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

Biochemistry I
Lecture 5

2009 (J.S.)

The pentose phosphate pathway

(also called the phosphogluconate pathway)
is one of the secondary pathways of glucose catabolism.

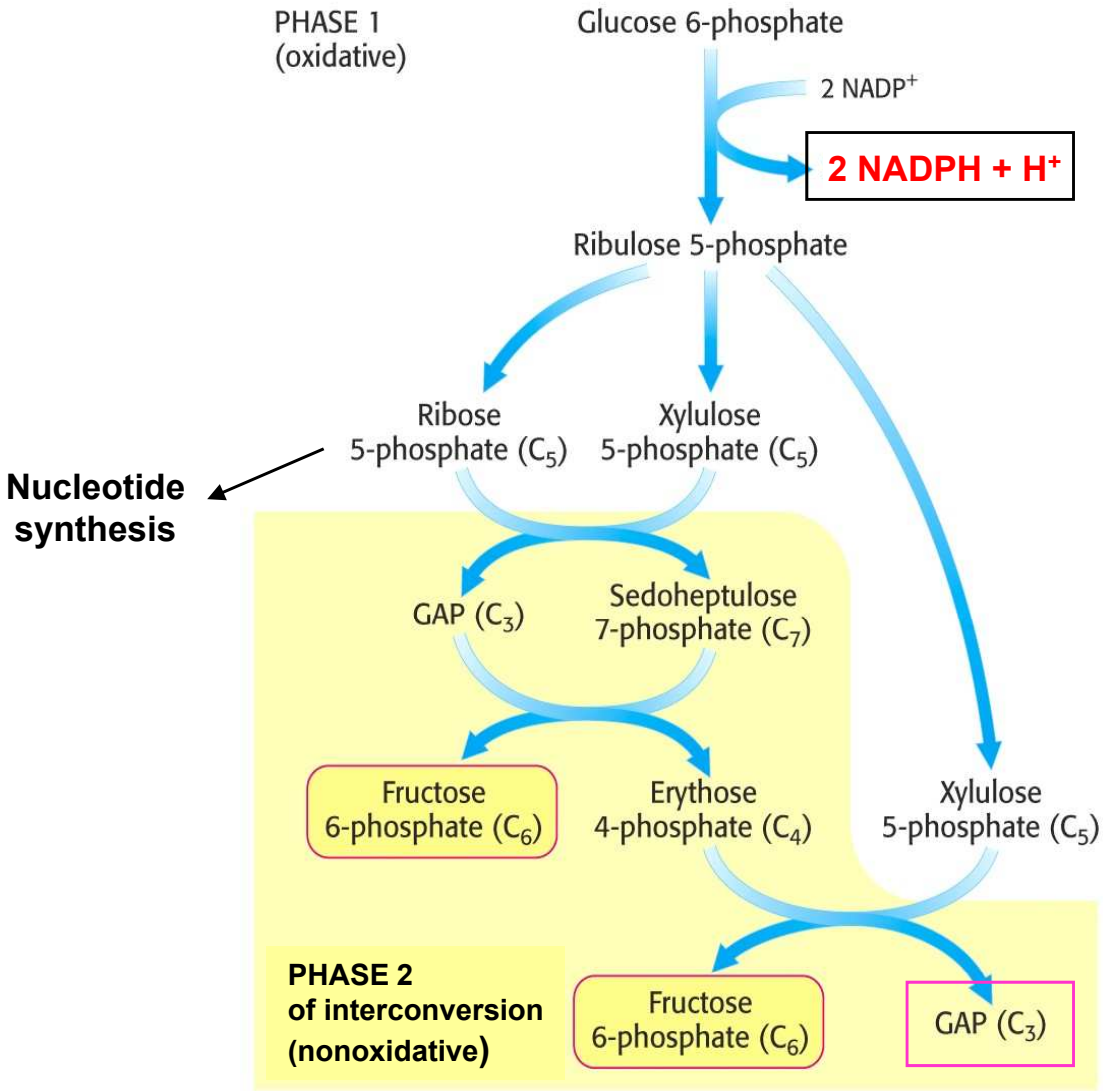
It leads to two special products in animal tissues:

NADPH is a carrier of chemical energy in the form of **reducing power for reductive syntheses** and **hydroxylations** catalysed by monooxygenases, and some other important reductions.

and **ribose 5-phosphate** used in the **biosynthesis of nucleic acids**.

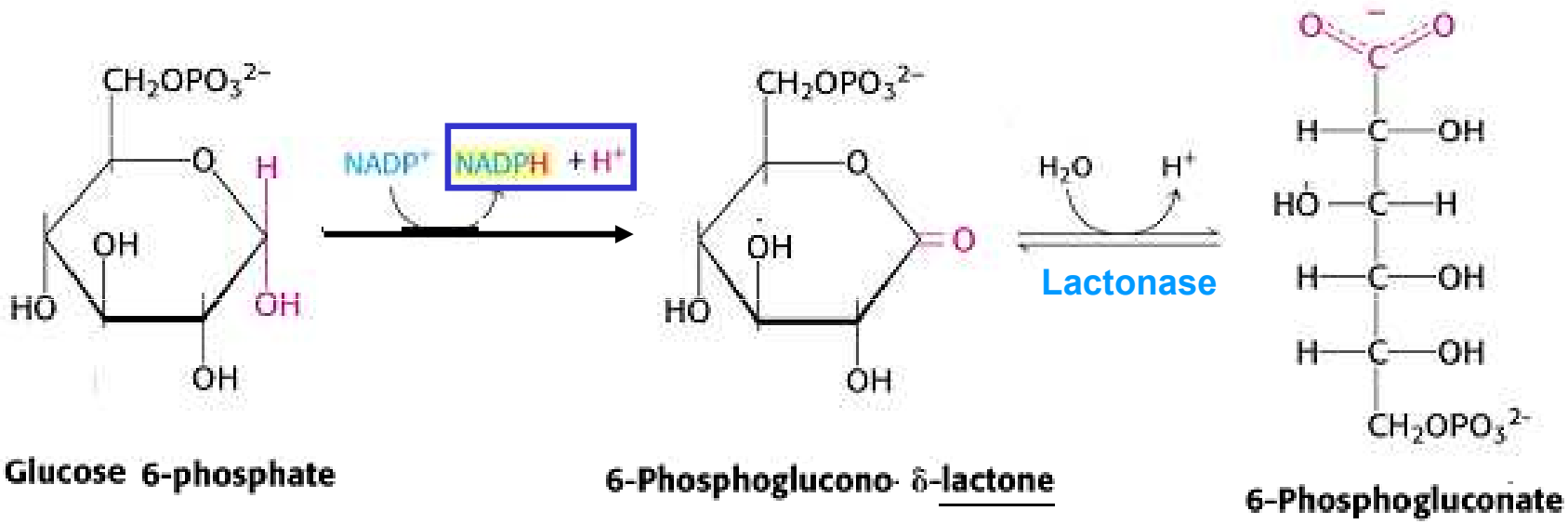
It does not serve to generate ATP energy.

The pathway is **highly active in the cytoplasm** of the **liver**, **adipose tissue, mammary gland,** and the **adrenal cortex**. Other tissues less active in synthesizing fatty acids, such as skeletal muscle, are virtually lacking in the pentose phosphate pathway.


PHASE 1
(oxidative)
Glucose 6-phosphate
2 NADP+
2 NADPH + H+
Ribulose 5-phosphate
Ribose
5-phosphate (C5)
Xylulose
5-phosphate (C5)
Nucleotide
synthesis
GAP (C3)
Sedoheptulose
7-phosphate (C7)
Fructose
6-phosphate (C6)
Erythose
4-phosphate (C4)
Xylulose
5-phosphate (C5)
PHASE 2
of interconversion
(nonoxidative)
Fructose
6-phosphate (C6)
GAP (C3)

1 The oxidative phase is irreversible

The first oxidative step is the dehydrogenation of the cyclic form of glucose 6-P (a hemiacetal) **to the lactone of 6-P gluconic acid:**



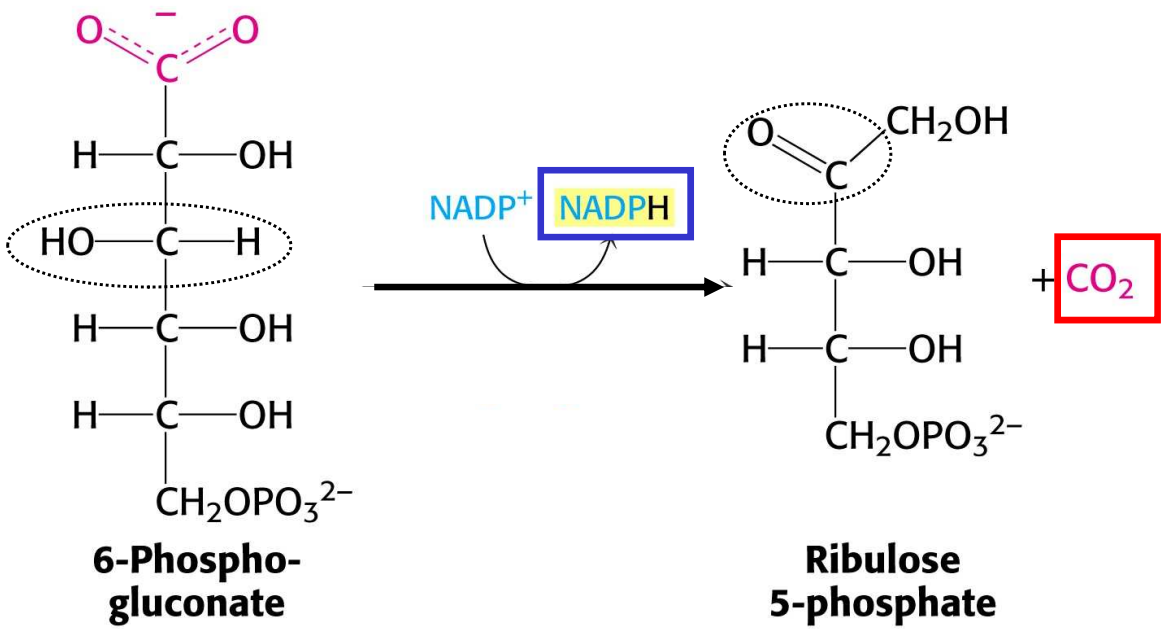
Glucose 6-phosphate

6-Phosphoglucono-δ-lactone

6-Phosphogluconate

Glucose 6-phosphate dehydrogenase
is the regulated key enzyme of the pathway: In the cytosol of a liver cell from a well fed rat the ratio $NADP^+$/NADPH is about 0.014. An increase of this ratio stimulates the activity of G-6-P dehydrogenase.
(For comparison, the ratio NAD^+/NADH is 700 under the same conditions, at much higher concentrations of NAD^+ + NADH.)

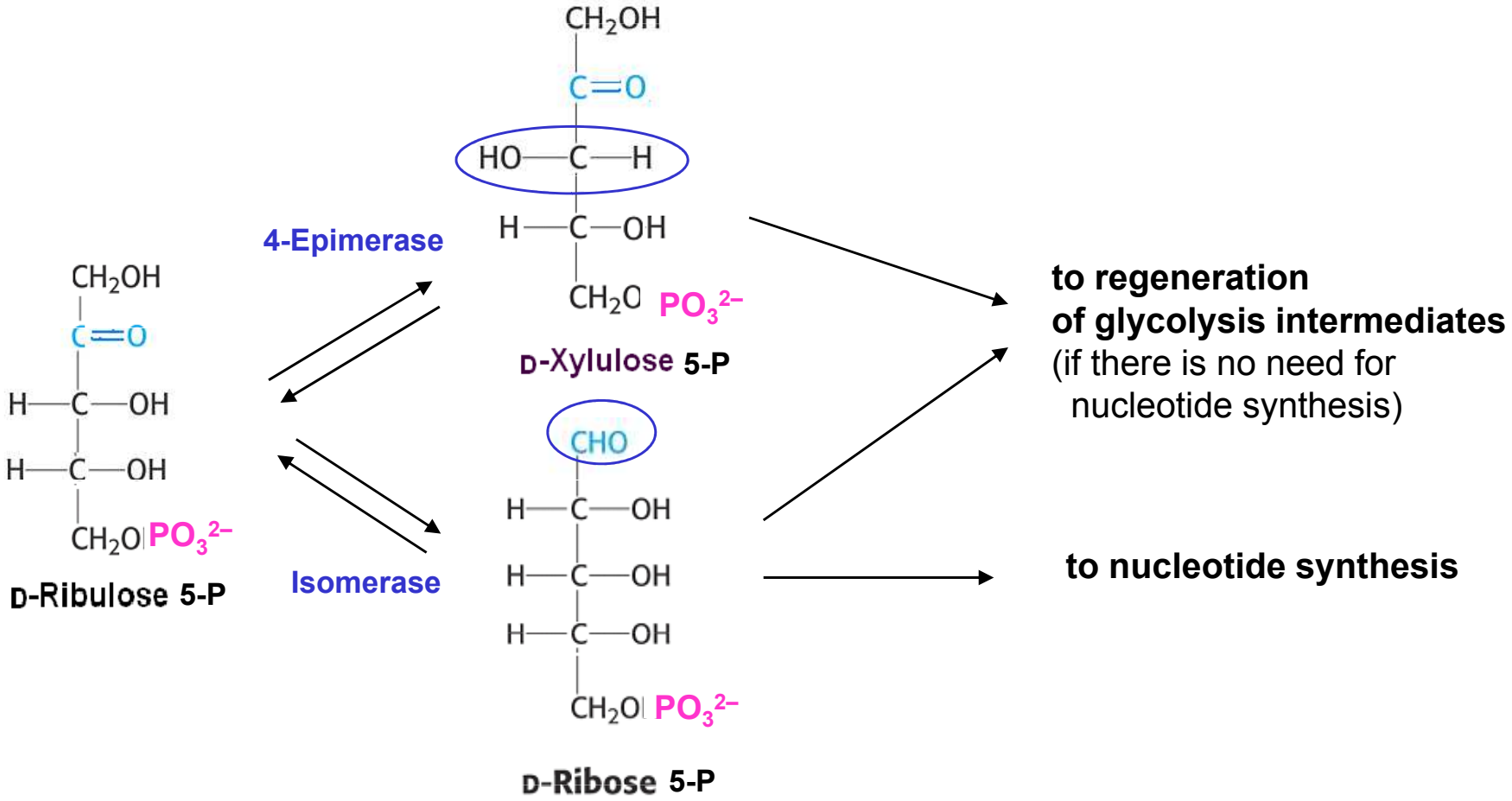
The second oxidative reaction (dehydrogenation at carbon 3) **is accompanied with decarboxylation:**



6-phosphogluconate dehydrogenase reaction

2 The interconversion phase is fully <u>reversible</u>

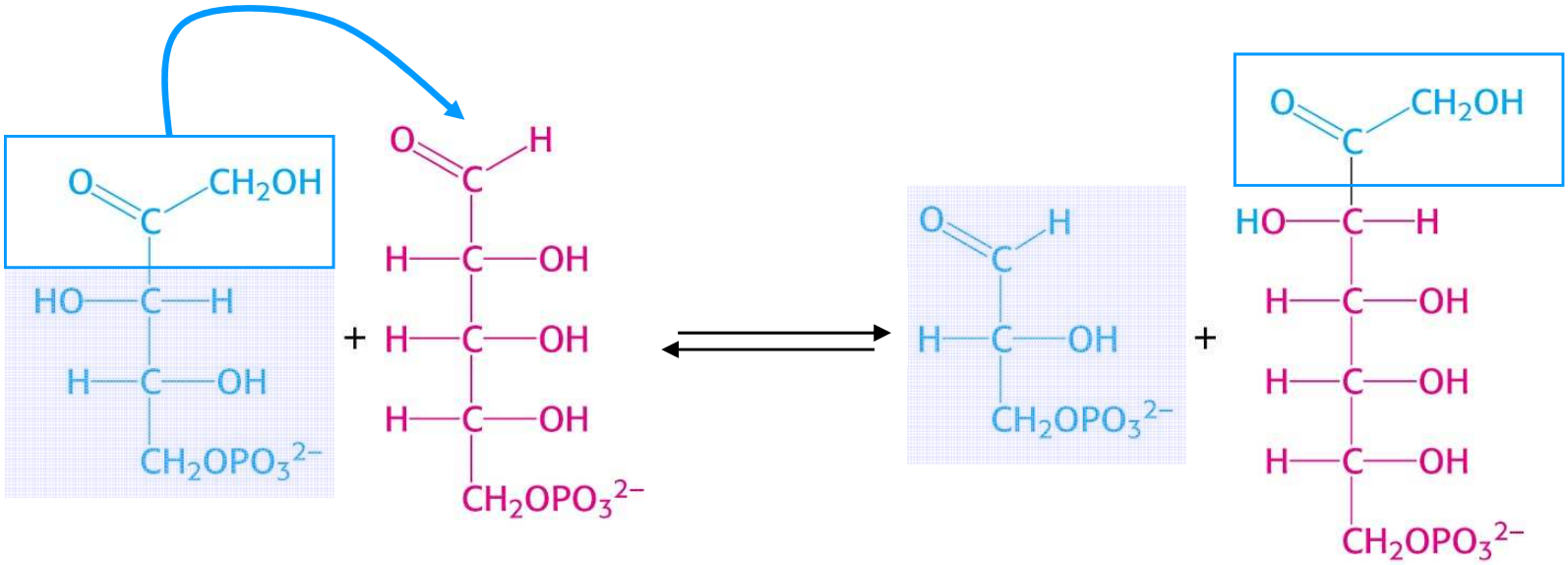
It begins with isomerization or epimerization of ribulose 5-phosphate:



Three pentose 5-phosphates are required for the regeneration of the glucose-pathway intermediates – two molecules of xylulose 5-phosphate and one molecule of ribose phosphate

The first transketolase reaction (the transfer of C_2 to ribose 5-P) **:**

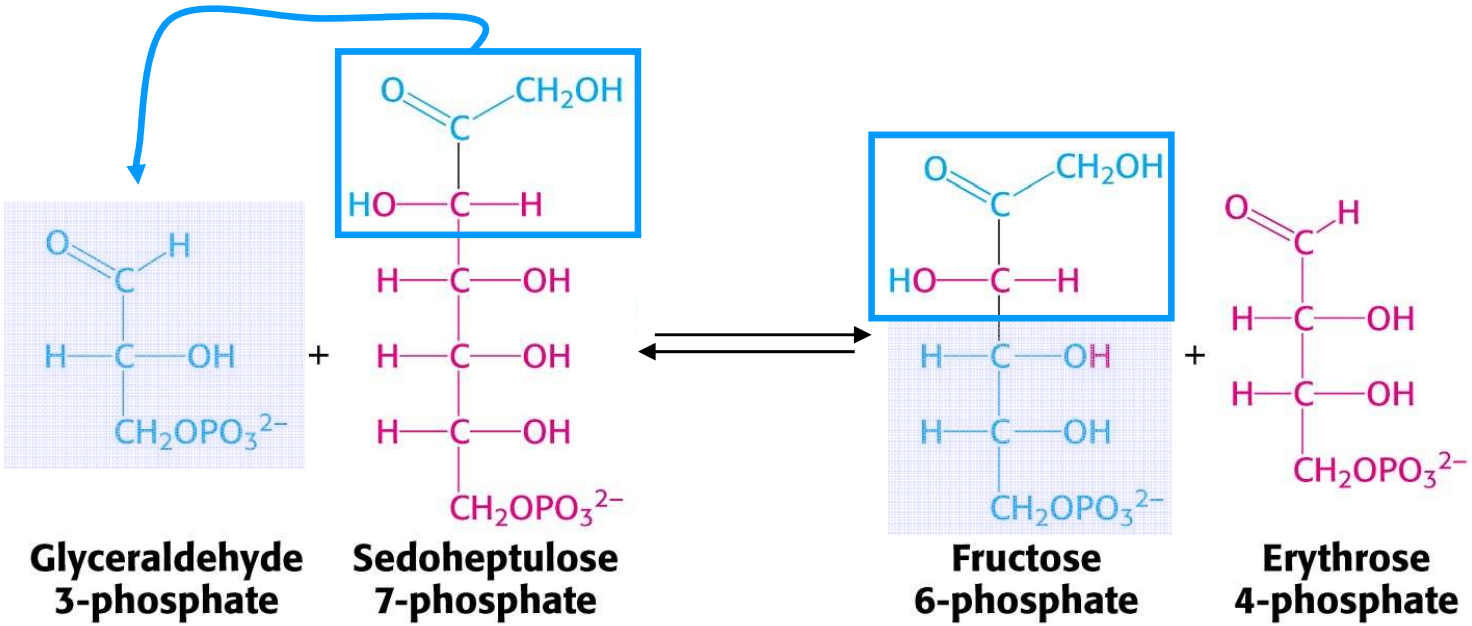
Transketolase has a **thiamine diphosphate** prosthetic group.



$$\underline{C_5} + \underline{C_5} \rightleftharpoons C_3 + C_7$$

The1st and the 2nd pentose 5-phosphate

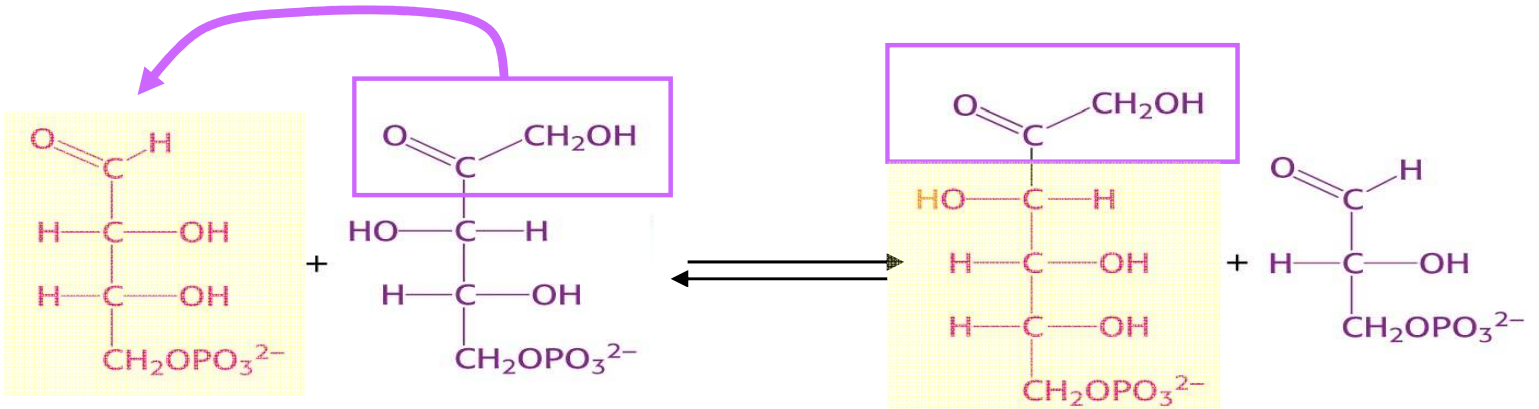
The transaldolase reaction (the transfer of C_3 to glyceraldehyde 3-P) :



$$C_3 + C_7 \rightleftharpoons C_6 + C_4$$

An intermediate of glycolysis

The second transketolase reaction (the transfer of C_2 to C_4):



Intermediates of the glycolytic pathway

$$C_4 + C_5 \rightleftharpoons C_6 + C_3$$

The 3rd pentose 5-phosphate

The summary of the pentose phosphate pathway:

Reaction	Enzyme
Oxidative phase	
Glucose 6-phosphate + $NADP^+$ ⟶ 6-phosphoglucono-δ-lactone + NADPH + H^+	Glucose 6-phosphate dehydrogenase
6-Phosphoglucono-δ-lactone + H_2O ⟶ 6-phosphogluconate + H^+	Lactonase
6-Phosphogluconate + $NADP^+$ ⟶ ribulose 5-phosphate + CO_2 + NADPH	6-Phosphogluconate dehydrogenase
Interconversion (non-oxidative) phase	
Ribulose 5-phosphate ⇌ ribose 5-phosphate	Phosphopentose isomerase
Ribulose 5-phosphate ⇌ xylulose 5-phosphate	Phosphopentose epimerase
Xylulose 5-phosphate + ribose 5-phosphate ⇌ sedoheptulose 7-phosphate + glyceraldehyde 3-phosphate	Transketolase
Sedoheptulose 7-phosphate + glyceraldehyde 3-phosphate ⇌ fructose 6-phosphate + erythrose 4-phosphate	Transaldolase
Xylulose 5-phosphate + erythrose 4-phosphate ⇌ fructose 6-phosphate + glyceraldehyde 3-phosphate	Transketolase

3 Glucose 6-P + 6 $NADP^+$ ⟶ 6 NADPH+H^+ + 2 Fructose 6-P + Glyceraldehyde 3-P + 3 CO_2

3 C_6 ⟶ 2 C_6 + C_3 + 3 C

or in the cells which need ribose 5-P for synthesis of nucleotides

3 Glucose 6-P + 6 $NADP^+$ ⟶ 6 NADPH + H^+ + 3 Ribose 5-P + 3 CO_2

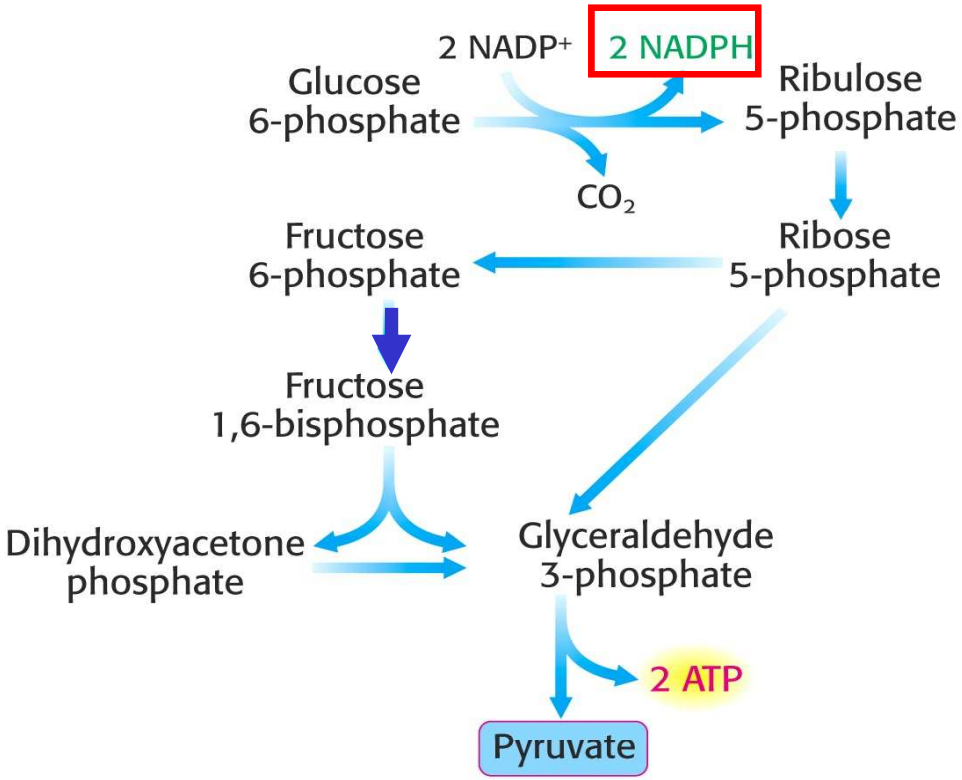
3 C_6 ⟶ 3 C_5 + 3 CO_2

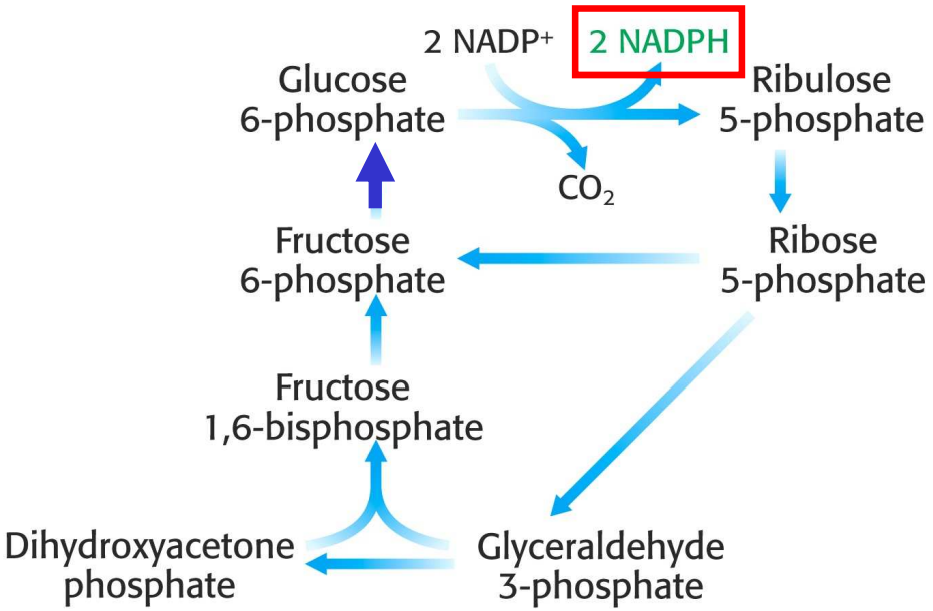
Some cells in certain states require much more NADPH (for reductive syntheses, e.g. in adipose tissue) than ribose 5-phosphate, but they do not require the intensive production of pentose 5-phosphates

- then **most of the pentose 5-phosphates is regenerated into glycolysis intermediates.**

With respect to the energy charge, the glycolysis intermediates are either **catabolized to gain energy,**

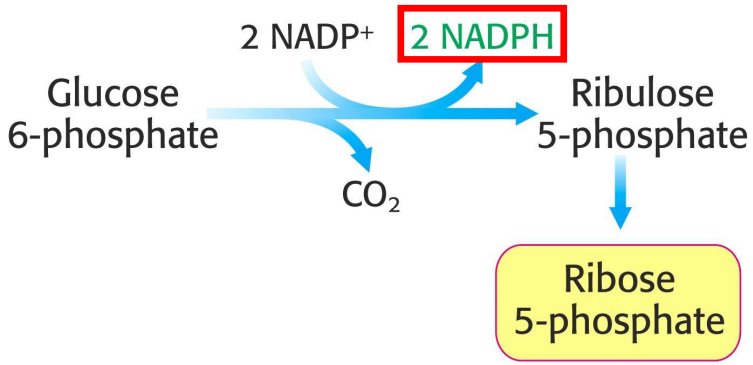
or they are used for **regeneration of glucose 6-phosphate** (e.g. for biosynthesis of glycogen) by the gluconeogenic pathway.





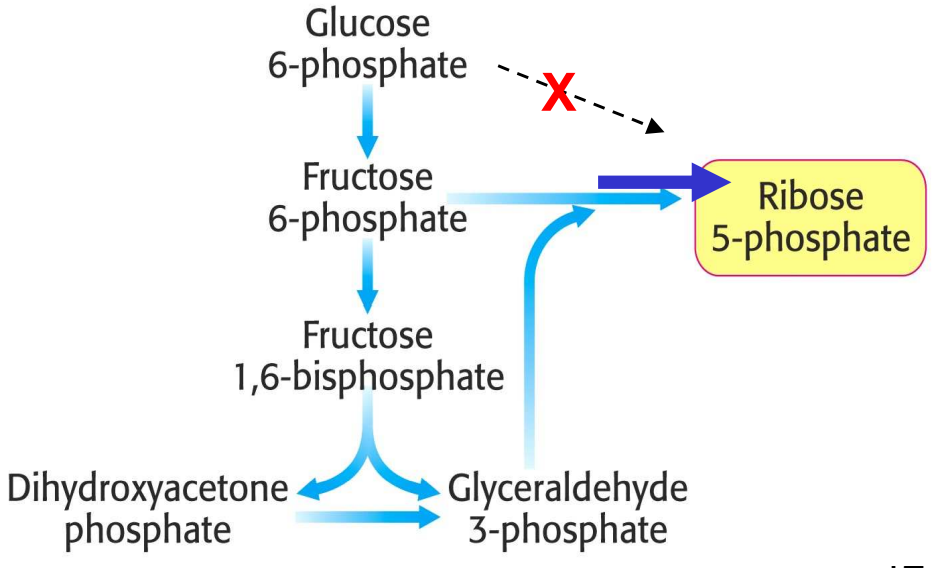
The cell spends NADPH very intensively in reductive syntheses
as well as in biosynthesis of nucleotides, the needs are balanced

- the pentose 5-phosphates do not enter the interconversion phase



The cell requires much more ribose 5-phosphate(to synthesize nucleotides) than NADPH
and does not require NADPH for reductive syntheses (e.g. skeletal muscle)

- the **reversal of the interconversion phase**



Tissues with active pentose phosphate pathways

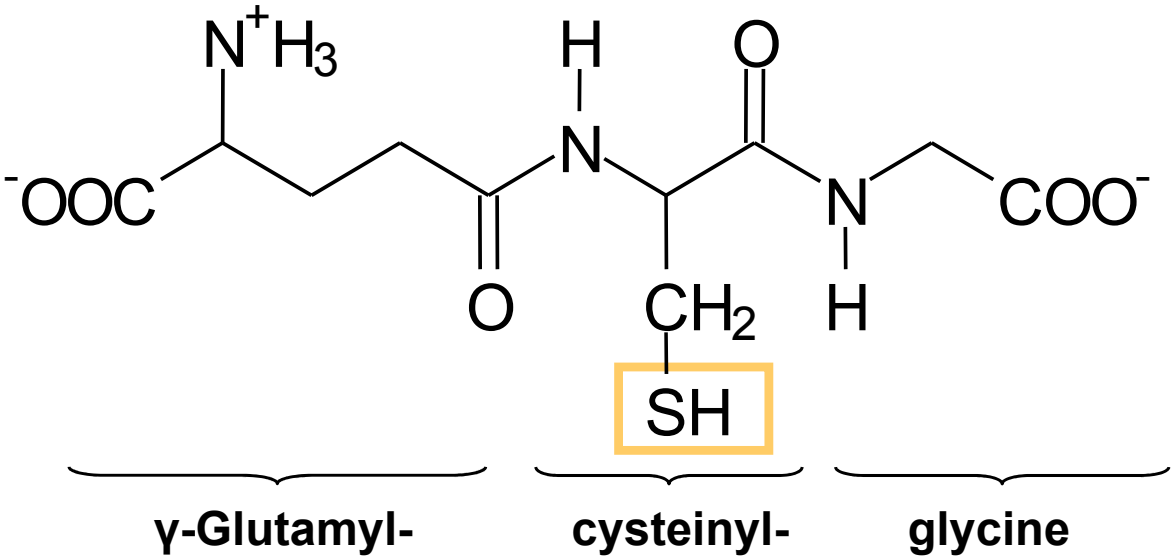
Tissue	Function
Liver	Fatty acid and cholesterol synthesis
Adipose tissue	Fatty acid synthesis
Mammary gland	Fatty acid synthesis
Adrenal gland	Steroid synthesis
Testes	Steroid synthesis
Ovary	Steroid synthesis
Red blood cells	Maintenance of reduced glutathione

Glucose 6-phosphate dehydrogenase

plays a role in protection against reactive oxygen species (ROS).

Reduced **glutathione** is required to "detoxify“ ROS with a free sulfanyl group, it maintains the normal reduced state in the cell.

Glutathione (reduced form, abbr. **GSH**)



Glutathione, oxidized by ROS (GSSH) is **reduced by NADPH** generated by Glc-6-P dehydrogenase in the pentose phosphate pathway.

Cells with reduced levels of Glc-6-P dehydrogenase are especially sensitive to oxidative stress. This stress is most acute in **red blood cells**.

Regeneration of the oxidized form of glutathione (GS-SG) is catalyzed by glutathione reductase:

$$GS\text{-}SG + NADPH + H^+ \rightarrow 2\ GSH + NADP^+$$

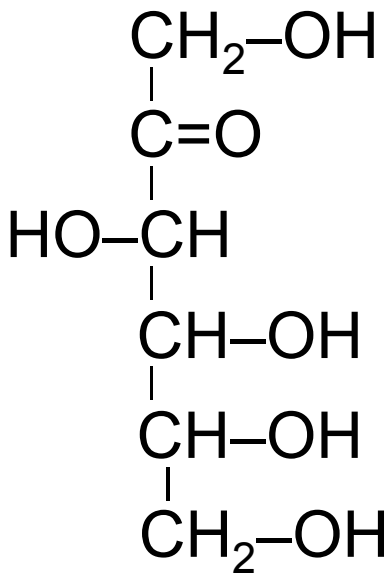
Glucose 6-phosphate dehydrogenase deficiency

in red blood cells is an inherited defect affecting hundred of millions of people (e.g. 11 % among Afroamericans). The deficiency is quite benign in the absence of oxidative stress.

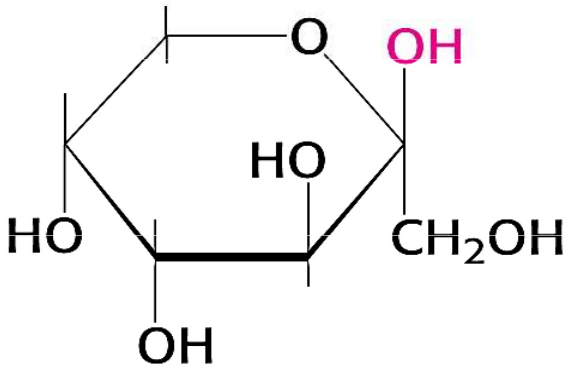
The generation of peroxides, e.g. after eating fava beans (of the Mediterranean plant *Vicia faba*) or taking an antimalarial drug pamaquine, may be a cause of **severe haemolysis**, destruction of red blood cells and **anaemia**.

On the other hand, **this enzyme deficiency protect against falciparum malaria.** The parasites causing this disease require reduced glutathione and the products of the pentose phosphate cycle for optimal growth.

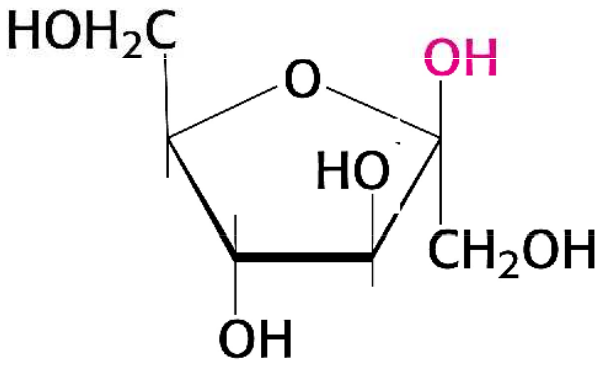
Metabolism of fructose



D-Fructose

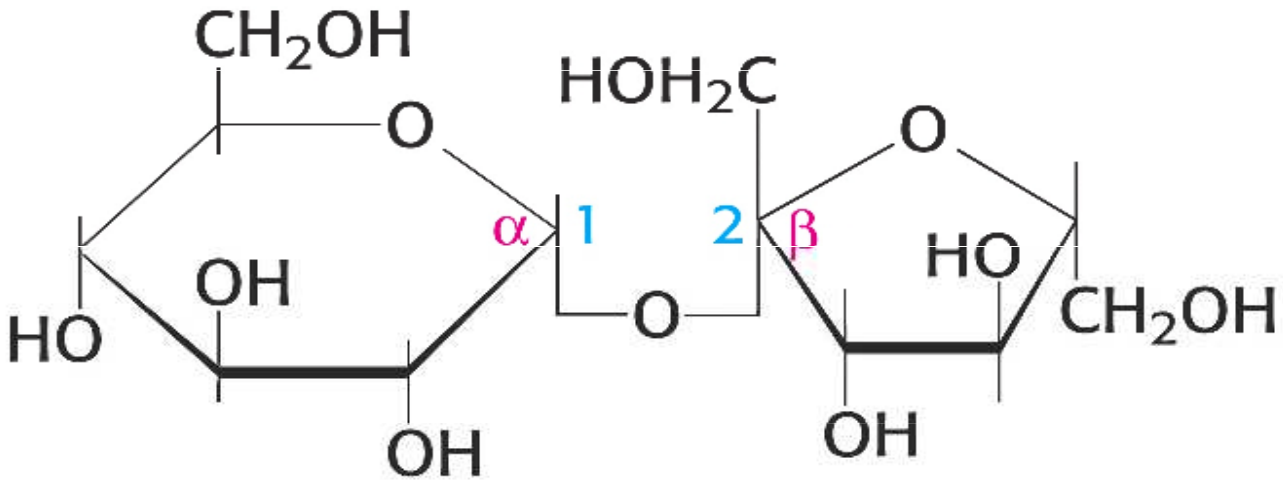


β-D-Fructopyranose



β-D-Fructofuranose

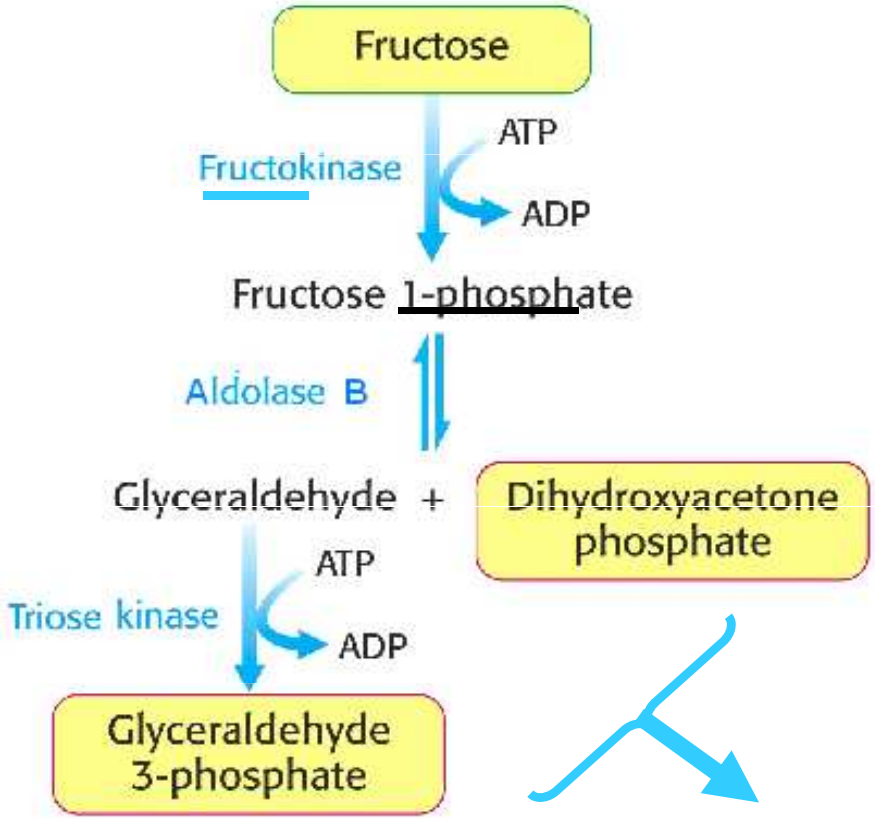
Fructose is present in many different fruits and in honey. A considerable quantities of this sugar are ingested chiefly in the form of sucrose:



Sucrose

α-D-Glucopyranosyl-(1 → 2)-β-D-fructofuranos ide

Fructose is metabolized mostly **in the liver**:

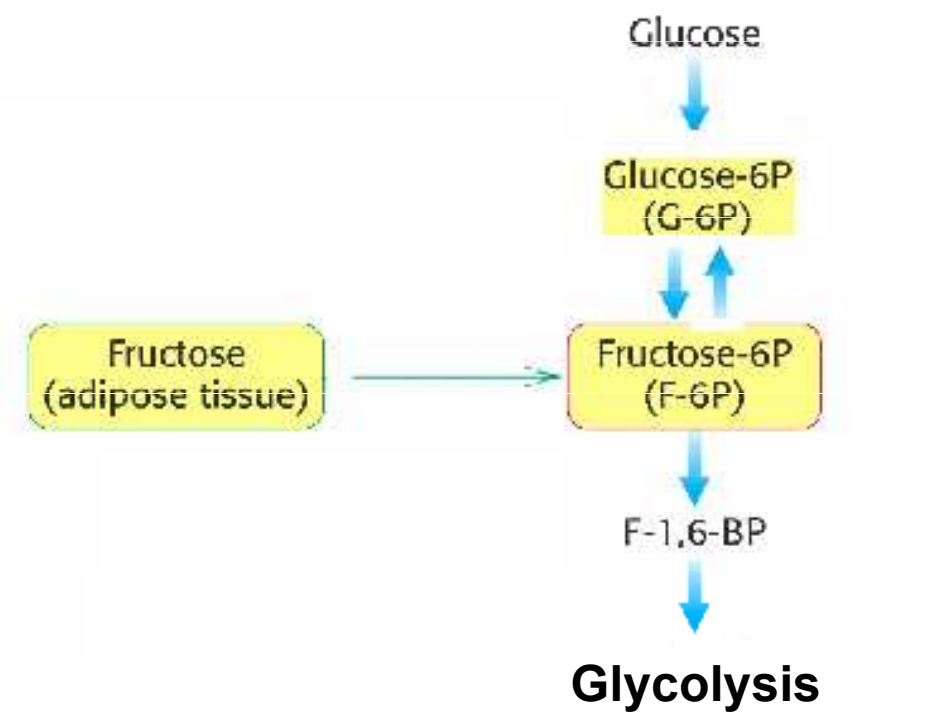


Fructose metabolism is **not subject to the insulin control** as that of glucose, it bypasses the phosphofructokinase reaction and is very rapid.

So unpredictable quantities of intermediates can enter the glycolytic pathway.

Glycolysis
or reconversion to glucose

In the intestinal mucosa, muscle, and adipose tissue, a part of fructose may enter directly into glycolysis:



Some tissues (e.g. gonads) are able to synthesize fructose from glucose through the **polyol metabolic pathway**:

If the blood concentration of a monosaccharide is very high (e.g. glucose in *diabetes mellitus* or galactose in *galactosaemia*), the polyol pathway produces alditols (glucitol and galactitol, resp.) that may cause **cataract** formation (a cataract is the clouding of the normally clear lens of the eye).

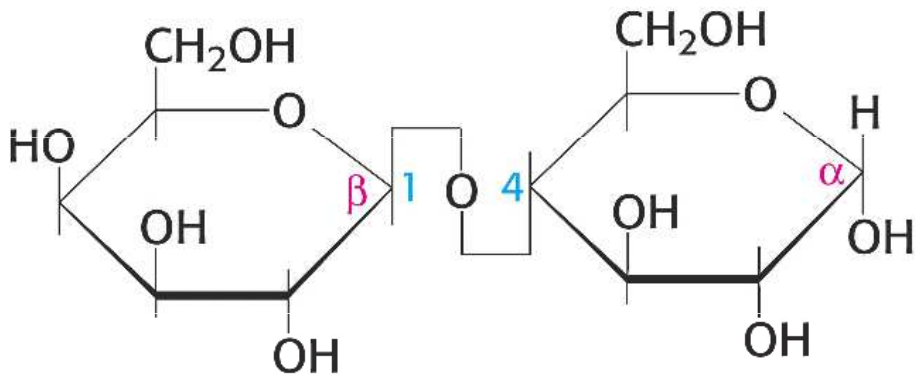
Defects in fructose metabolism

Essential fructosuria – lack in **fructokinase**
is without any serious consequences: blood fructose concentration is abnormally high and fructose is excreted into the urine.

Hereditary fructose intolerance – low activity of **aldolase B**;
fructose 1-phosphate may accumulate in the liver to such an extent that most of the **inorganic phosphate is removed from the cytosol**. Oxidative phosphorylation is inhibited and hypoglycaemia also appears (Fru-1-P inhibits both glycolysis and gluconeogenesis).
The intake of fructose and sucrose must be restricted.

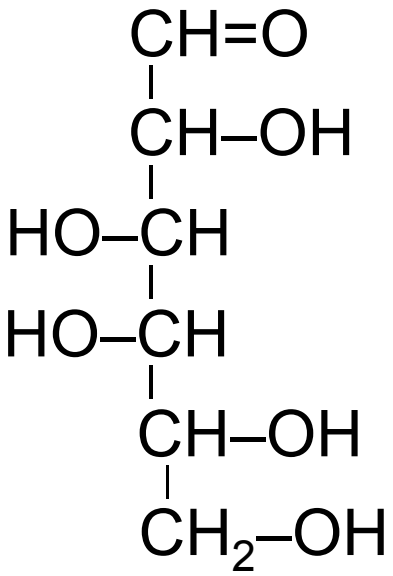
Metabolism of galactose

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

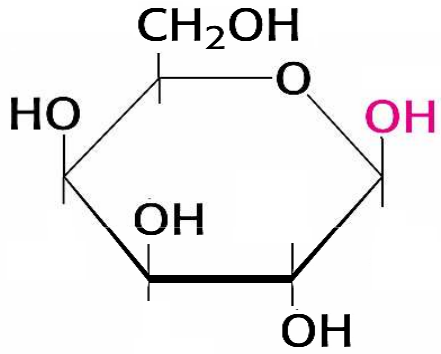


α-Lactose

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

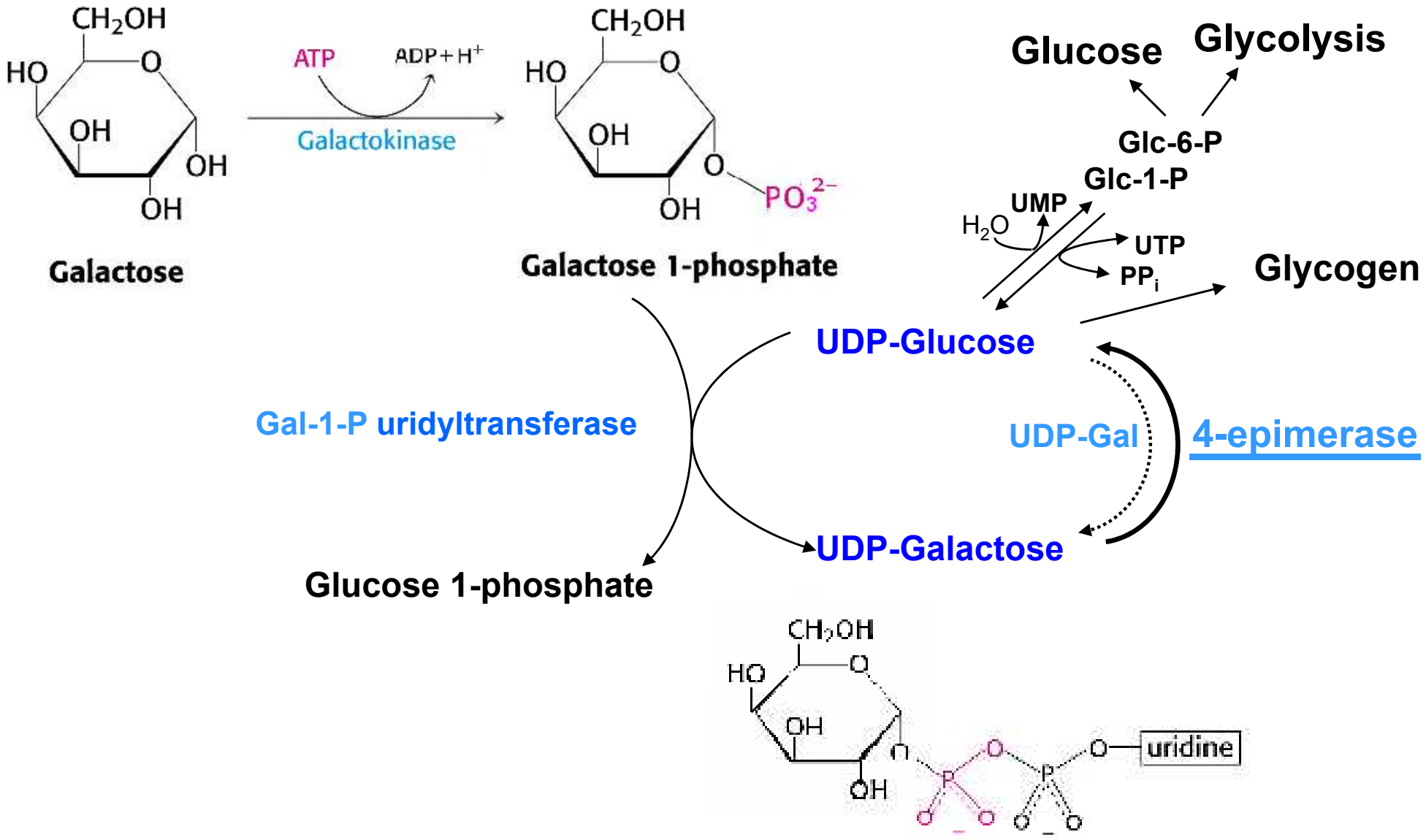


D-Galactose



β-D-Galactopyranose

Transformation of galactose into glucose in the liver



Defect in galactose catabolism

Galactosaemia is the hereditary deficiency of either galactokinase or (mostly) Gal-1-P uridyltransferase. It can have **fatal results** for children if they are not quickly put on a lactose-free diet.

Afflicted infants fail to thrive, they vomit after consuming milk, very common is the disturbance of the liver function and retarded mental development. A cataract formation is caused by accumulation of galactitol in the lens of the eye.

Lactose intolerance

Many adults are intolerant of milk because they are "deficient" in lactase bound in the membrane of cells in the intestinal mucosa. The decrease in lactase is normal during development in all mammals, usually to about 5 – 10 % of the level at birth (this decrease is not as pronounced with some groups of people, most notably Northern Europeans.)

Micro organisms in the colon ferment the unresorbed lactose to lactic acid that is osmotically active and causes diarrhoea, while also generating methane and hydrogen – the gas creates uncomfortable feeling of gut distension.

Galactose and *N*-acetylgalactosamine

are important constituents of

glycoproteins, proteoglycans, and glycolipids.

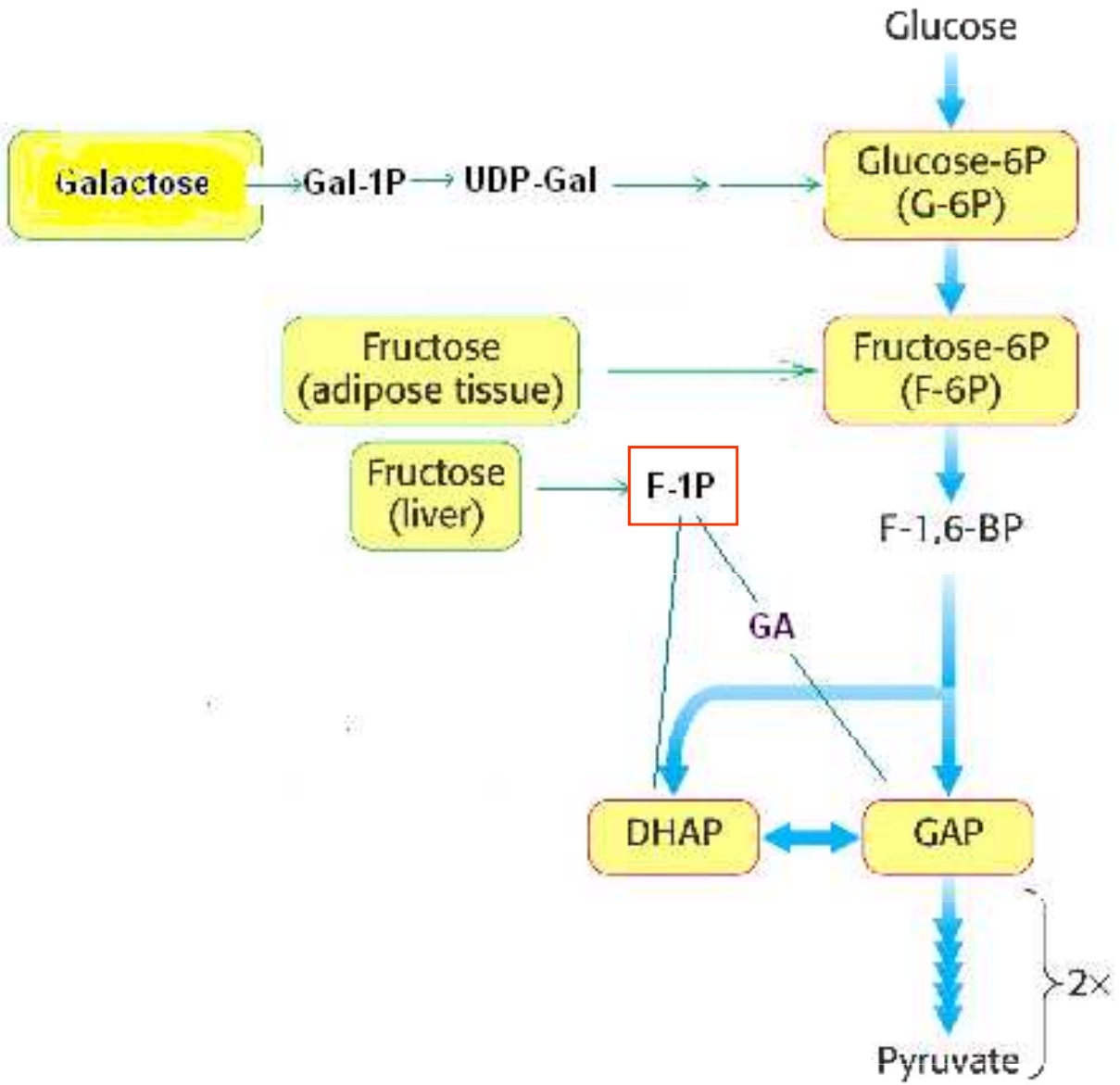
In the synthesis of those compounds **in all types of cells**, the galactosyl and *N*-acetylgalactosyl groups are transferred from UDP-galactose and UDP-*N*-acetyl-galactose by the action of **UDP-galactosyltransferase**.

Biosynthesis of lactose

occurs **only in the lactating mammary gland**

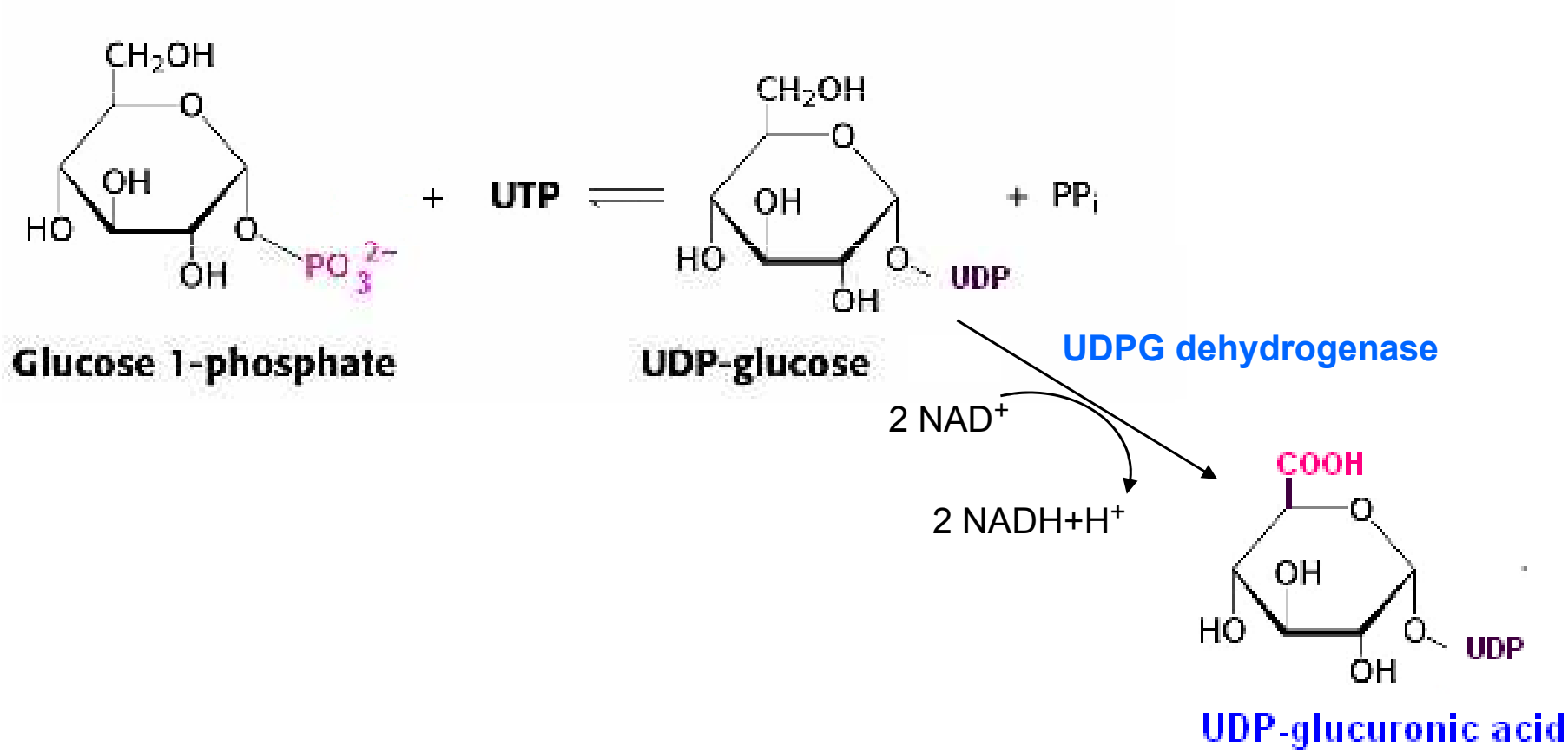
The specificity of **UDP-galactosyltransferase** is modified by a regulatory protein **α-lactalbumin**, which is synthesized in the mammary gland due to steep decrease of hormonal levels just before the birth. α-Lactalbumin binds onto the transferase and changes its specificity so that it begins to catalyze the **transfer of galactosyl from UDP-Gal to glucose** and production of lactose.

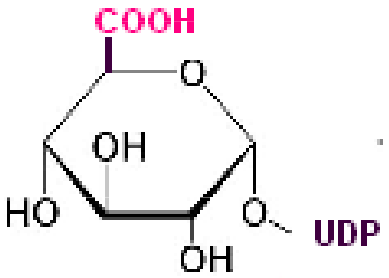
Entry points in glycolysis for fructose and galactose



The uronic acid pathway

is an alternative oxidative pathway for glucose.
It supplies **glucuronic acid**, and in most animals (not in humans, other primates, and guinea pigs) **ascorbic acid**.
Glucuronic acid is finally metabolized to the **pentoses** which can be reconverted to intermediates of glycolysis.





UDP-glucuronic acid

Synthesis of glycoseaminoglycans

Conjugation with xenobiotics

H_2O

UDP

CH=O
HC–OH
HO–CH
HC–OH
HC–OH
COOH

D-Glucuronic acid
(free)

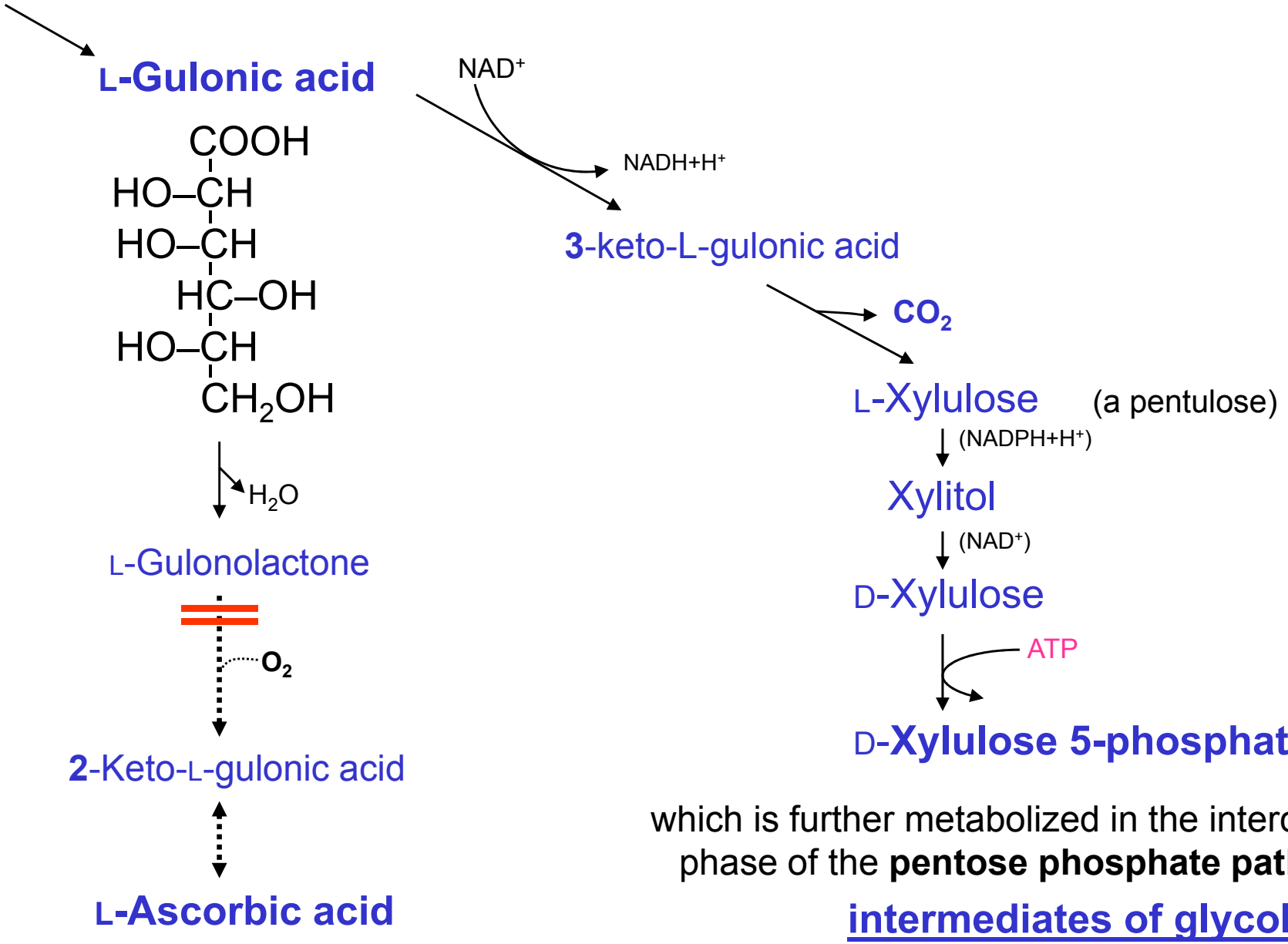
NADP**H**+**H**$^+$

CH_2OH
HC–OH
HO–CH
HC–OH
HC–OH
COOH

≡

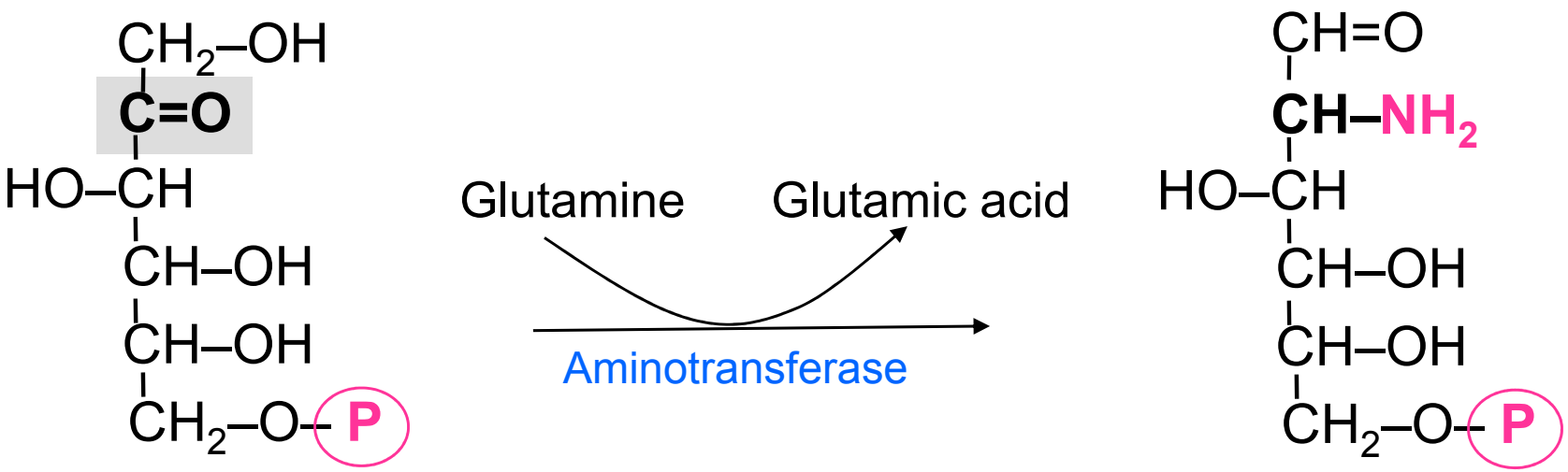
COOH
HO–CH
HO–CH
HC–OH
HO–CH
CH_2OH

L-Gulonic acid



which is further metabolized in the interconversion phase of the **pentose phosphate pathway** to the **intermediates of glycolysis**.

Synthesis of amino sugars



Fructose 6-phosphate

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

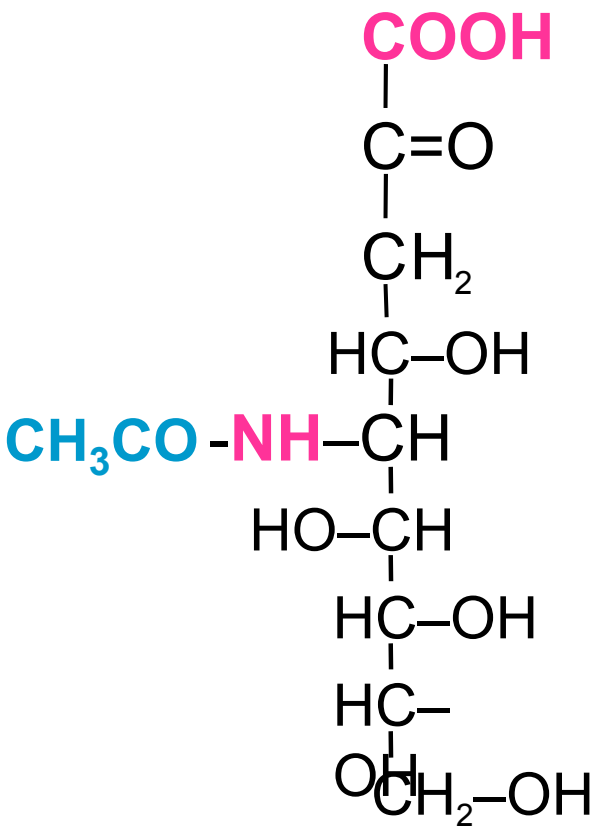
The basic amino groups **–NH_2** of amino sugars are nearly always "neutralized“ by acetylation in the reaction with acetyl-coenzyme A, so that they exist as ***N*-acetylhexosamines**.
Unlike amines, **amides** (**acetamido groups) are nor basic**.

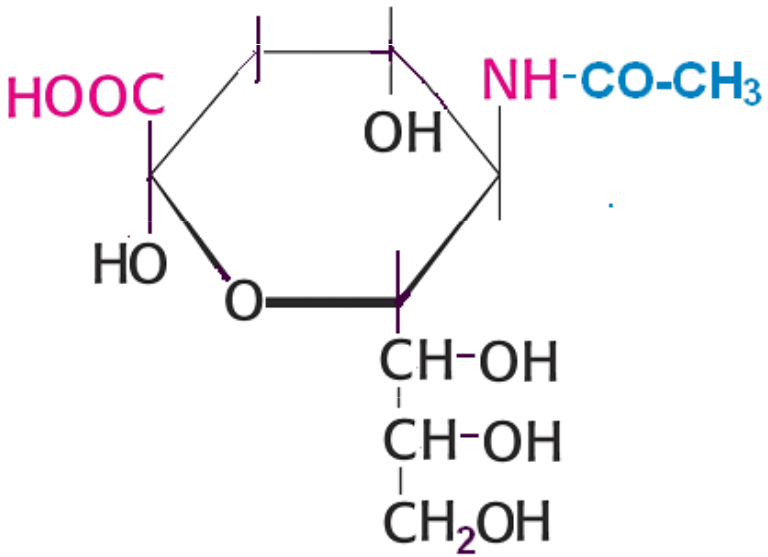
Synthesis of sialic acids

Sialic acids is the group name used for various
acylated derivatives of neuraminic acid (*N*- as well as *O*-acylated).

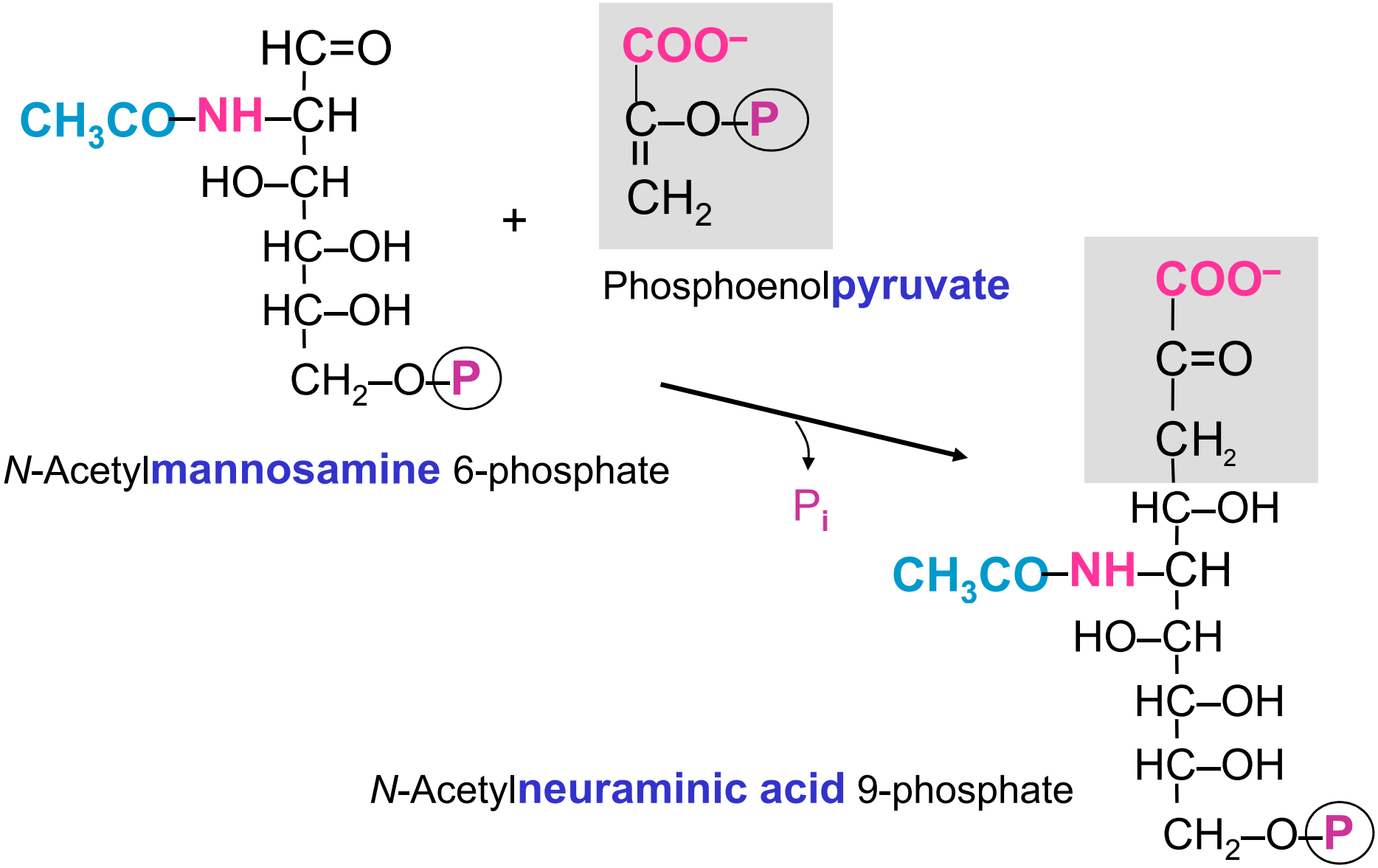
(Neuraminic acid is 5-amino-3,5-dideoxy-nonulosonic acid.)

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





Synthesis of sialic acid:

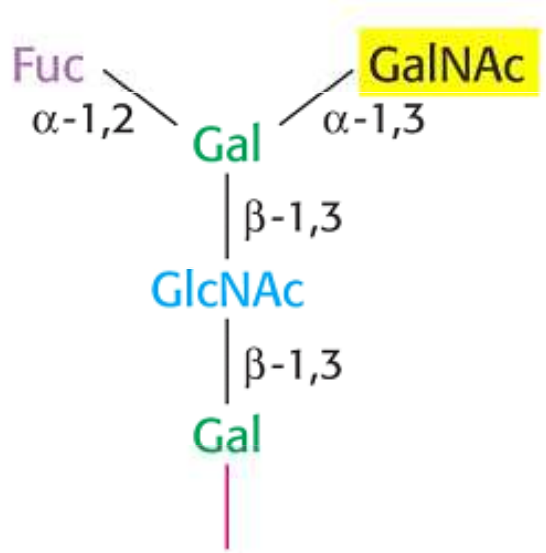


Saccharides found in glycoproteins and glycolipids

		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**

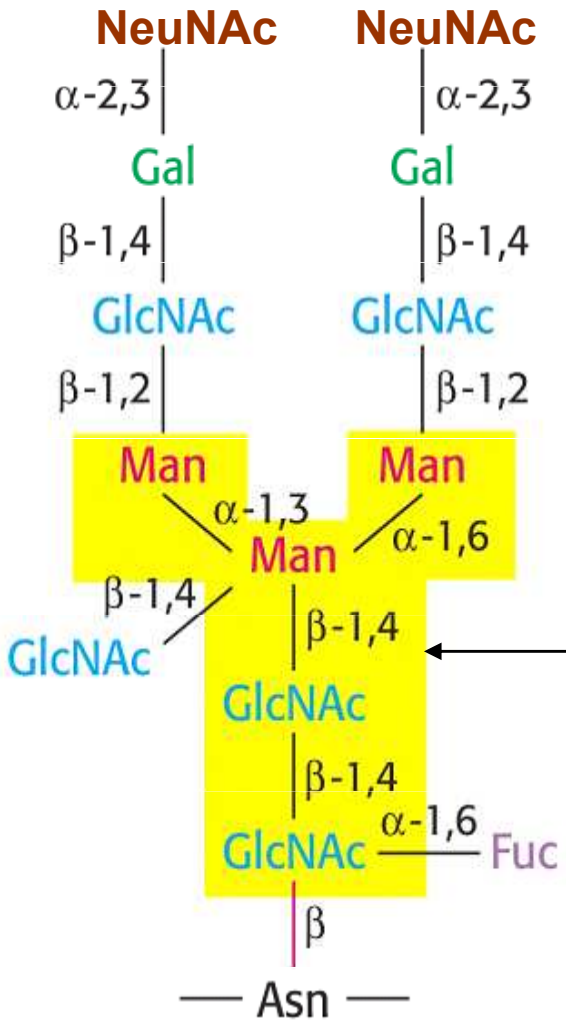
Examples of saccharidic component of glycolipids or glycoproteins:

Blood group substance A



Ceramide (**sphingolipid**) or protein

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

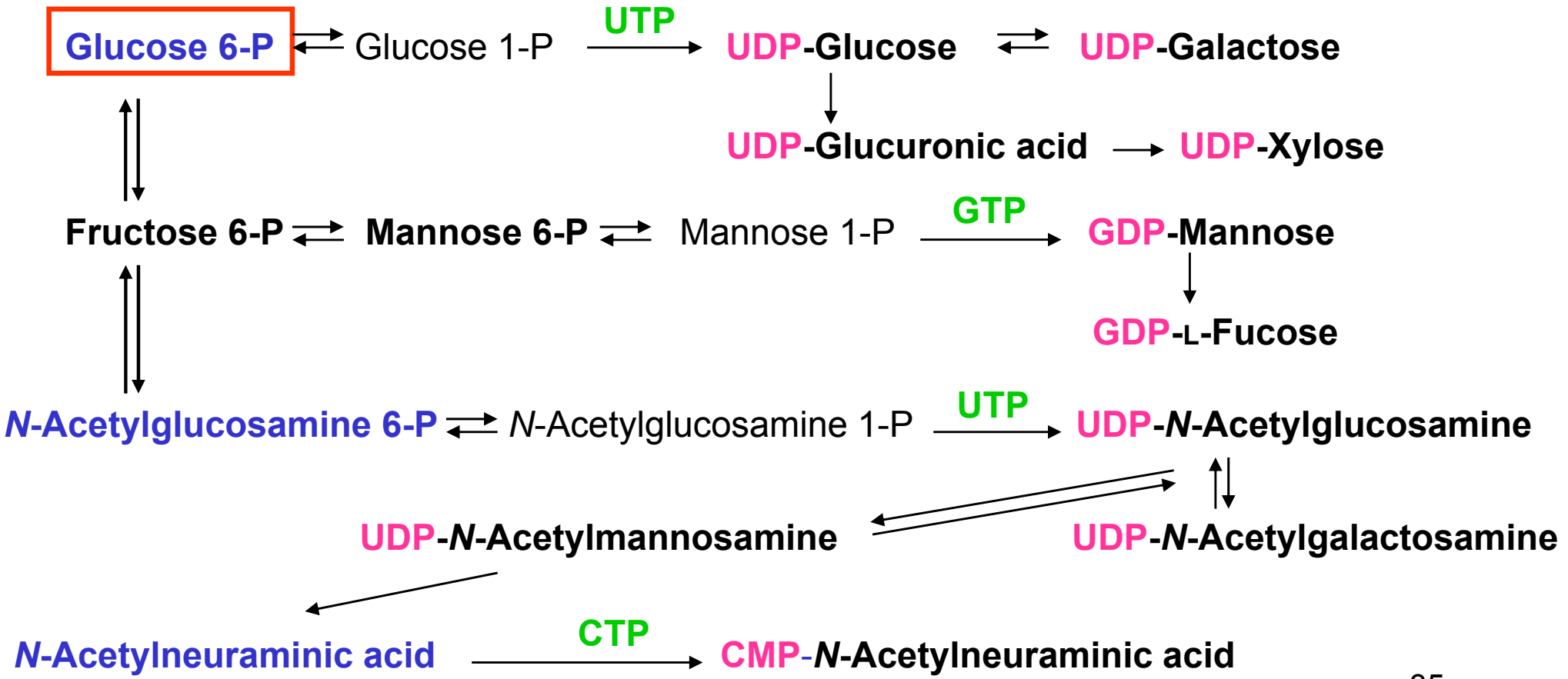


The boxed area encloses the pentasaccharide core common to all *N*-linked glycoproteins.

Glycosyl donors in glycoprotein synthesis

Before being incorporated into the oligosaccharide chains, monosaccharides involved in the synthesis of glycoproteins are **activated by formation of nucleotide sugars,** similarly to formation of UDP-glucose in the reaction of glucose 1-phosphate with UTP.

The glycosyls of these compounds can be transferred to suitable acceptors provided appropriate transferases are available.



A brief survey of major pathways in saccharide metabolism