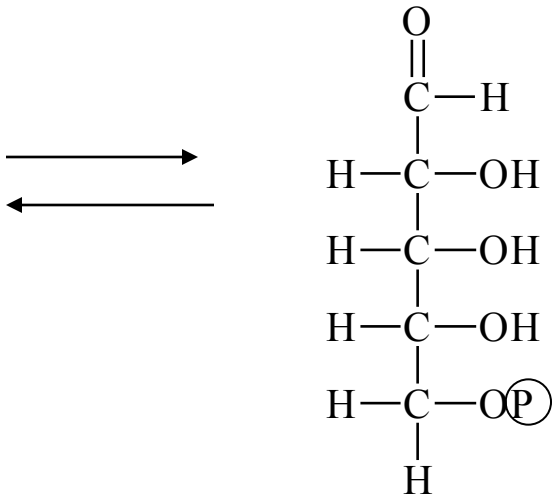
Some cells require many NADPH. Its production in oxidative phase is associated with formation of large amount of pentoses, that the cell does not need. The pentoses are converted to fructose-6-phosphate and glyceraldehyde-3-P that are inermediates of glycolysis.

Enzymes in reversible phase of pentose phosphate pathway

Isomerase



Ribulose-5-P

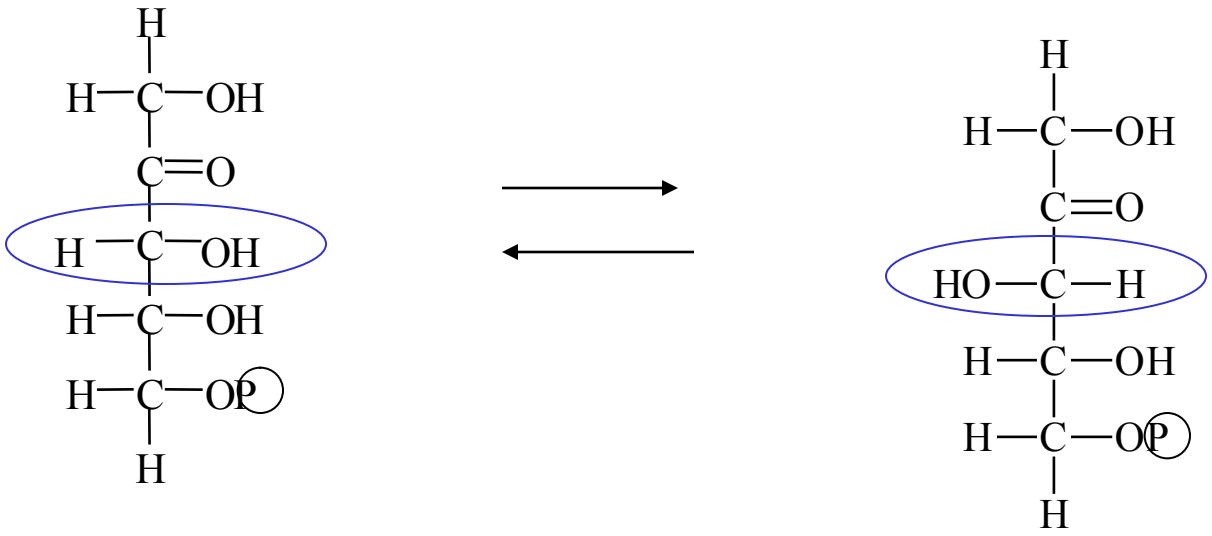


Ribose-5-P

Synthesis of nucleotides and nucleic acids

Reactions of nonoxidative phase of pentose phosphate pathway

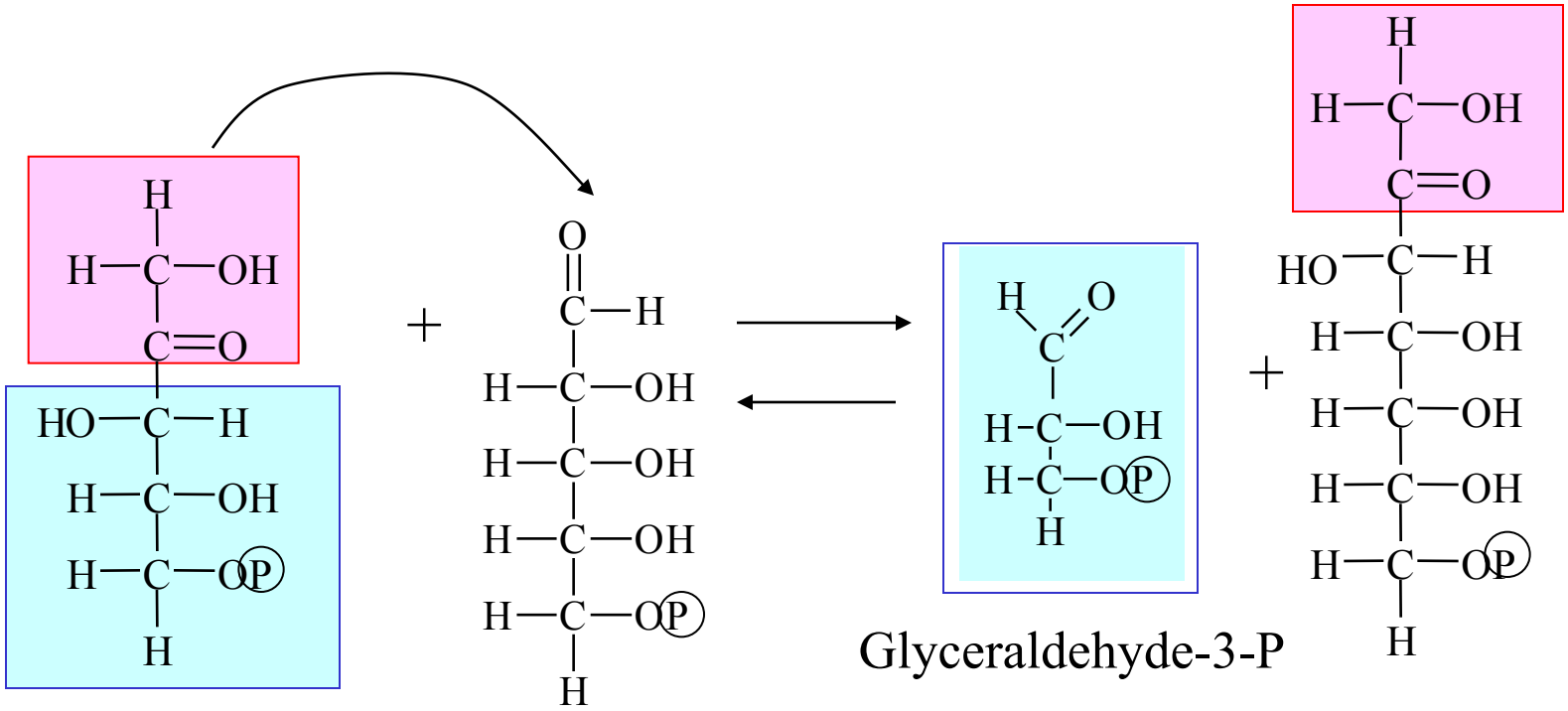
Epimerase



Ribulose-5-P

Xylulose-5-P

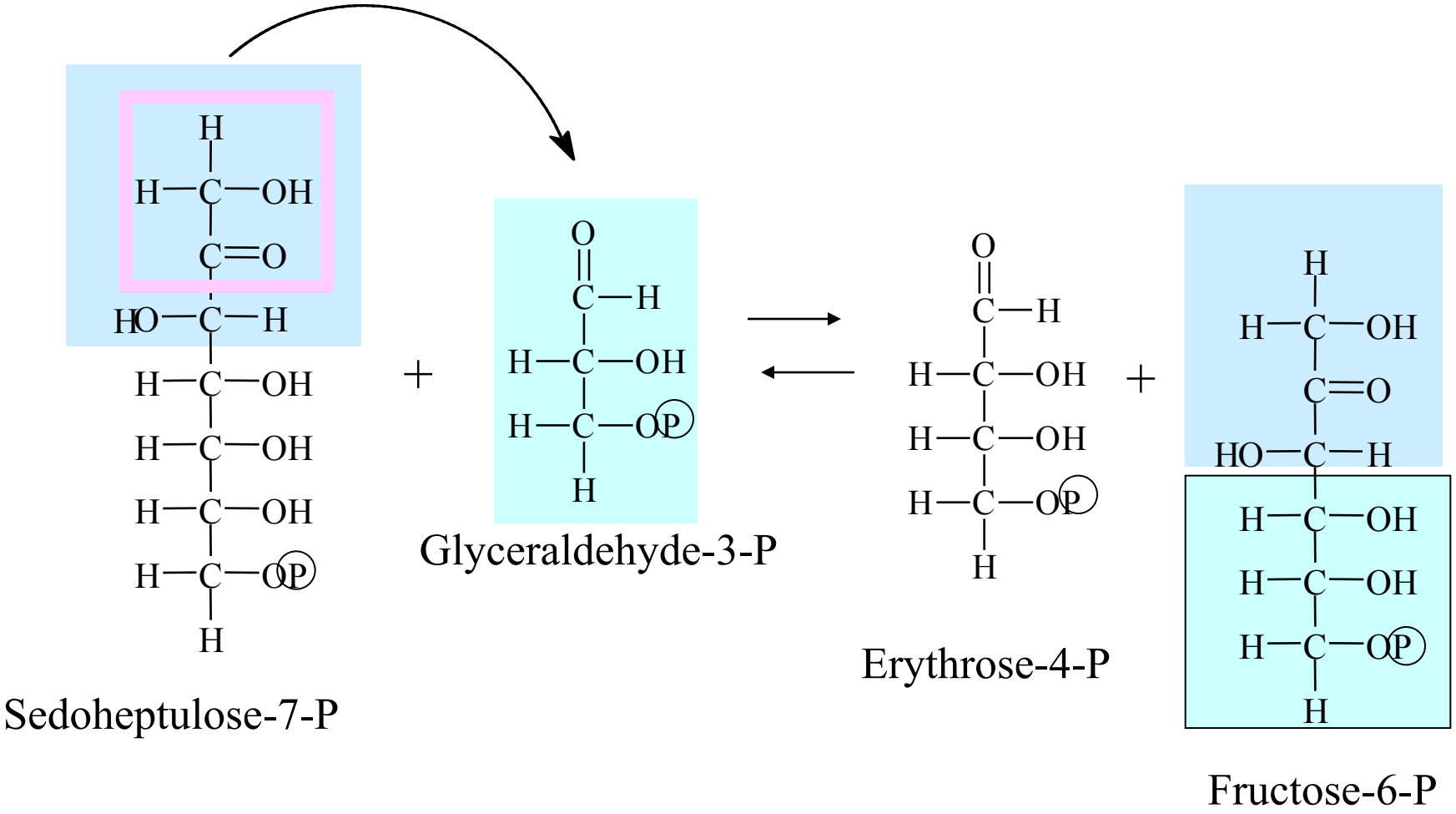
Transketolase – it transfers two-carbon units



5C + 5C ⇄ 3C + 7C

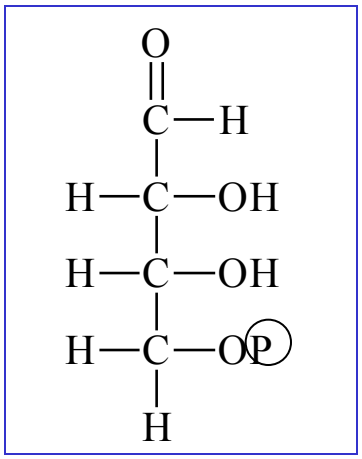
Prostetic group of transketolase: thiamine diphosphate

Transaldolase – it transfers three-carbon units



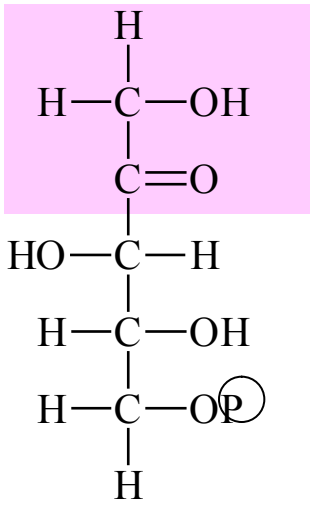
7C + 3C ⇄ 4C + 6C

Transketolase – it transfers two-carbon units

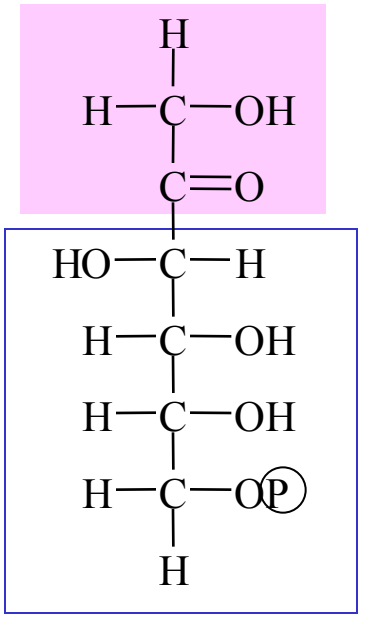


Erythrose-4-P

+

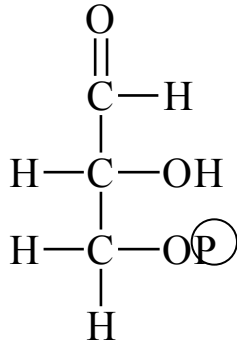


Xylulose -5-P



Fructose-6-P

+



Glyceraldehyde-3-P

4C + 5C ⇄ 6C + 3C

The summary of pentose phosphate pathway

Ribulose-5-P ⇌ Ribose -5-P

2 Ribulose-5-P ⇌ 2 Xylulose -5-P

Xylu-5-P + Rib-5-P ⇌ Glyc-3-P + Sed-7-P

Sed-7-P + Glyc-3-P ⇌ Ery-4-P + Fru-6-P

Xylu-5-P + Ery-4-P ⇌ Glyc-3-P + Fru-6-P

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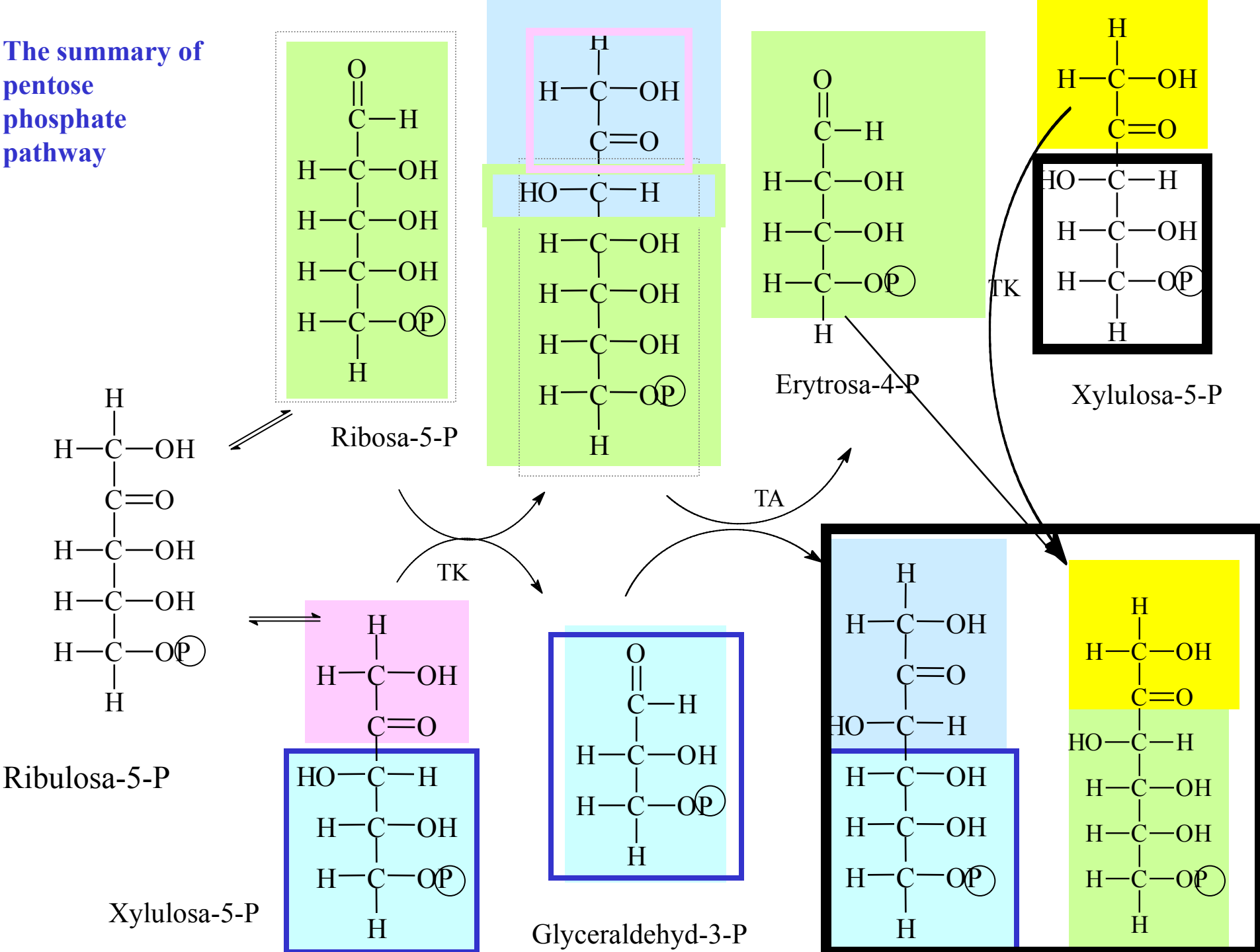
3 Ribulose-5-P ⇌ Glyceraldehyde-3-P + 2 Fru-6-P

3 x 5C ⇌ 3C + 2 x 6C

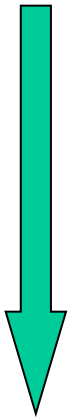
The summary of pentose phosphate pathway



Generation of ribose phosphate from intermediates of glycolysis

The reactions of nonoxidative phase are reversible.

This enables that ribose-5-phosphate can be generated from intermediates of glycolytic pathway in case when the demand for ribose for incorporation into nucleotides and nucleic acids is greater than the need for NADPH.



Transketolase reaction in opposite direction

fructose-6-P + glyceraldehyde-3-P ⟷ erytrosa-4-P + xylulosa-5-P

(from glycolysis)

Transaldolase reaction in opposite direction

erytrose-4-P + fructose-6-P⟷ sedoheptulose-7-P

+ glyceraldehyde-3-P

(from glycolysis)

Transketolase reaction in opposite direction

sedoheptulose-7-P + glyceraldehyde-3-P ⟷ 2 pentose phosphates

Cellular needs dictate the direction of pentose phosphate pathway

Cellular need	Direction of pathway
NADPH only	Oxidative reactions produce NADPH, nonoxidative reactions convert ribulose 5-P to glucose 6-P to produce more NADPH
NADPH + ribose-5-P	Oxidative reactions produce NADPH and ribulose 5-P, the isomerase converts ribulose 5-P to ribose 5-P
Ribosa-5-P only	Only the nonoxidative reactions. High NADPH inhibits glucose 6-P dehydrogenase, so transketolase and transaldolase are used to convert fructose 6-P and glyceraldehyde 3-P to ribose 5-P
NADPH and pyruvate	Both the oxidative and nonoxidative reactions are used. The oxidative reactions generate NADPH and ribulose 5-P, the nonoxidative reactions convert the ribulose 5-P to fructose 5-P and glyceraldehyde 3-P, and glycolysis converts these intermediates to pyruvate

Most important reactions using NADPH

- reduction of oxidized glutathion
- monooxygenase reactions with cytP450
- respiratory burst in leukocytes
- reductive synthesis:

  synthesis of fatty acids

  elongation of fatty acids

  cholesterol synthesis

  nucleotide synthesis

  NO synthesis from arginine

NADH x NADPH / comparision

Characteristics	**NADH**	**NADPH**
formation	Mainly in dehydrogenation reactions of substrates in catabolic processes	In dehydrogenation reactions other than catabolic
utilization	Mainly respiratory chain*	Reductive synthesis and detoxication reactions Cannot be oxidized in resp. chain
Form that is prevailing in the cell	NAD^+	NADH

* Transhydrogenase in mitochondrial membrane can catalyze transfer of H from NADH to $NADP^+$

Significance of pentose phosphate pathway for red blood cells

Pentose phosphate pathway is the only source of NADPH for erc

It consumes about 5-10% of glucose in erc

NADPH is necessary for maintenance of reduced glutathione pool

$$GS\text{-}SG + NADPH + H^{+} \xrightarrow[\text{glutathionreductase}]{} 2GSH + NADP^{+}$$

Oxidized form of glutathione is generated during the degradation of hydrogen peroxide and organic peroxides in red blood cells

glutathionperoxidase

$$2GSH + HO\text{-}OH \rightarrow GSSG + 2H_2O$$

$$2GSH + ROOH \rightarrow GSSG + ROH + H_2O$$

Accumulation of peroxides in the cell triggers the haemolysis

Deficiency of glucose 6-P dehydrogenase in red blood cells

Inherited disease

It is caused by point mutations of the gene for glucose 6-P dehydrogenase in chromosome X in some populations (400 different mutations)

More than 400 milions of individuals worldwide

Erythrocytes suffer from the lack of reduced glutathione

Most individuals with the disease do not show clinical manifestations. Some patients develop hemolytic anemia if they are treated with an oxidant grug, ingest favabeans or contract a severe infetion (*AAA)

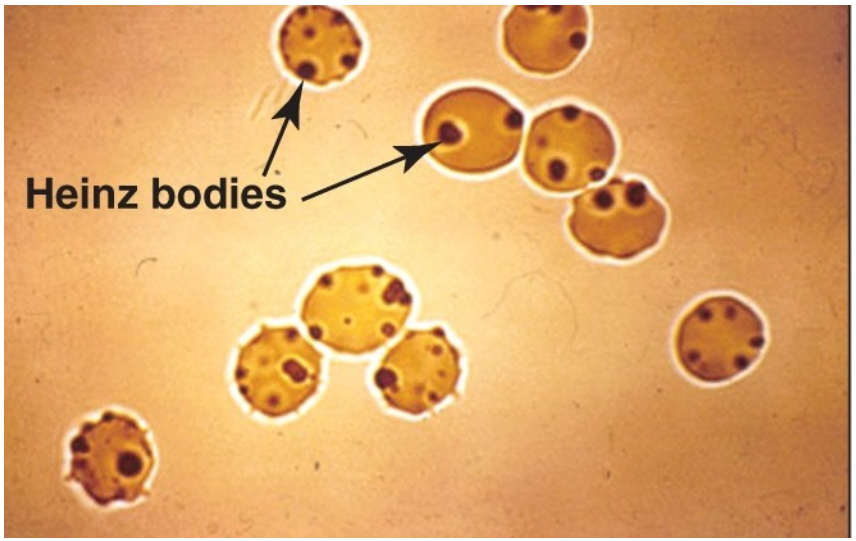
The highest prevalence in the Middle East, tropical Afrika and Asia, parts of Mediterranean

AAA* - antimalarials, antibiotics, antipyretics

Heinz bodies are present in red blood cells with glucose-6-P-dehydrogenase deficience

Deficiency of reduced glutathion results in protein damage – oxidation of sulfhydryl groups in proteins leads to the formation of denaturated proteins that form insoluble masses (Heinz bodies)

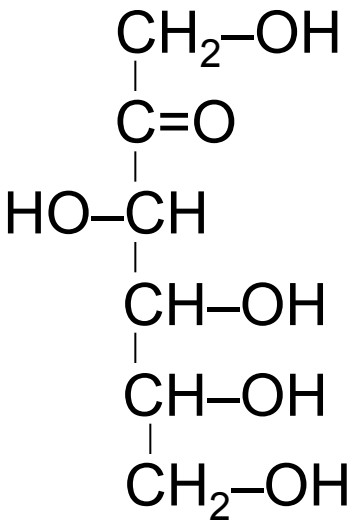
Erytrocytes are rigid and nondeformable – they are removed from circulation by macrophages in spleen and liver.

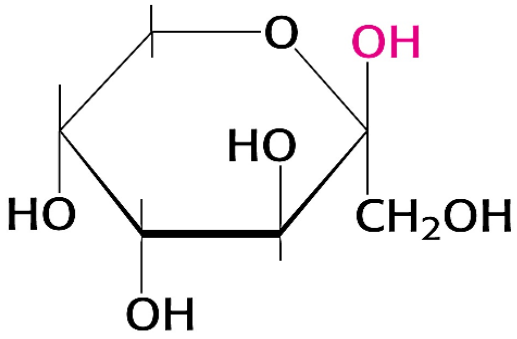


Favism

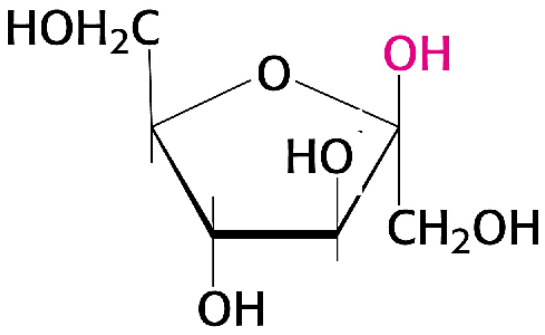
Some people with GHPD deficiency are susceptible to the fava bean (Vicia fava). Eating them results in hemolysis.

Metabolism of fructose





β-D-Fructopyranose



β-D-Fructofuranose

Sources of fructose

Source fructose: sucrose from diet, fruits, honey, high fructose corn syrup*

For thousands of years humans consumed fructose amounting to 16–20 grams per day, largely from fresh fruits. Westernization of diets has resulted in significant increases in added fructose, leading to typical daily consumptions amounting to 85–100 grams of fructose per day.

Fructose enters most of the cells by facilitated diffusion on the GLUT V

* High-fructose corn syrup is used as a sweetener in many soft drinks, yogurts, saladd dressings etc.

Obesity and high intake of HFCS

High-fructose corn syrup (commonly abbreviated HFCS) is a sweetening food ingredient produced by adding enzymes to corn syrup, which is mostly glucose, to create fructose. The result is a cheaper alternative to sugar that also functions as a preservative. As such, high fructose corn syrup is a common ingredient in a variety of foods,

HFCS is in nearly everything: jelly, juice, sodas, whole-grain breads, cereals, ketchup, crackers, yogurt, sweet pickles, applesauce, salad dressing, ice cream, cough syrup and lots more.

The biggest problem is that HFCS is being added to food items that don't normally have sugar and that you wouldn't even describe as sweet -- crackers, for instance. So, not only are we chugging down lots of sugars with our sodas, but your PBJ sandwich could have HFCS in each of its three ingredients. Meal after meal, day after day, all of this extra sugar adds up, and that, and not necessarily the qualities of HFCS itself, is likely one reason why rates for obesity and diabetes have climbed since the introduction of HFCS.

Probably, the increase in consumption of HFCS has a temporal relation to the epidemic of obesity, and the overconsumption of HFCS in calorically sweetened beverages may play a role in the epidemic of obesity.

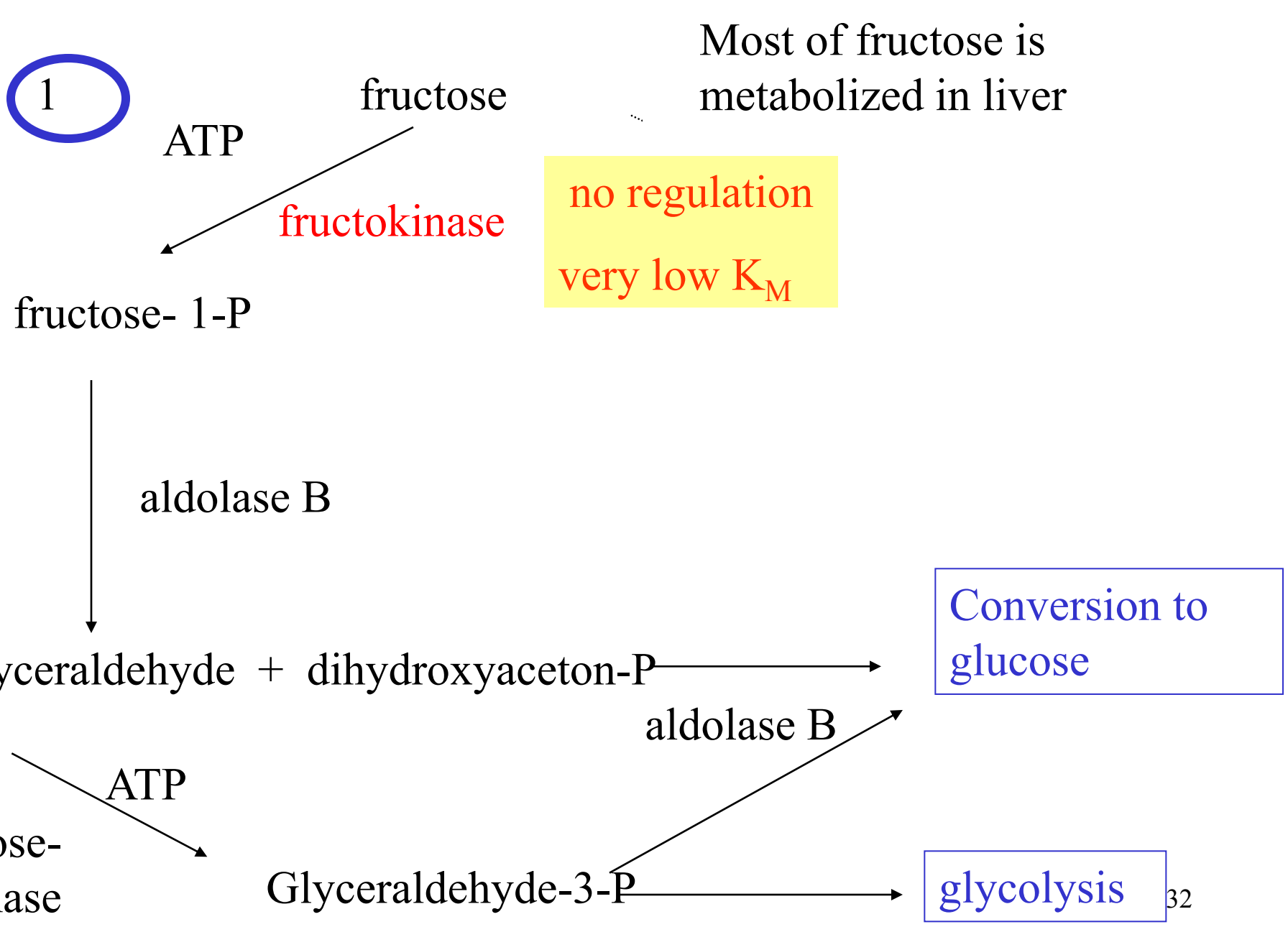
Fructose and glucose – comparison of metabolic features

	glucose	fructose
Intestinal absorption	rapid	slower
Metabolism	slower	more rapid
Half-life in blood	43 min	18 min
Place of metabolism	Most of tissues	mainly liver, kidneys, enterocytes
K_M for hexokinase	0,1 mmol/l	3 mmol/l
K_M pro fructokinase	-	0,5 mmol/l
Effect on insulin release	↑	no

Important differences between metabolism of glucose and fructose

- fructose is metabolized mainly in liver by fructokinase
- hexokinase phosphorylates fructose only when its concentration is high
- fructose is metabolized more rapidly then fructose in the liver
- fructose do not stimulate release of insulin
- hepatic metabolism of fructose favors de novo lipogenesis.

Metabolismus of fructose



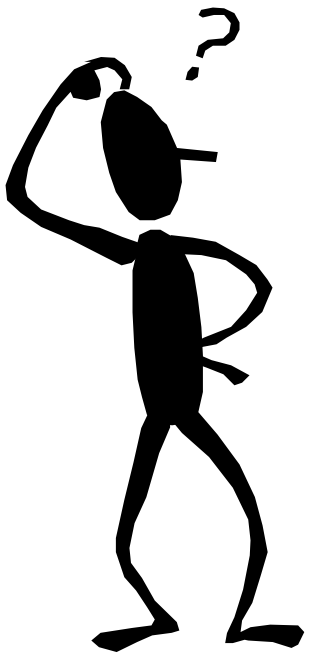
Aldolase A a aldolase B

- isoenzymes (also aldolase C is known)
- aldolase A : glycolysis (cleavage of Fru 1,6-bisP)
- aldolase B: cleavage of fructose1-P

  gluconeogenesis (synthesis of Fru-1,6-bisP)

Fructose is very rapidly metabolised in comparison with glucose.

Why ?



Metabolism of fructose

fructokinase and aldolase B (liver):

metabolismus bypasses the regulated enzymes, fructose can *continuously* enter the glycolytic pathway

⇒ rapid degradation

☺ fructose is rapid, on insulin independent source of energy

☹ high intake of fructose results in increased production of fatty acids and consequently increased production of triacylglycerols

☹ at very high fructose intake, phosphate is sequestrated in fructose -1-phosphate and synthesis of ATP is diminished

fructose alone spikes blood sugar fairly slowly, high fructose corn syrup raises blood sugar levels rapidly. One of the main reasons that fructose alone does not raise blood sugar levels quickly, and therefore, is often encouraged for diabetics is that it is often eaten in its natural form in fruits. Fruits also have fiber, which slows sugar absorption.

Fructose and diabetics

Fructose was formerly recommended as harmless sweetener replacing glucose in diabetics' diets

Current recommendations

- excessive consumption of fructose is not recommended

- a small amount of fructose, such as the amount found in most vegetables and fruits, is not a bad

Defects in metabolism of fructose

Lack of fructokinase

- essential fructosuria

fructose accumulates in blood and is excreted into the urine

Disease is without any serious consequences.

Fructose free diet.

Diagnostics: positive reduction test with urine

negativ result of specific test for glcose

Lack of aldolase B

- hereditary fructose intolerance (fructose poisoning)

Very serious for newborns

Fructose-1-P accumulates in the liver cells to such an extent that most of the **inorganic phosphate is removed from the cytosol**.

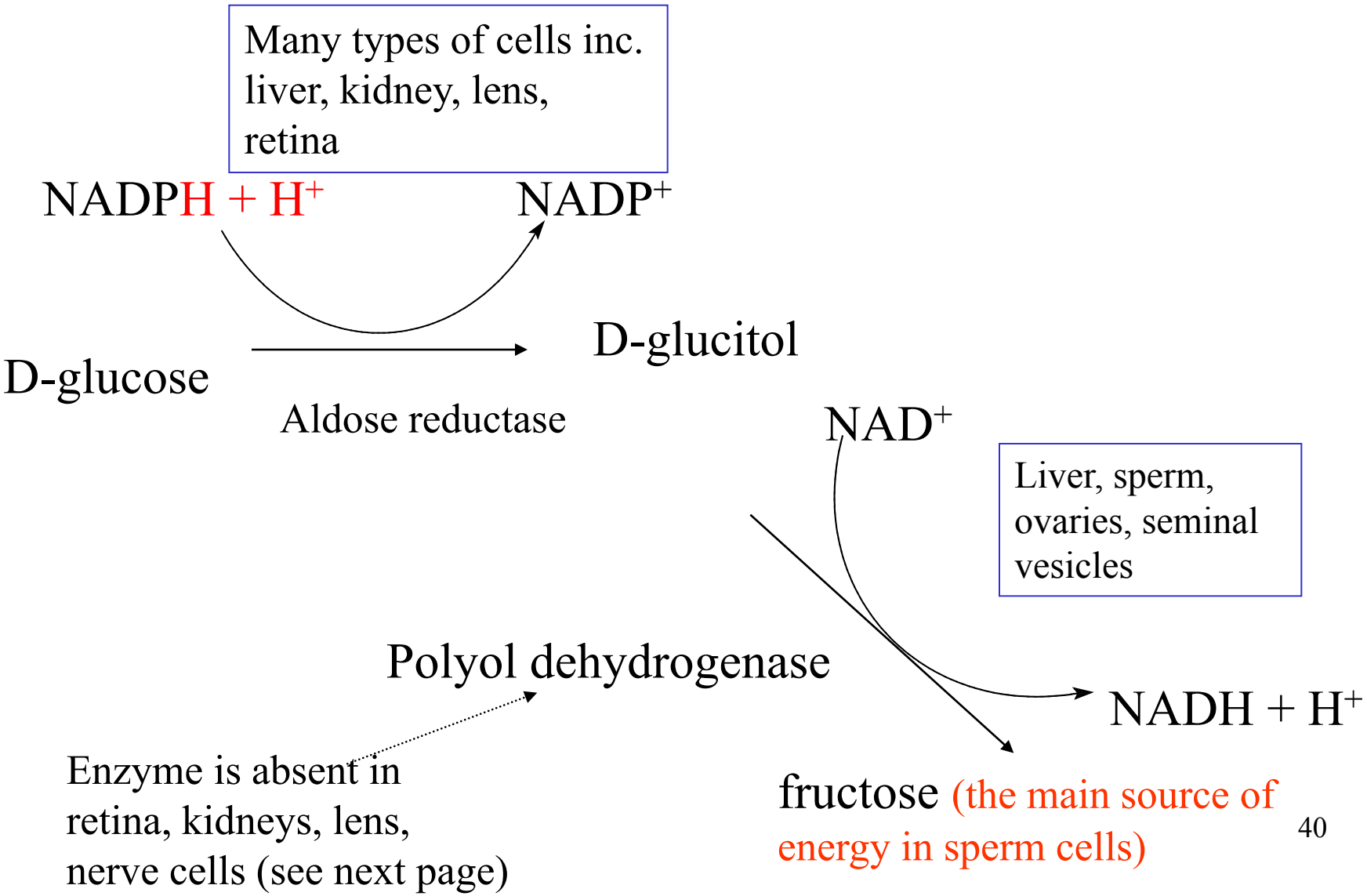
Phosphate is needed for function of glycogen phosphorylase, oxidative phosphorylation is inhibited and hypoglycaemia also appears (Fru-1-P inhibits both glycolysis and gluconeogenesis).

Symptoms are vomiting, hypoglycemia, jaudice, hepatomegaly.

Symptoms can be seen after a baby starts eating food or formula.

Treatment: the intake of fructose and sucrose must be restricted.

Synthesis of fructose in polyol pathway



Polyol metabolism in diabetics

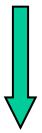
• If the blood concentration of glucose is very high (e.g. in *diabetes mellitus*), large amount of glucose enter the cells

• The polyol pathway produces glucitol.

•It cannot pass efficiently through cytoplasmic membrane

it remains „trapped“inside the cells

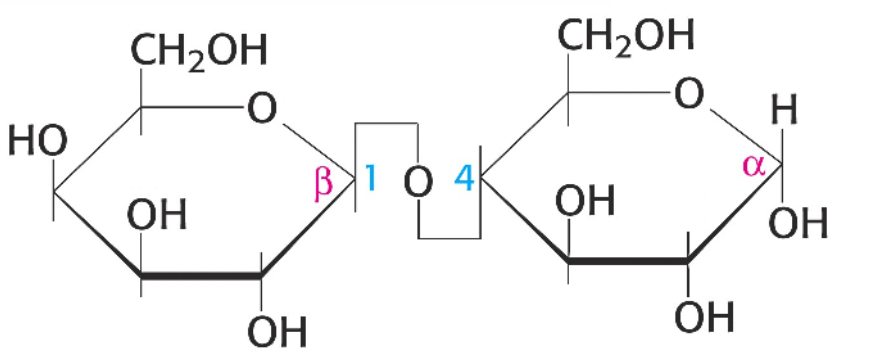
•When sorbitol dehydrogenase is absent (lens, retina, kidney, nerve cells), sorbitol cannot be converted to fructose and accumulates in the cell



•Some of the pathologic alterations of diabetes are attributed to this process (e.g. cataract formation, peripheral neuropathy, retinopathy and other)

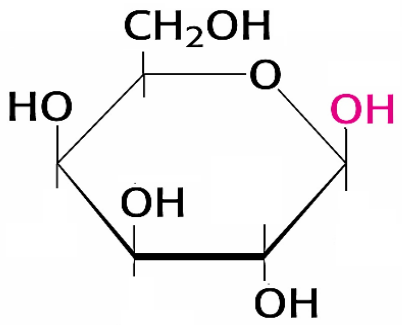
Metabolism of galactose

Galactose occurs as component of lactose in milk and in dairy products. Hydrolysis of lactose in the gut yields glucose and galactose.



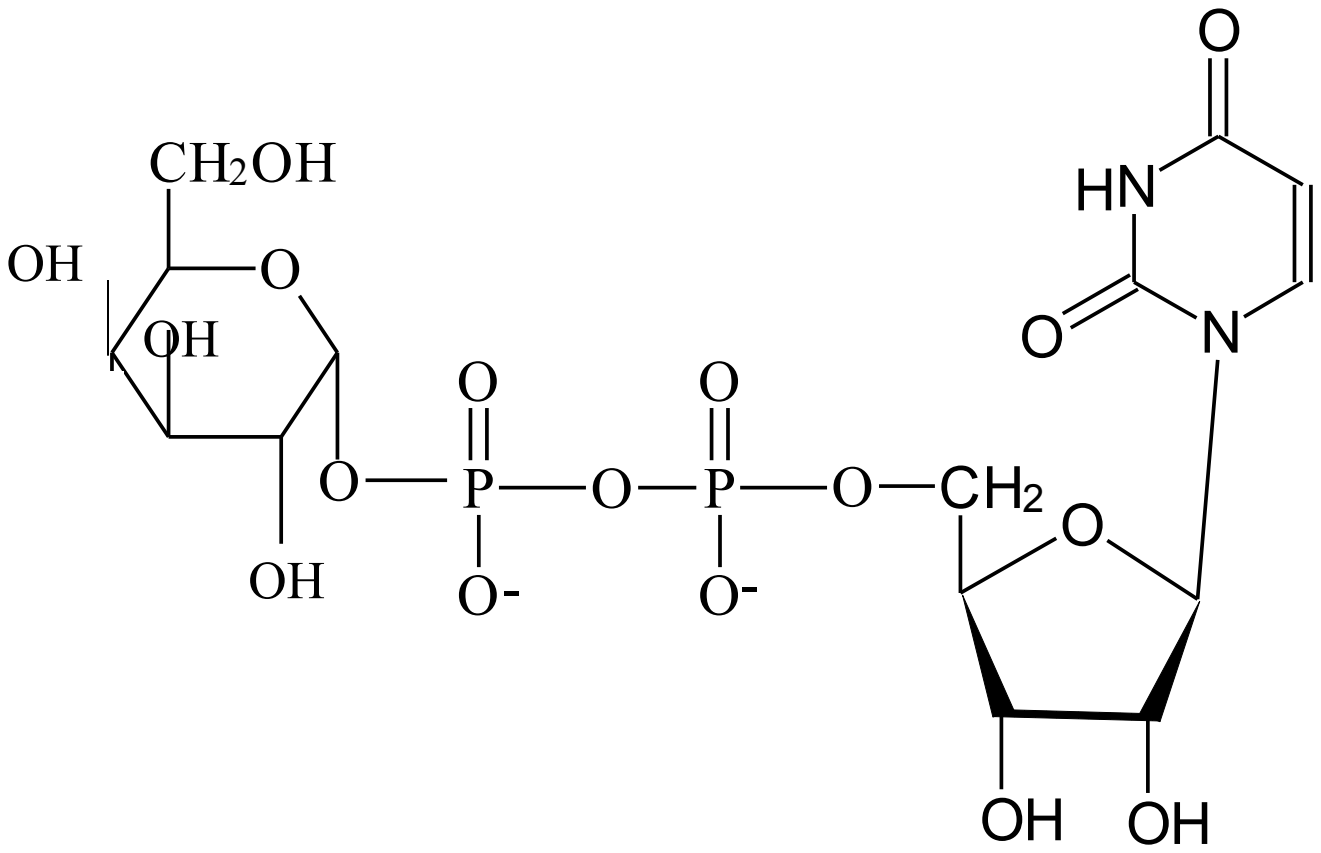
α-Lactose

β-D-Galactopyranosyl-(1→4)-α-D-glucopyranose



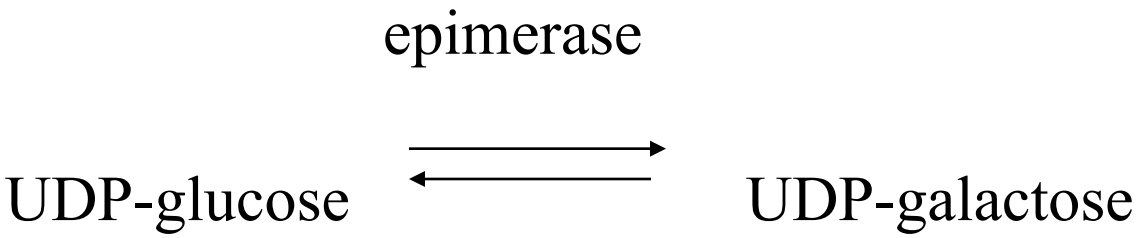
β-D-Galactopyranose

UDP-galactose (active form of galactose)



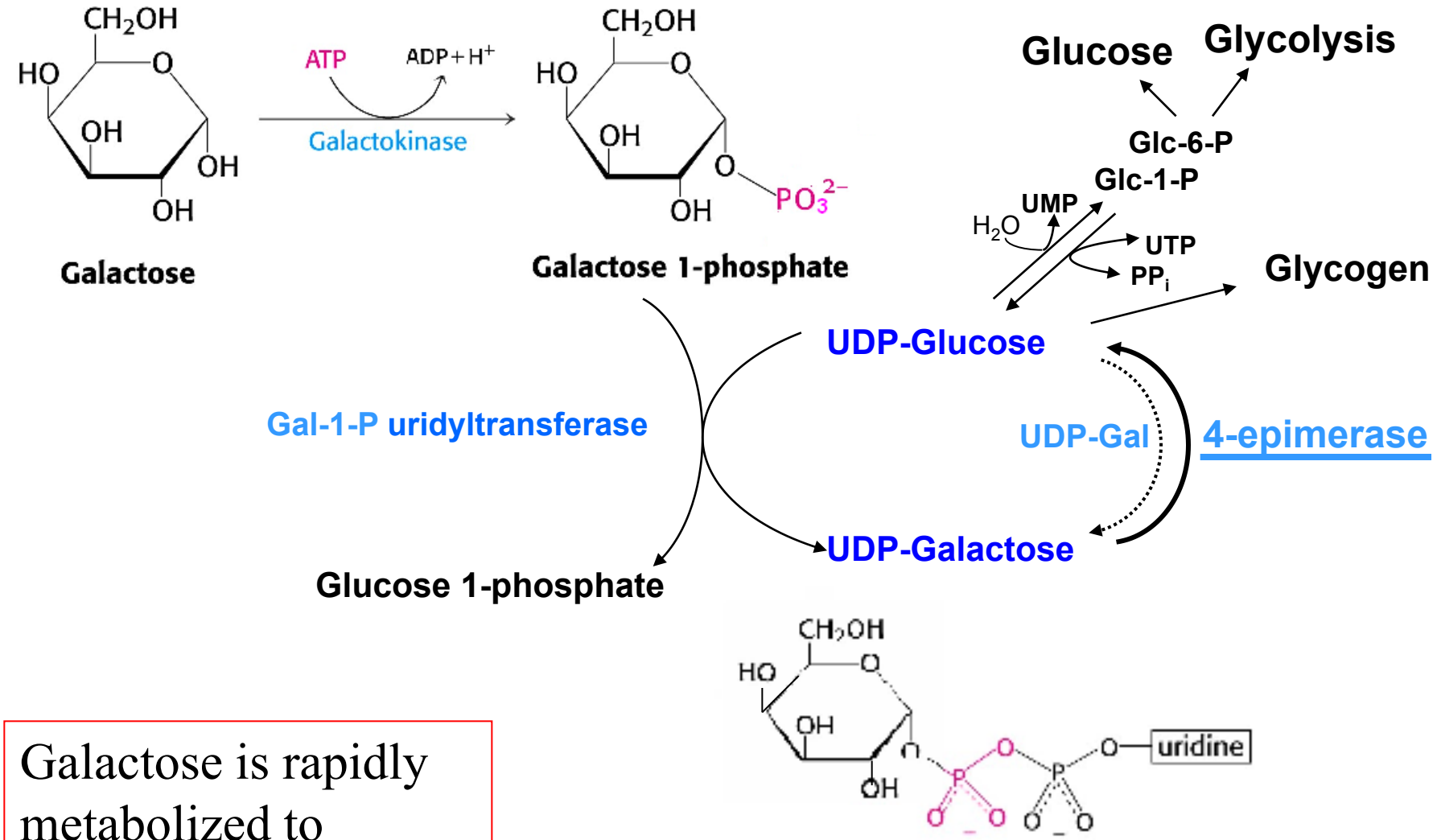
It is formed in reaction with UDP-glucose

Izomeration of glucose to galactose



reaction is reversible, can be used also for formation of glucose

Transformation of galactose into glucose in the liver



Galactose is rapidly metabolized to glucose

Utilization of galactose

- Synthesis of lactose
- Synthesis of glycolipids, proteoglycans and glycoproteins

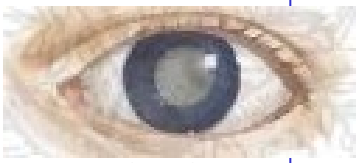
Galactosemia

•the hereditary deficiency of Gal-1-P uridyltransferase

•Acummulation of galactose-1-P

•Interferention with metabolism of phosphates and glucose

•Conversion of galactose to galactitol in lens – kataracta



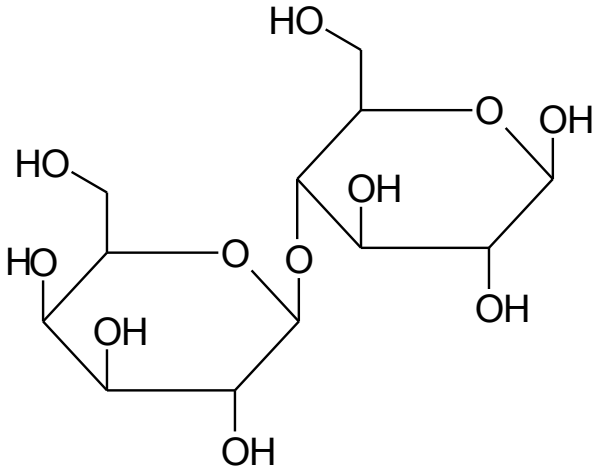
• Dangerous for newborns

•Non treated galactosemia leads to liver damage and retarded mental development

•Restriction of milk and milk-products in the diet

Biosynthesis of lactose

Unique for lactating mammary gland



UDP-galactose

glucose

Lactose synthase

Lactose (galactosyl-1,4-glucose)

Laktose synthase is a complex of two proteins:

- galactosyl transferase (present in many tissues)
- α-lactalbumin (present only in mammary gland during lactation, the synthesis is stimulated by hormone prolactin)

Metabolismus of galactose in other cells

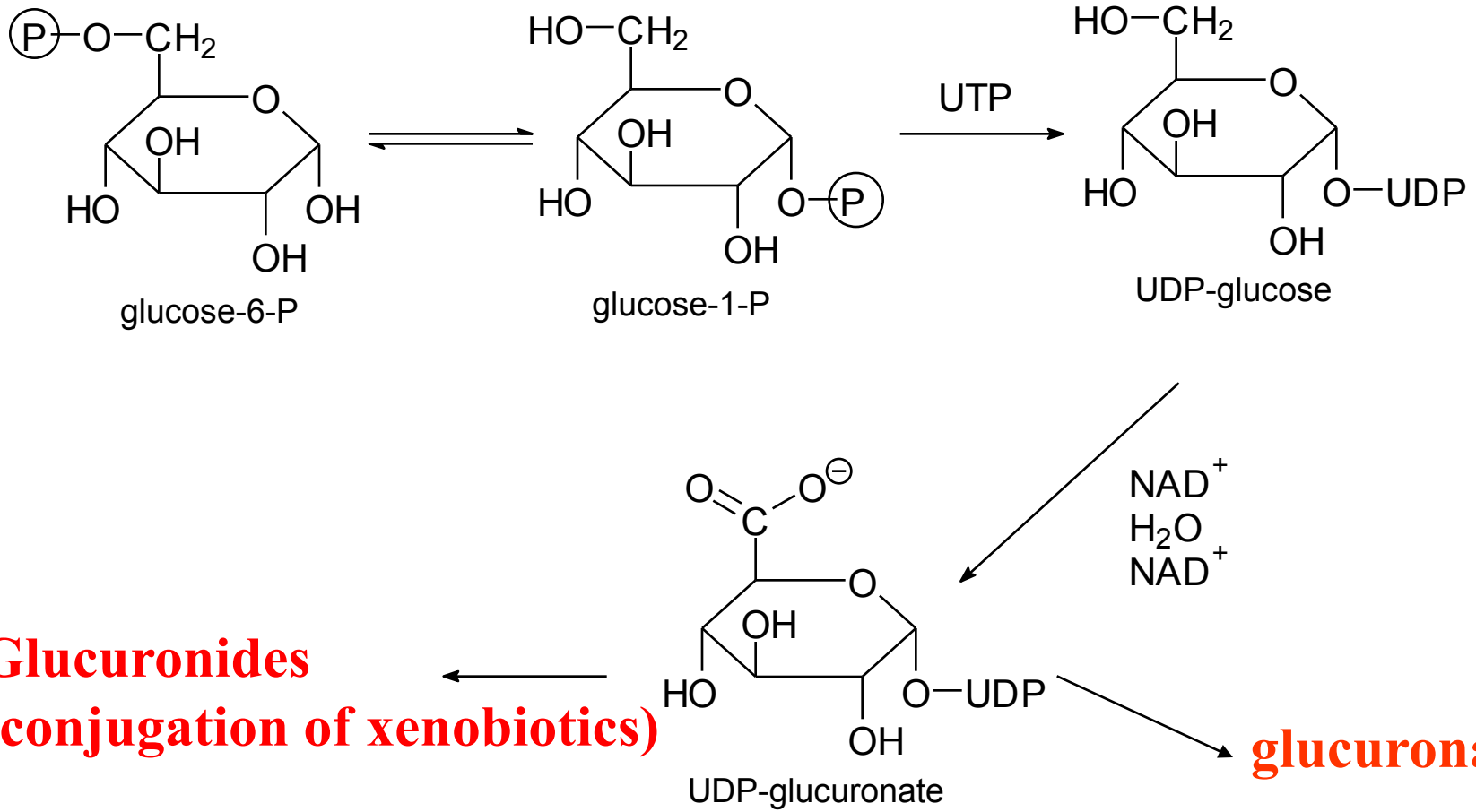
Galactose and *N*-acetylgalactosamine
are important constituents of
glycoproteins, proteoglycans, and glycolipids.

In the synthesis of those compounds **in all types of cells**, the galactosyl and *N*-acetylgalactosyl groups are transferred from UDP-galactose and UDP-*N*-acetyl-galactose by the action of **UDP-galactosyltransferase**.

The uronic acid pathway – synthesis and utilization of glucuronic acid

- An alternative oxidative pathway for glucose.
- It supplies **glucuronic acid**, and in most animals (not in humans, other primates, and guinea pigs) **ascorbic acid**.

Biosynthesis and utilization of UDP-glucuronate



Examples of compound degraded and excreted as urinary glucuronides

Estrogen

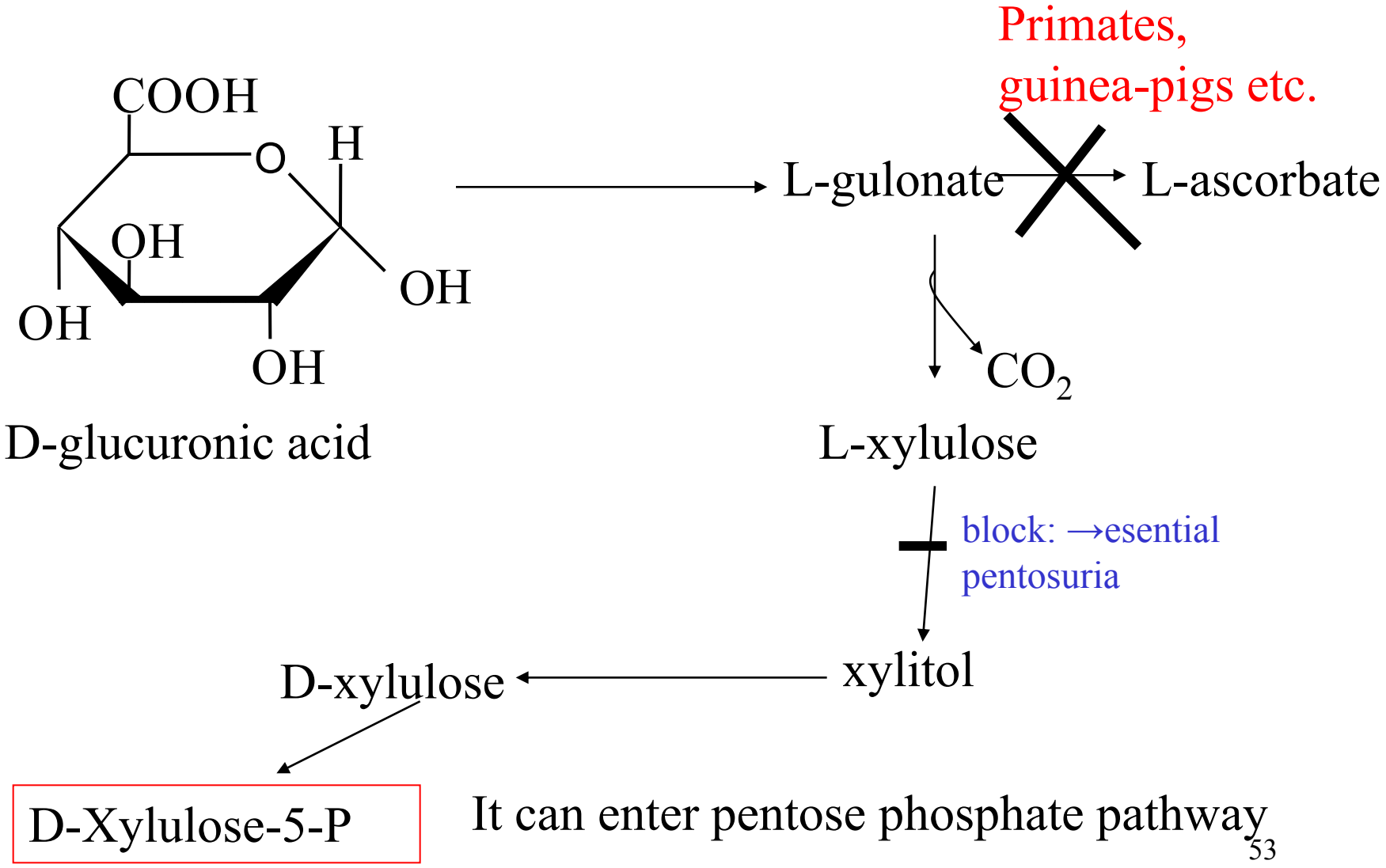
Bilirubine

Progesterone

Meprobamate

Morphine

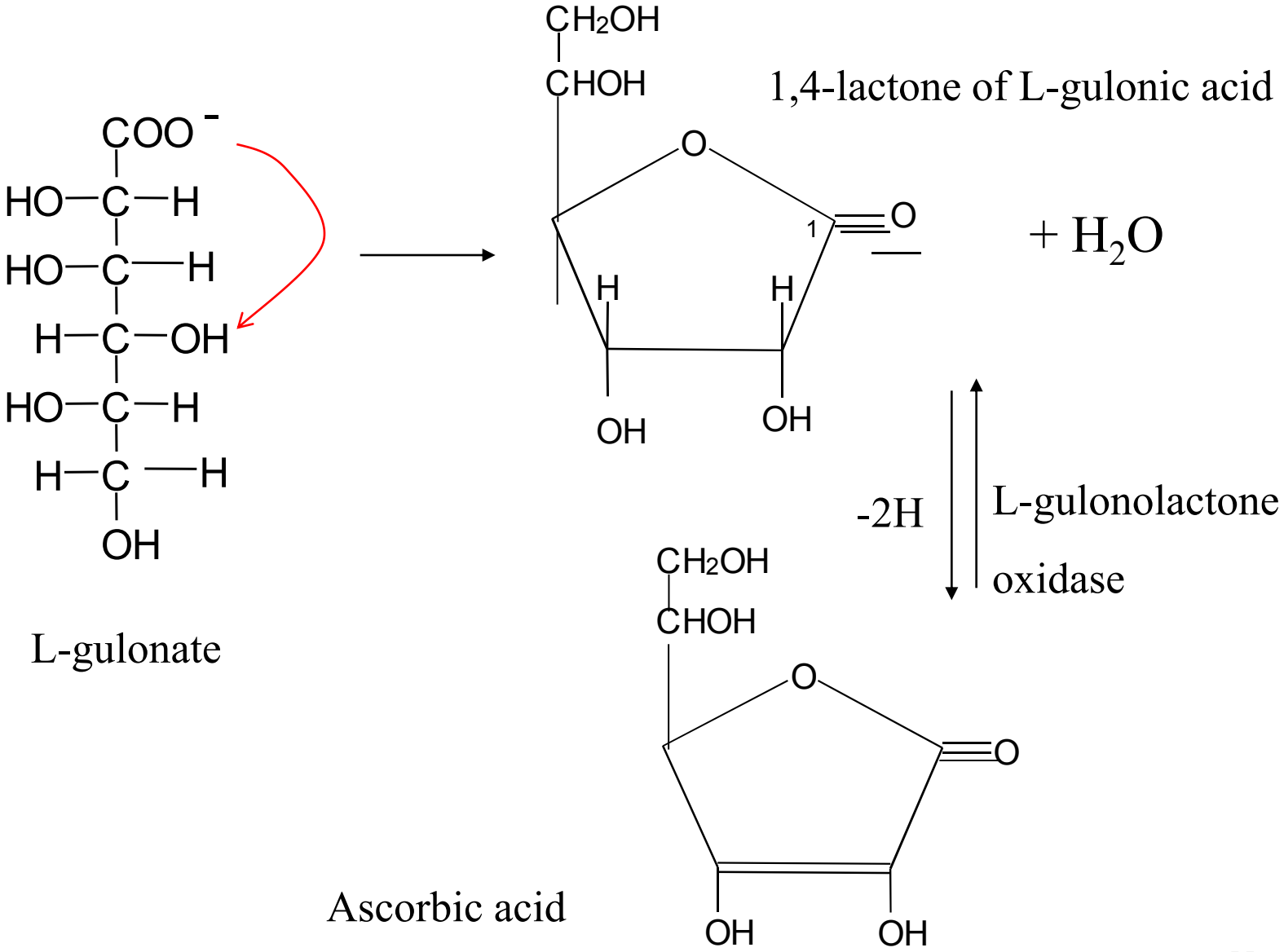
Degradation of D-glucuronic acid



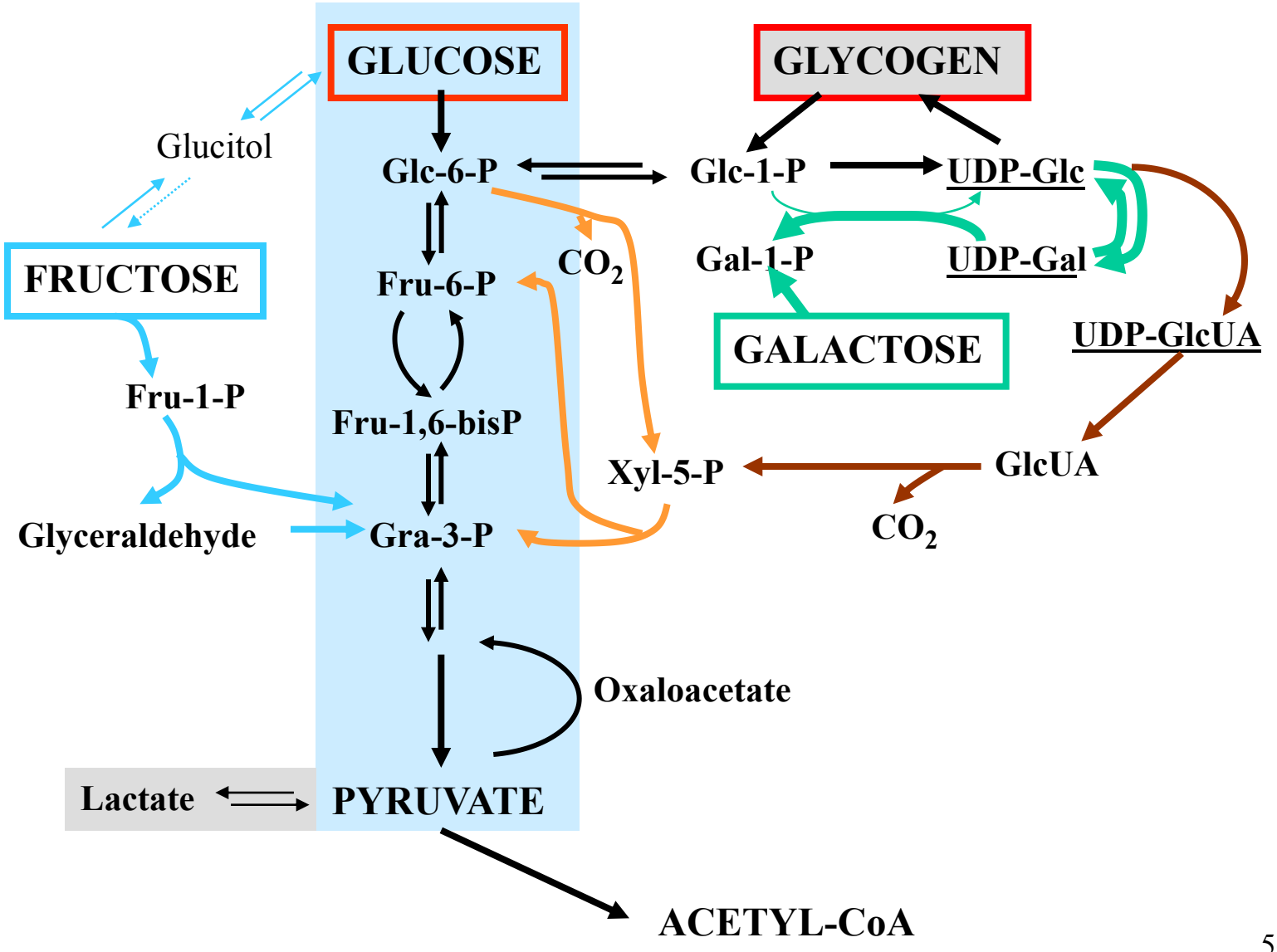
Ascorbate

- Ascorbate is required for a range of essential metabolic reactions in all animals and plants. It is made internally by almost all organisms; the main exceptions are bats, guinea pigs, capybaras and primates. Ascorbate is also not synthesized by some species of birds and fish. These animals all lack the L-gulonolactone oxidase
- All species that do not synthesize ascorbate require it in the diet.
- Deficiency causes the disease scurvy in humans
- In human body it is necessary for the hydroxylation proline and lysine in the synthesis of collagen, synthesis of carnitine, and synthesis of noradrenaline from dopamine.

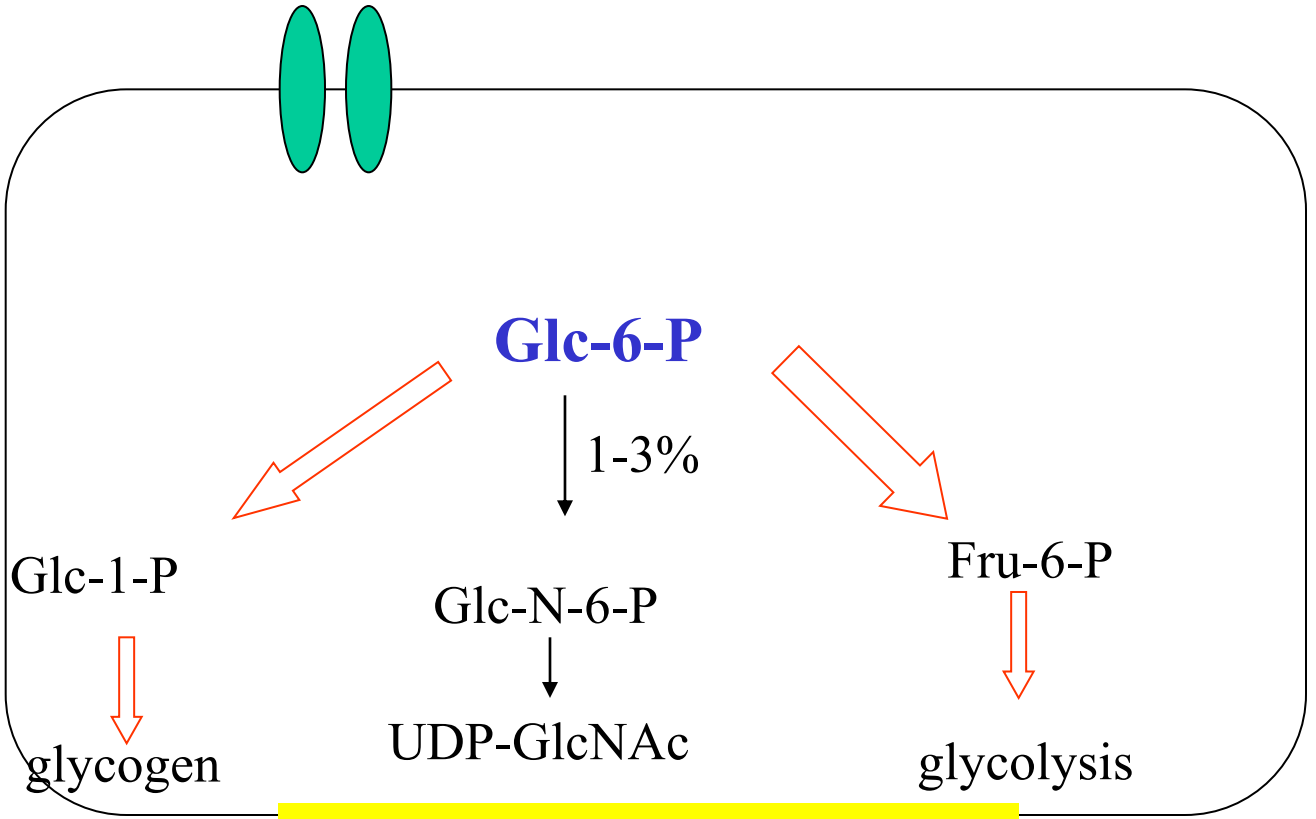
Synthesis of L-ascorbate



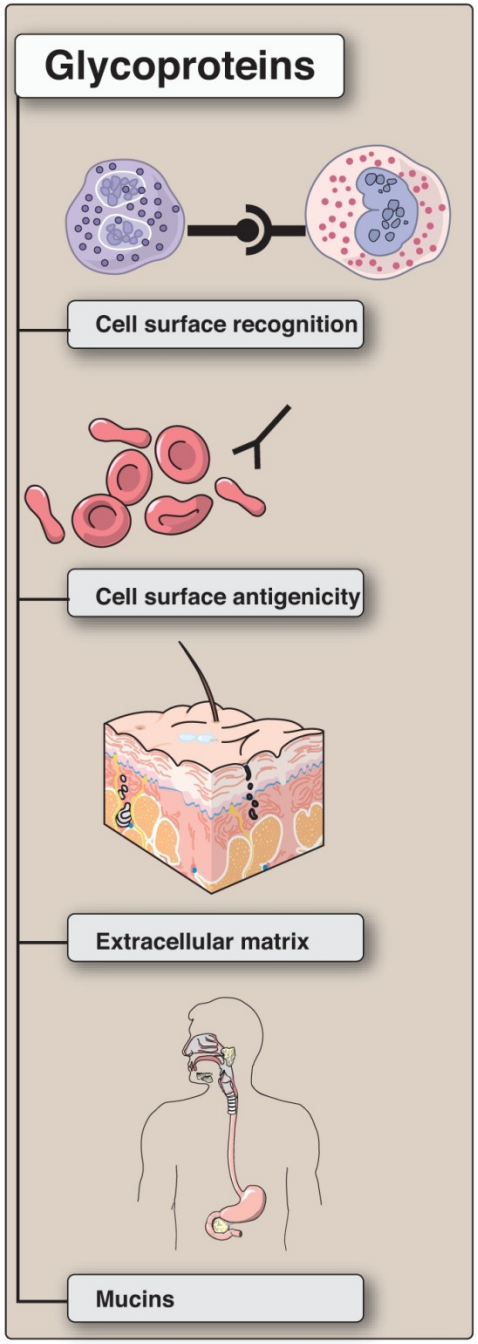
A brief survey of major pathways in saccharide metabolism



Hexosamine biosynthetic pathway - HBP



Glycosylation (formation of glycoproteins, glycolipids, proteoglycans)



Functions of glycoproteins

Interaction between the cells, interaction with hormones, viruses

Antigenicity (ABO groups etc.)

Components of extracelular matrix

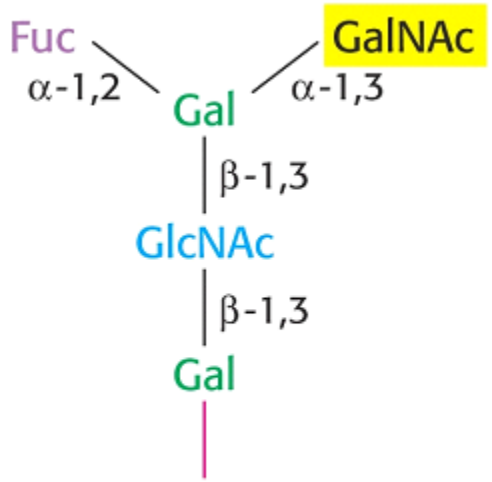
Mucines (protective effect in digestion and urogenitary systém)

Saccharides found in glycoproteins and glycolipids

		Abbreviation:
Hexoses:	**Glucose**	**Glc**
	Galactose	**Gal**
	Mannose	**Man**
Acetyl hexosamines:	***N*-Acetylglucosamine**	**GlcNAc**
	***N*-Acetylgalactosamine**	**GalNAc**
Pentoses:	**Xylose**	**Xyl**
	Arabinose	**Ara**
Deoxyhexose (Methyl pentose):	**L-Fucose**	**Fuc**
Sialic acids:	***N*-Acetylneuraminic acid** (predominant)	**NeuNAc**

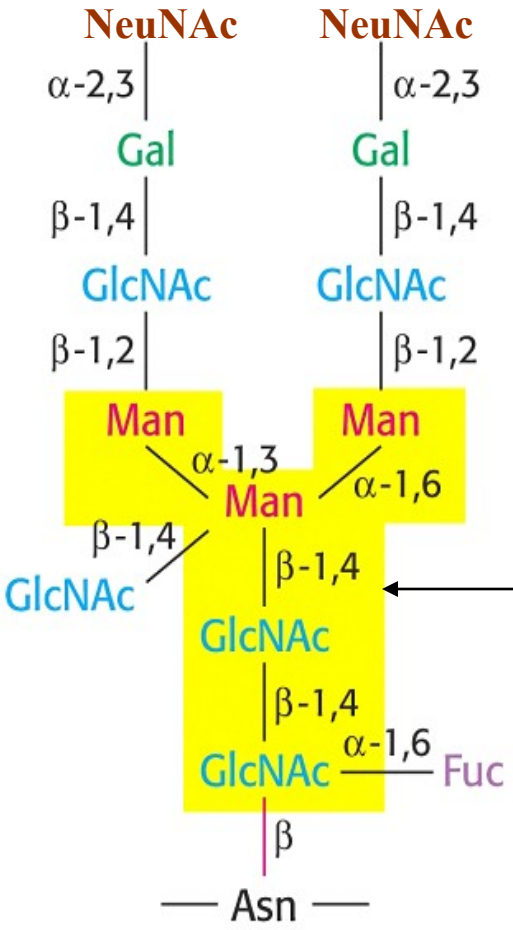
Examples of saccharidic component of glycolipids or glycoproteins:

Blood group substance A



Ceramide (**sphingolipid**) or protein

Bi-antennary component of a plasma-type (*N*-linked) oligosaccharide



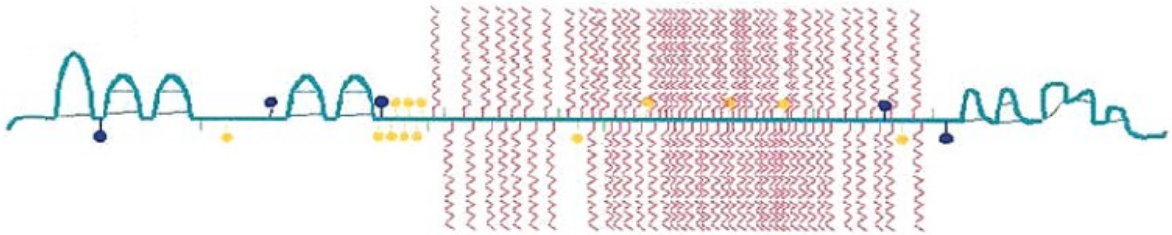
The boxed area encloses the pentasaccharide core common to all *N*-linked glycoproteins.

Glycosaminoglycans (mucopolysacharides)

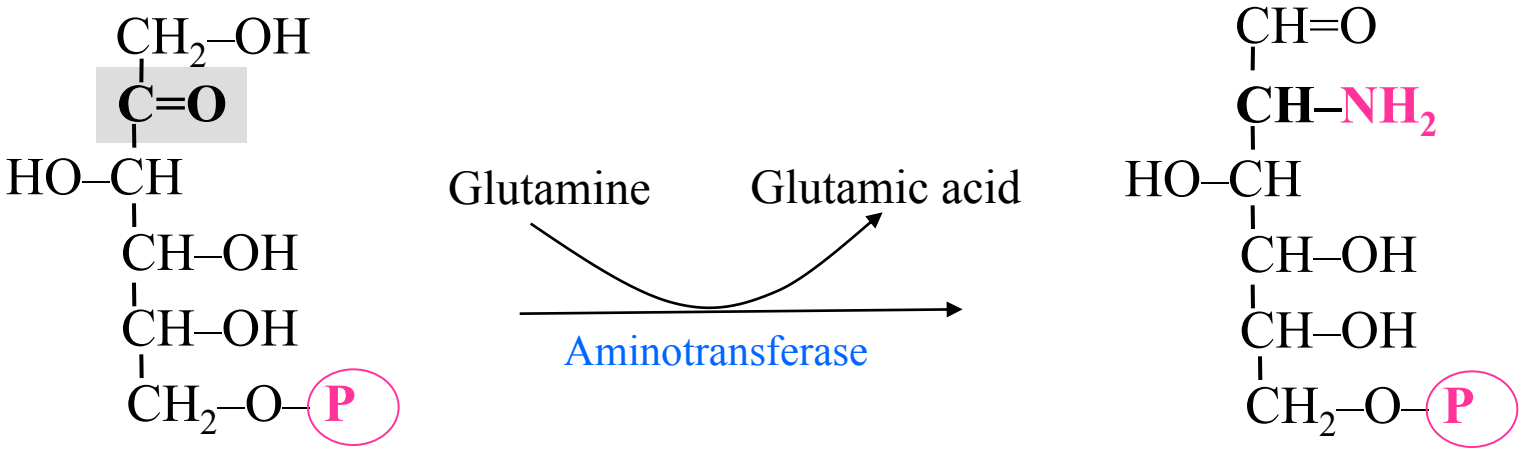
- non branched heteropolysaccharides
- they are components of proteoglycans and peptidoglycans
- formed of repeated disaccharide units:

$$[\text{ glycosamine} - \text{uronic acid}]_n$$

Present in intracelular matrix and cell surfaces (glycokalix)
They increase viscosity, support integrity of tissue
Examples: hyaluronate, dermatansulfate, heparansulfate, keratansulfate etc.



Synthesis of amino sugars



Fructose 6-phosphate

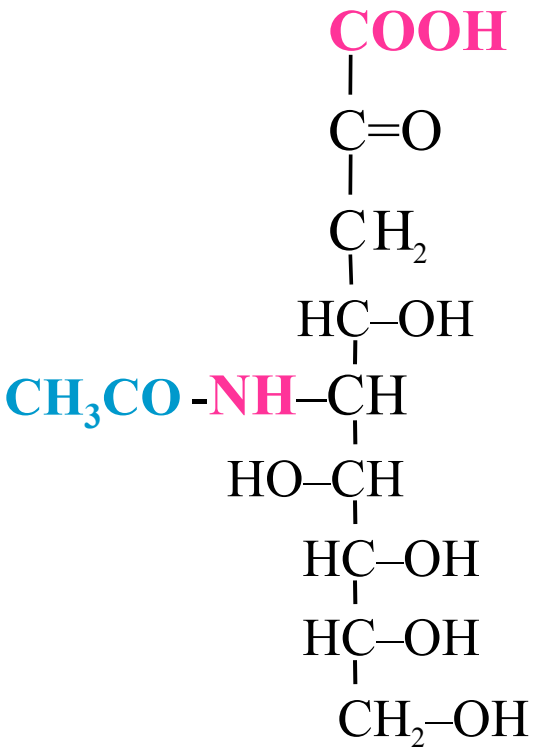
Glucosamine 6-phosphate
(2-Amino-2-deoxyglucosamine 6-phosphate)

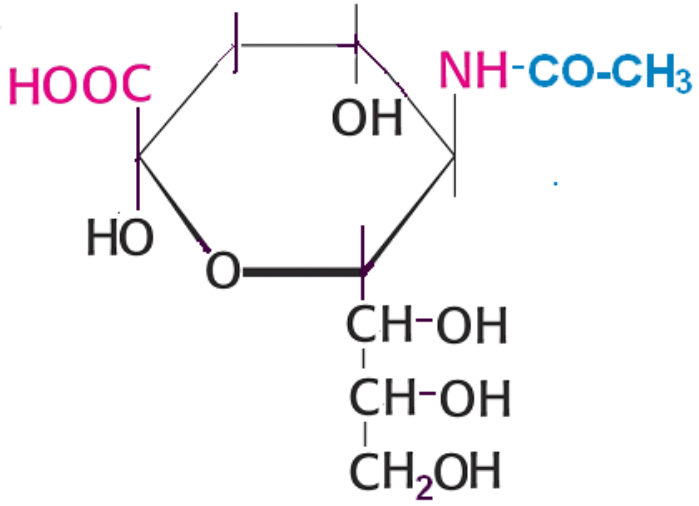
The basic amino groups **–NH_2** of amino sugars are nearly always "neutralized“ by acetylation in the reaction with acetyl-coenzyme A, so that they exist as ***N*-acetylhexosamines**.
Unlike amines, **amides (acetamido groups) are nor basic**.

Sialic acids

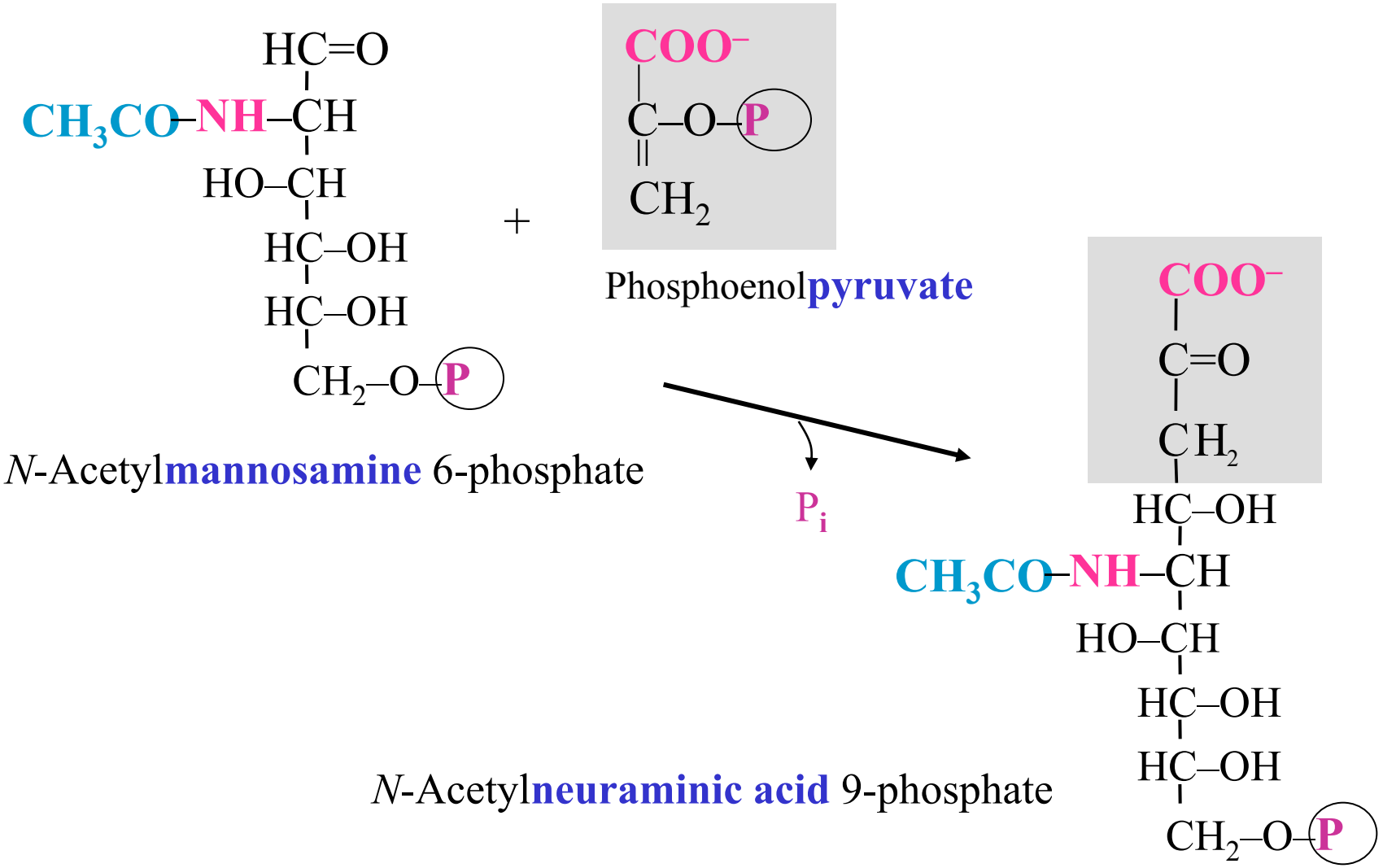
Sialic acids is the group name used for various **acylated derivatives of neuraminic acid**.

The most common sialic acid is *N*-acetylneuraminic acid:

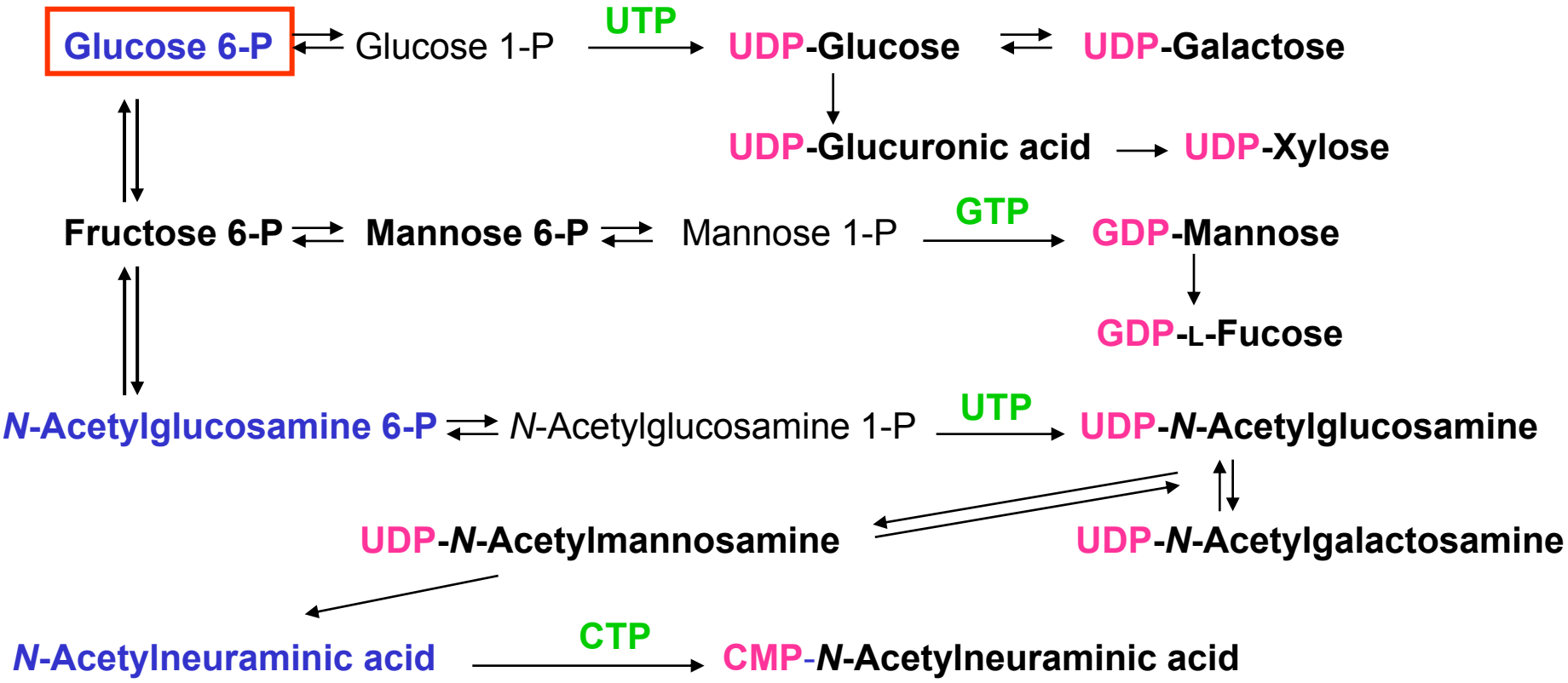




Synthesis of sialic acid:



Glycosyl donors in glycoprotein synthesis



Mucopolysaccharidoses

- metabolic disorders caused by the absence or malfunctioning of lysosomal enzymes needed to break down **glycosaminoglycans**
- **belong among lysosomal storage disease**
- Enzymes necessary for breakdown of glycosaminoglycans are either not produced enough or do not work properly.
- Over time, these glycosaminoglycans collect in the cells, blood and connective tissues. The result is permanent, progressive cellular damage which affects appearance, physical abilities, organ and system functioning, and, in most cases, mental development.
- 7 types are known, they share many clinical features but have varying degrees of severity

Disturbance in metabolism of glycoproteins - oligosaccharidoses

- Lysosomal storage disease
- Accumulation of oligosaccharides in lysosmes caused by lack of enzymes breaking down oligosaccharides of glycoproteins
- Mannosidose, fucosidose, sialidose