Ministry of Public Health of Ukraine
Poltava State Medical University

Carbohydrate metabolism - 1. Glycolysis, aerobic oxidation of glucose, alternate pathways of monosaccharide metabolism.

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Lecture plan

- Pathways of glucose metabolism
- General characteristics of anaerobic and aerobic oxidation of glucose.

- Sequence of reactions and enzymes of glycolysis.

- Glycolytic oxidoreduction.

- Regulation of glycolysis.

-The reasons and consequences of hyperlactatemia and hyperpyruvatemia.

- Stages of aerobic oxidation of glucose.

- Oxidative decarboxylation of pyruvate.

- Multienzymatic pyruvate dehydrogenase complex.

- The comparative characteristic of aerobic and anaerobic oxidation of glucose.

- - Shuttle mechanisms of glycolytic NADH transportation.

- Pentose phosphate pathway.
- Glucuronic acid pathway of glucose.
- Metabolism of fructose.
- Metabolism of galactose.

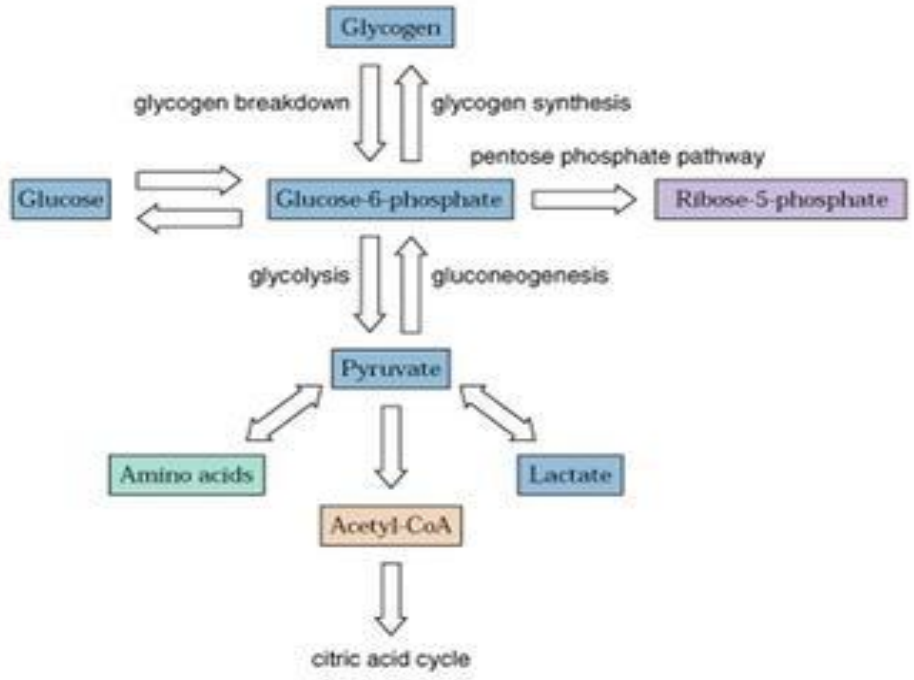
Pathways of glucose metabolism

- Aerobic glucose oxidation
- Anaerobic glucose oxidation

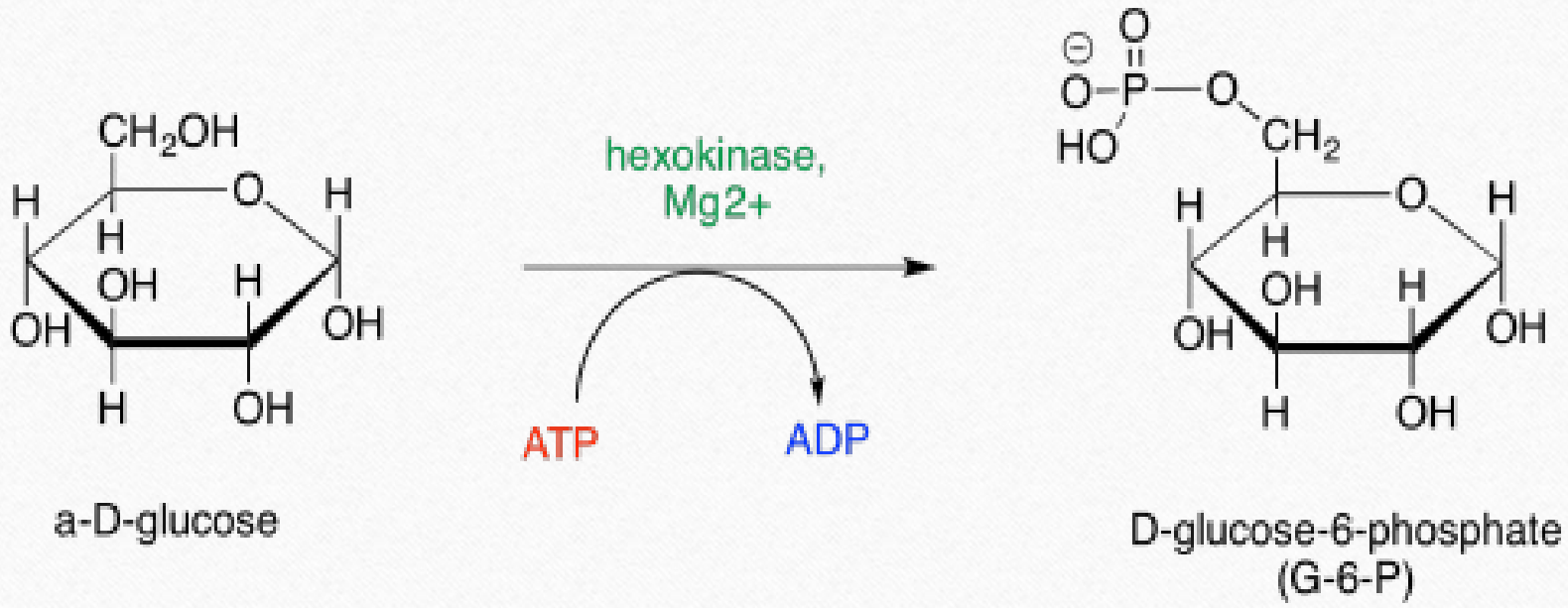
- Glycogenesis
- Lipogenesis

- Aminoacidogenesis
- Pentose phosphate pathway
- Glucuronic acid pathway

Overview of Glucose Metabolism



phosphorylation of glucose is catalyzed by hexokinase and glucokinase.



Glucose phosporylarion

Hexokinase - the first enzyme of glucose metabolism The presence of glucose in the cell is provided, first of all, by its facilitated diffusion from the blood into the cytosol with the participation of special transport proteins - glucose transporters (GluT).

Glucose activation

After moving through the membranes, glucose in the cytosol is immediately phosphorylated by the enzyme hexokinase, in connection with which the enzyme is figuratively called the "glucose trap".

Phosphorylation of glucose is important, because:

- glucose phosphate ester is unable to leave the cell, since the molecule is negatively charged and repels from the phospholipid surface of the membrane,
- the presence of a charged group ensures the correct orientation of the molecule in the active center of the enzyme,
- the concentration of free (non-phosphorylated) glucose in the cell decreases, which facilitates the diffusion of its new molecules from the blood.

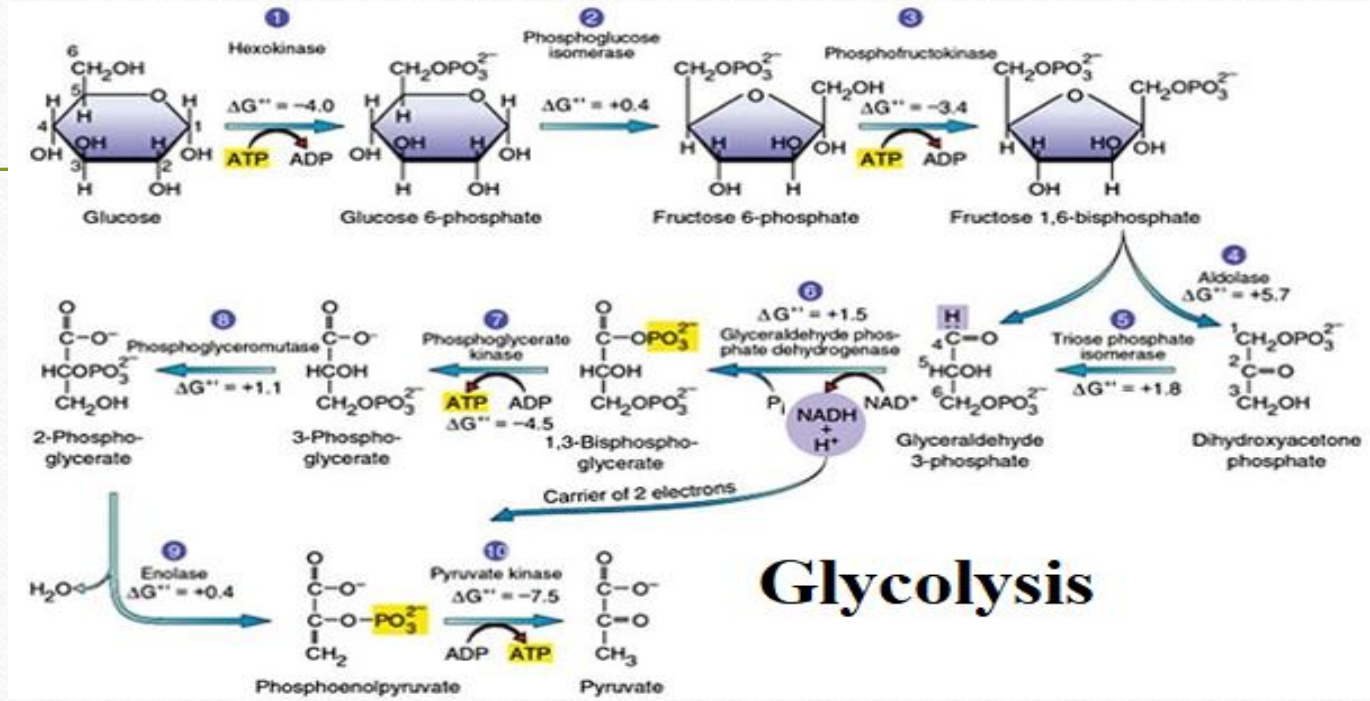
Glucose is phosphorylated in all cells, but there are fundamental differences in the metabolism of glucose in the liver from other tissues and organs.
This is due to the presence in tissues of various isoenzymes of hexokinase.
The liver is characterized by a special isoenzyme hexokinase IV, which received its own name - glucokinase.
The differences between this enzyme and hexokinases of other tissues are:

Difference between hexokinase and glucokinase

Hexokinase	*Glucokinase*
Present in extrahepatic tissue	Present in liver
High affinity for its substrate glucose (low K_m)	Low affinity for its substrate glucose (high K_m)
Inhibited by its product glucose-6-phosphate	No inhibition by its product glucose-6-phosphate
Its function is to ensure supply of glucose for the tissues, irrespective of blood glucose concentration	Its function is to remove glucose from the blood, when the blood glucose level increases (following meal)
Catalyze the phosphorylation of other hexoses like fructose, galactose, etc.	Specific for glucose
Its activity is not affected by insulin	It is an inducible enzyme that increases its synthesis in response to insulin

Glycolysis

- Glycolysis is the central pathway for the glucose catabolism in which glucose (6-carbon compound) is converted into pyruvate (3-carbon compound) through a sequence of 10 steps.
- Glycolysis is an oxygen-independent metabolic pathway.
- The first five steps of Glycolysis are regarded as the preparatory (or investment) phase, since they consume energy to convert the glucose into two three-carbon sugar phosphates
- The second half of glycolysis is known as the pay-off phase, characterized by a net gain of the energy-rich molecules ATP and NADH. Since glucose leads to two triose sugars in the preparatory phase, each reaction in the pay-off phase occurs twice per glucose molecule. This yields 2 NADH molecules and 4 ATP molecules, leading to a net gain of 2 NADH molecules and 2 ATP molecules from the glycolytic pathway per glucose.



https://www.biosciencenotes.com/glycolysis/

- **Functions of anaerobic Glycolysis :**

- **ATP** production

- **2,3 bisphosphoglycerate** as powerful effector of O_2 binding with haemoglobin in RBC is formed from 1,3 bisphosphoglycerate (glycolysis intermediate)

- **Anaerobic Glycolysis reactions:**

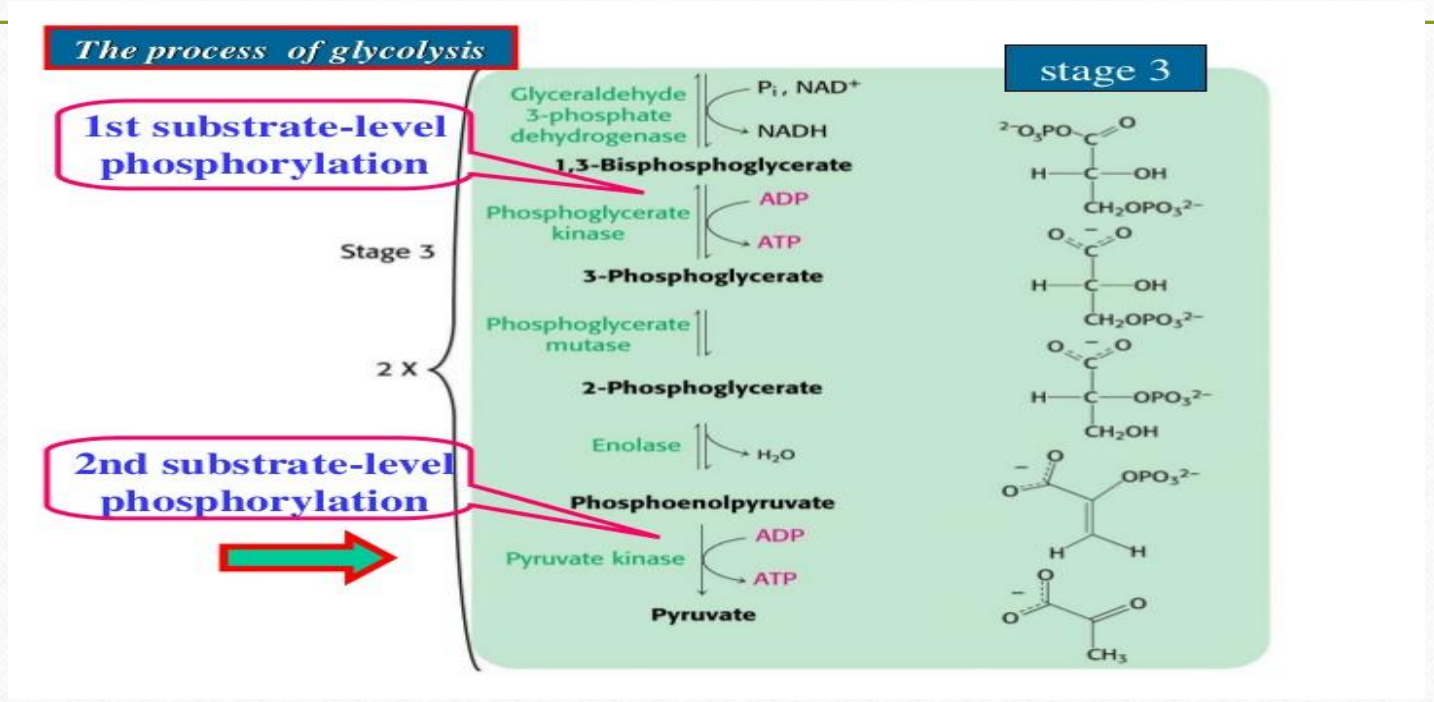
  All reactions of anaerobic glycolysis to pyruvate are the same as they are in aerobic glycolysis but one reaction is added else : Pyruvate is reduced by NADH to lactate

Net reaction for anaerobic Glycolysis:

$$C_6H_{12}O_6 + 2ADP + 2P_i \longrightarrow 2\ CH_3\text{-}CHOH\text{-}COOH + 2ATP$$

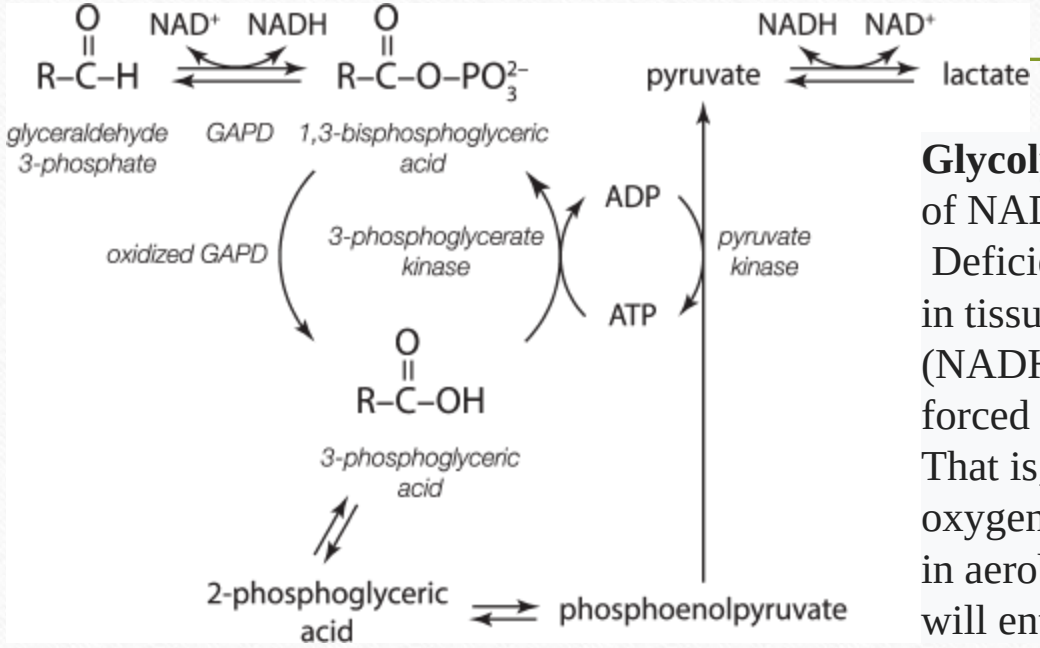
https://ppt-online.org/438129

Reactions of substrate-level phosphorylation



https://www.toppr.com/ask/question/respiratory-formation-of-atp-during-the-reactions-13diphosphoglyceric-acid3phosphoglyceric-acid/

Glycolytic oxidoreduction



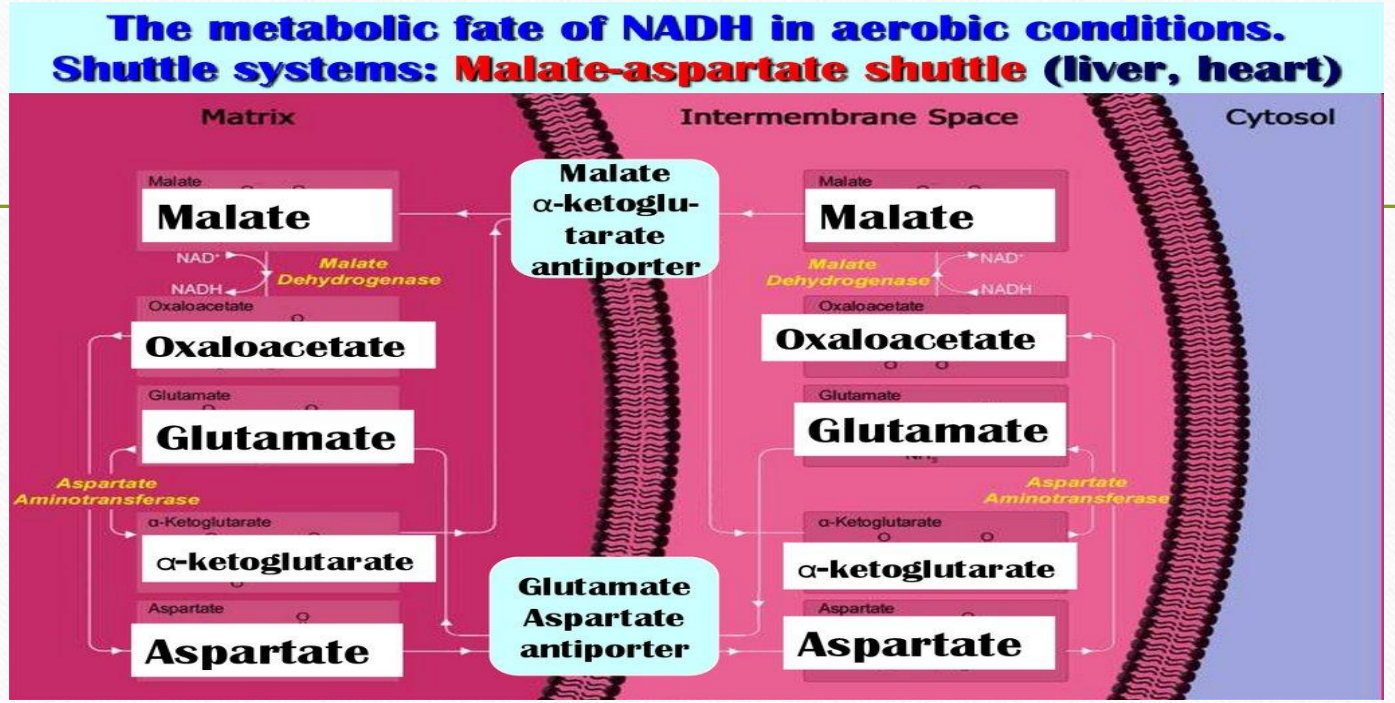
Glycolytic oxidoreduction is a process of cyclic reduction of NAD+ and oxidation of NADH under anaerobic conditions. Deficiency of oxygen as the ultimate acceptor of hydrogen in tissue respiration, prevents the oxidation of the reduced equivalent (NADH), which was formed in 6 reactions, so there is a forced reduction of pyruvate to lactate.

That is, the reduction of pyruvate to lactate is a forced in conditions of oxygen deficiency, because in aerobic conditions NADH, through the shuttle system, will enter the mitochondria in the respiratory chain.

Shuttle systems of glycolytic NADH transportation

Cytosolic NADH (reaction 6 of glycolysis) cannot
transfer hydrogen to the respiratory chain,
because the mitochondrial membrane is not permeable to it.
The transfer of hydrogen through the membrane occurs
using special systems called "shuttle" systems.
Hydrogen is transported across the membrane with the
participation of pairs of substrates bound by corresponding dehydrogenases,
on both sides of the mitochondrial membrane there is a specific dehydrogenase.
2 shuttle systems:

- malate-aspartate
- glycerole-3-phosphate.



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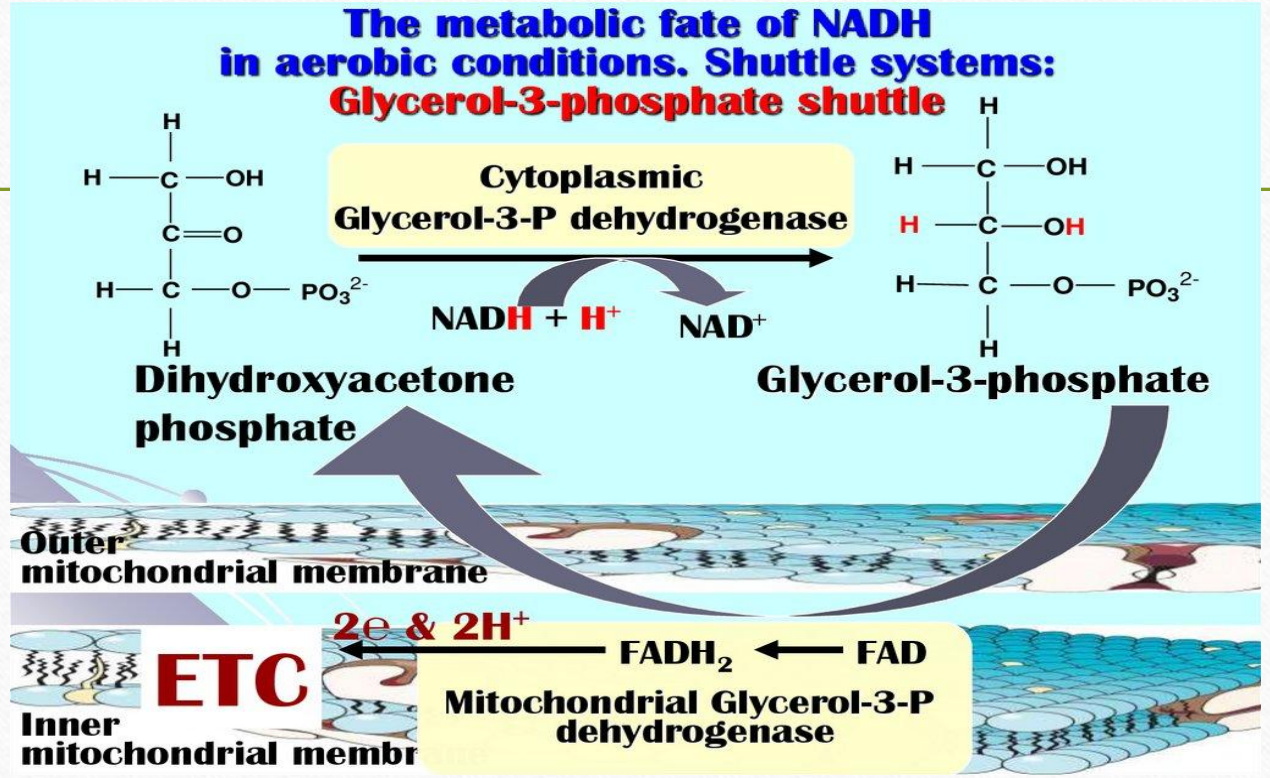

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Glycolysis is regulated at 3 steps involving non equilibrium reactions

- Step 1: **Hexokinase**
- Step 3: **Phosphofructokinase 1**
- Step 10: **Pyruvate kinase**

These three enzymes are key enzymes for Glycolysis

Specific effectors of Glycolysis

Enzyme	Activator	Inhibitor
Hexokinase (muscle) Glucokinase (liver)		G 6-P
PFK1	ADP, AMP (muscle), Pi, NH_4^+ ↑ F-2,6-biP (in the liver due to insulin)	ATP Citrate, PEP H^+ (low pH) ↓ F-2,6-biP (in the liver due to glucagon)
Pyruvate kinase	F-1,6-biP	ATP Acetyl-CoA Fatty acids Alanine -c-AMP dependent PK (in the liver due to glucagon)

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Allosteric Regulation of Glycolysis

Fast-acting & transient mechanism

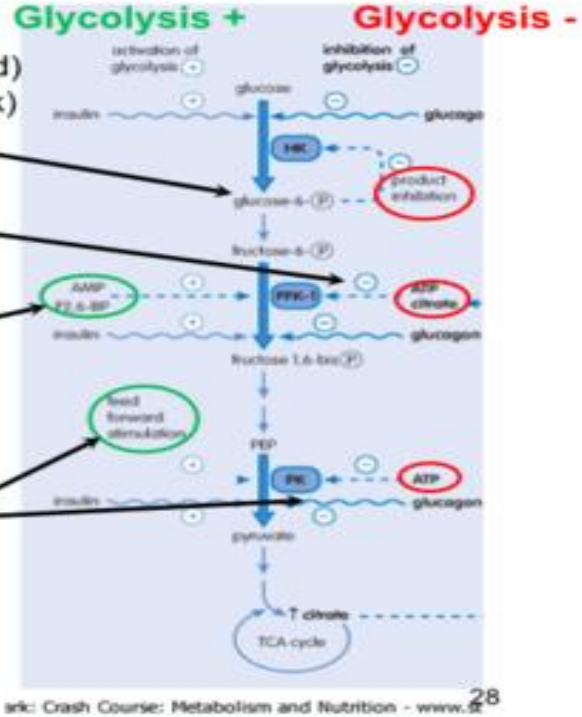
- Glucokinase activated by glucose (feed-forward)
- Hexokinase inhibited by glucose-6P (feed-back)

- PFK-1 inhibited by:
  - ATP (high energy charge)
  - citrate (from TCA; feed-back)

- PFK-1 activated by:
  - AMP (low energy charge)
  - fructose-2,6-BP

- Pyruvate kinase inhibited by:
  - ATP (high energy charge)

- Pyruvate kinase activated by
  - fructose 1,6-BP (feed-forward)

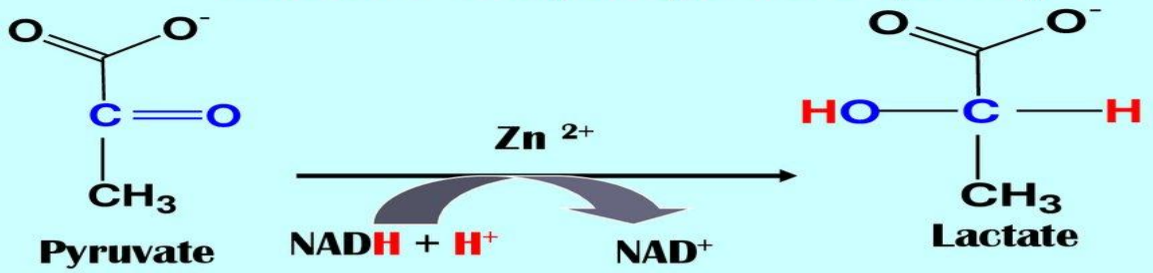


src: Crash Course: Metabolism and Nutrition - www.s

https://quizlet.com/278765967/biochemistry-chapter-22-flash-cards/

Anaerobic glycolysis last step

Lactate dehydrogenase (LDH)



Functional LDH are homo or hetero tetramers composed of M and H protein subunits:

LDH-1 (4H) - in the heart (at hypoxia), renal cortex & RBCs
LDH-2 (3H1M) - in the reticuloendothelial system
LDH-3 (2H2M) - in the lungs
LDH-4 (1H3M) - in the kidneys, placenta and pancreas
LDH-5 (4M) - in the liver and skeletal muscles

Causes and consequences of hyperlactatemia

Normal blood lactate concentration: 0,55-2,22 mmol/L

- In health, blood lactate concentration is maintained within the approximate range of 0.5-1.5 mmol/L . Exercise represents a physiological process in which this balance is temporarily upset due to the rapid increase in lactate production by muscle cells in temporary oxygen debt. In severe exercise, blood lactate may rise to levels in excess of 20 mmol/L but due to the capacity for rapid lactate disposal, in health this rise is only transitory.

Causes of hyperlactatemia:

- Hypoxia
- Liver failure (hepatitis, cirrhosis)
- Prolonged physical activity
- Decompensated diabetes mellitus
- Glycogenosis
- Anemia
- Leukemia
- Convulsions (epilepsy, tetanus)
- Acute blood loss
- Malignant neoplasms (Warburg effect)
- Intoxication
- Chronic alcoholism

Consequences of hyperlactatemia:

Development of metabolic acidosis (lactic acidosis) •

Ways of utilization of lactate:

The liver is the main organ responsible for the disposal of lactate from the bloodstream. In hepatocytes, mainly lactate through oxidation to pyruvate under conditions hyperlactatemia is used in gluconeogenesis. Also lactate is utilized by the kidneys.

Aerobic Glycolysis

- **Definition:**
  Aerobic Glycolysis is the **metabolic pathway** in which monosaccharides (mainly **glucose**) are split **into** two molecules of **pyruvate**
- **Location** in the body : **all type cells**
- **Location** within the cell : **cytosol**
- **Substrates:** Glucose, galactose, fructose
- **Products:** 2 pyruvates & 2 ATP & 2 NADH

Net reaction for aerobic Glycolysis:

$$C_6H_{12}O_6 + 2\ NAD^+ + 2ADP + 2P_i \rightarrow 2\ CH_3\text{-}CO\text{-}COOH + 2\ NADH + 2H^+ + 2ATP$$

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Functions of aerobic Glycolysis :

1) to convert glucose to **pyruvate** which can be:
   - burned for **energy** (due to PDH and TCA)
   - or converted to **fatty acids, cholesterol, amino acids** synthesis, etc.

2) such intermediate as **dihydroxyacetone phosphate** can be reduced to glycerol phosphate either
   - for use in the **biosynthesis** of **lipids** or
   - for **reducing equivalents transfer** from cytosolic NADH into mitochondrion (glycerol phosphate shuttle)

3) the reversible reactions of glycolysis in opposite direction of duration are used for **gluconeogenesis**

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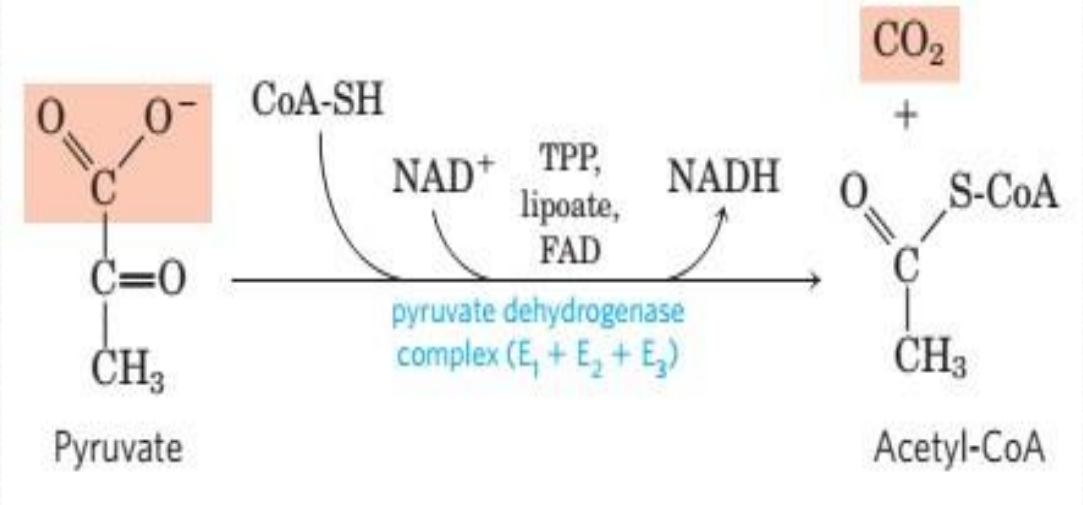
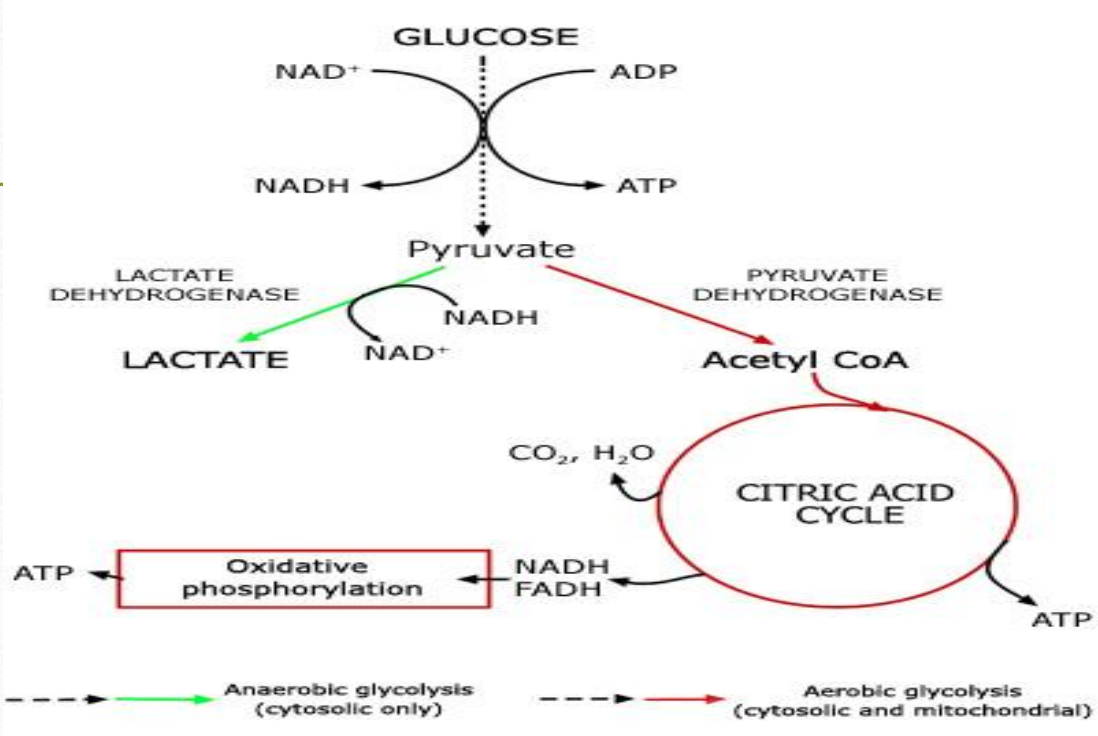
Oxidative decarboxylation of pyruvate



http://www.biosciencenotes.com/oxidative-decarboxylation-of-pyruvate/



https://acutecaretesting.org/en/articles/lactate-and-lactic-acidosis

Structure of pyruvate dehydrogenese complex

Enzymes	Abbrev.	Cofactors	# subunits prokaryotes	# subunits eukaryotes
pyruvate dehydrogenase (EC 1.2.4.1)	E1	TPP (thiamine pyrophosphate)	24	30
dihydrolipoyl transacetylase (EC 2.3.1.12)	E2	lipoate coenzyme A	24	60
dihydrolipoyl dehydrogenase (EC 1.8.1.4)	E3	FAD NAD^+	12	12

https://en.wikipedia.org/wiki/Pyruvate_dehydrogenase_complex#:~:text=Pyruvate%20dehydrogenase%20complex%20(PDC)%20is,a%20process%20called%20pyruvate%20decarboxylation.&text=This%20multi-enzyme%20complex%20is,acid%20dehydrogenase%20multi-enzyme%20complexes.

Causes of hyperpyruvatemia

Normal blood pyruvate concentration: 0,03-0,1 mmol/L

Causes of hyperpyruvatemia:

- **Primary:**

- Mitochondrial diseases
- Pyruvate dehydrogenase deficiency
- Pyruvate carboxylase deficiency
- Congenital lactic acidosis

- **Secondary:**

- Decompensated diabetes mellitus
- Hypo – and avitaminosis of thiamine, riboflavin, pantothenate, lipoate, niacin.
- Hepatic failure
- Hypoxia

Comparative characteristics of aerobic oxidation of glucose (to CO_2&H_2O) and anaerobic glycolysis energy balance

Aerobic oxidation of glucose (to CO_2&H_2O)

I. Glycolysis stage:

- 2 ATP (used for phosphorylation of glucose & fructose 6-P)

+ 4 ATP (produced by 1,3 bis P-glycerate and pyruvate kinases)

+ 6 ATP (if malate-aspartate shuttle translocates electrons from 2 NADH for oxidative phosphorylation (OP))

or + 4 ATP (if glycerol-3-phosphate shuttle translocates electrons from 2 NADH for OP)

= 8 (or 6)

II. Oxidative decarboxylation of pyruvate stage (2 pyruvates enter) :

+ 6 ATP (due to utilization of 2 NADH: OP)

III. Krebs cycle (2 acetyl CoA enter) **stage:**

+ 18 ATP (due to utilization of 6 NADH for OP)
+ 4 ATP (due to utilization of 2 $FADH_2$ for OP)
+ 2 ATP (due to 2 GTP conversion)
= 24

In all = 38 (or 36) ATP

Anaerobic glycolysis:

- 2 ATP (used for phosphorylation of glucose & fructose 6-P)

+ 4 ATP (produced by 1,3 bis-P-glycerate kinase and pyruvate kinase)

2 NADH are not used for oxidative phosphorylation but are consumed in LDH reaction

In all = 2 ATP

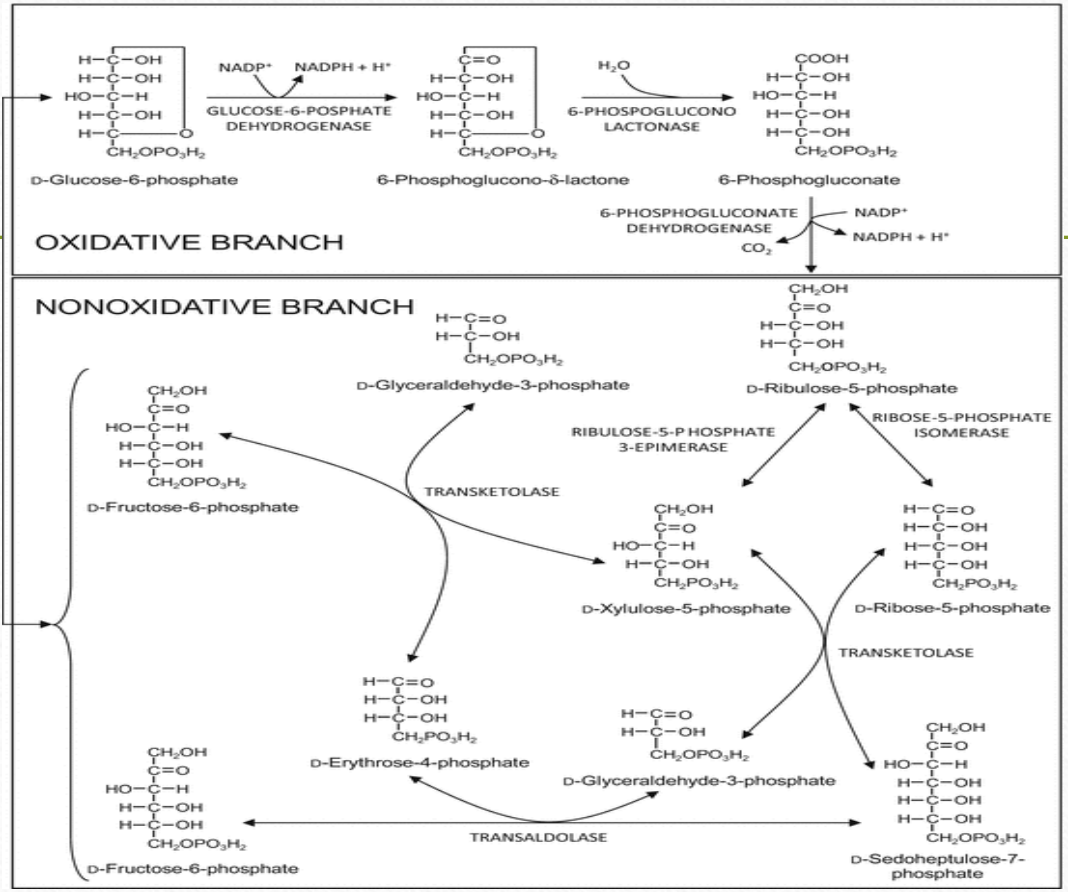
Glycolysis is normally faster than the TCA cycle capacity, and lactate is the usual product of glycolysis even in resting muscle.

The lactate/pyruvate ratio is about 10 in resting muscle, but in working muscle this ratio may hit 200

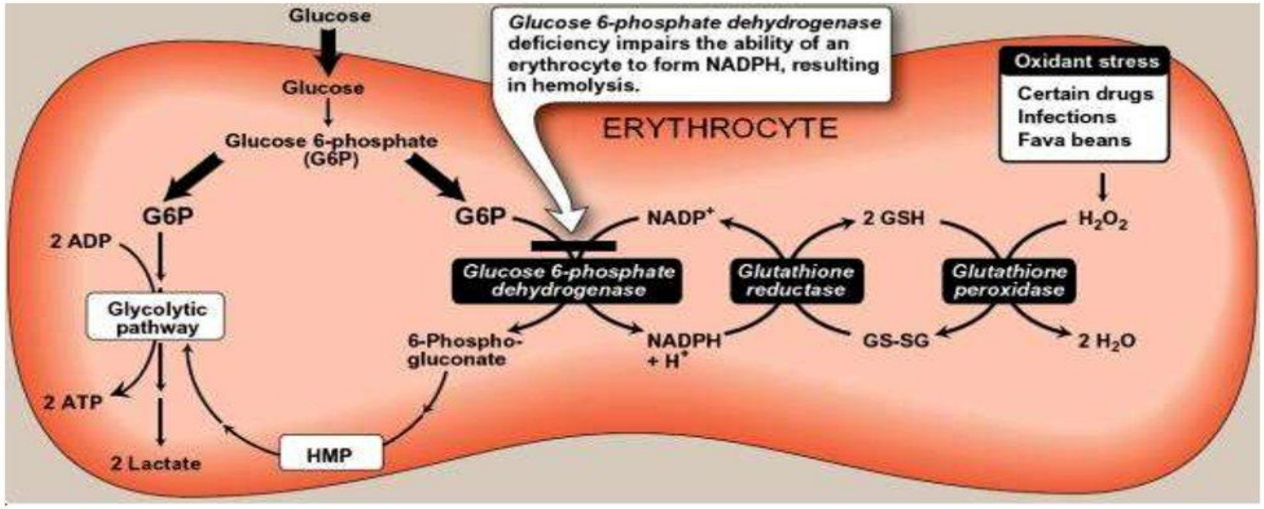
Pentose phosphate pathway

- Production of reduced NADPH2 for reductive biosynthesis (fatty acids, cholesterol, etc.).
- Synthesis of pentose phosphates for the formation of nucleotides, nucleic acids and some coenzymes.
- Synthesis of monosaccharides with the number of carbon atoms from 3 to 8.
- Detoxification of xenobiotics - NADPH2 is required.
- NADPH2 is a coenzyme of glutathione reductase, which is part of the glutathione antioxidant system of cells to protect against reactive oxygen species, which prevents the peroxidation of membrane lipids. A deficiency of glucose-6-phosphate dehydrogenase increases hemolysis, which leads to the development of hemolytic jaundice.

Pentose phosphate pathway



Violation of pentose phosphate pathway



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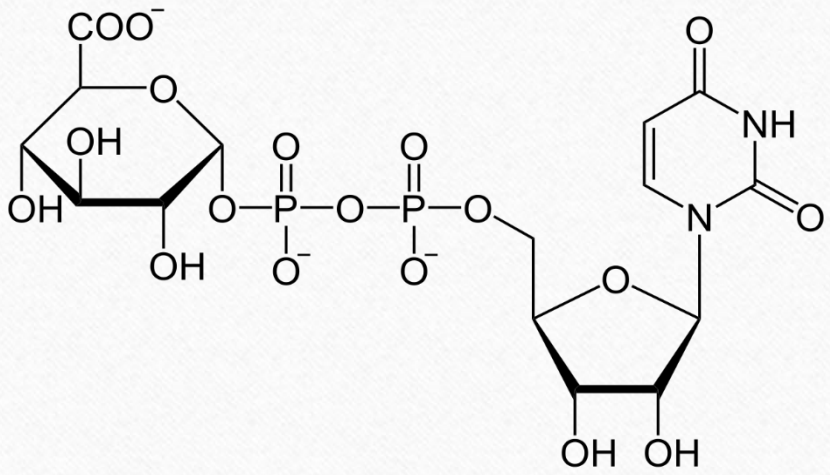
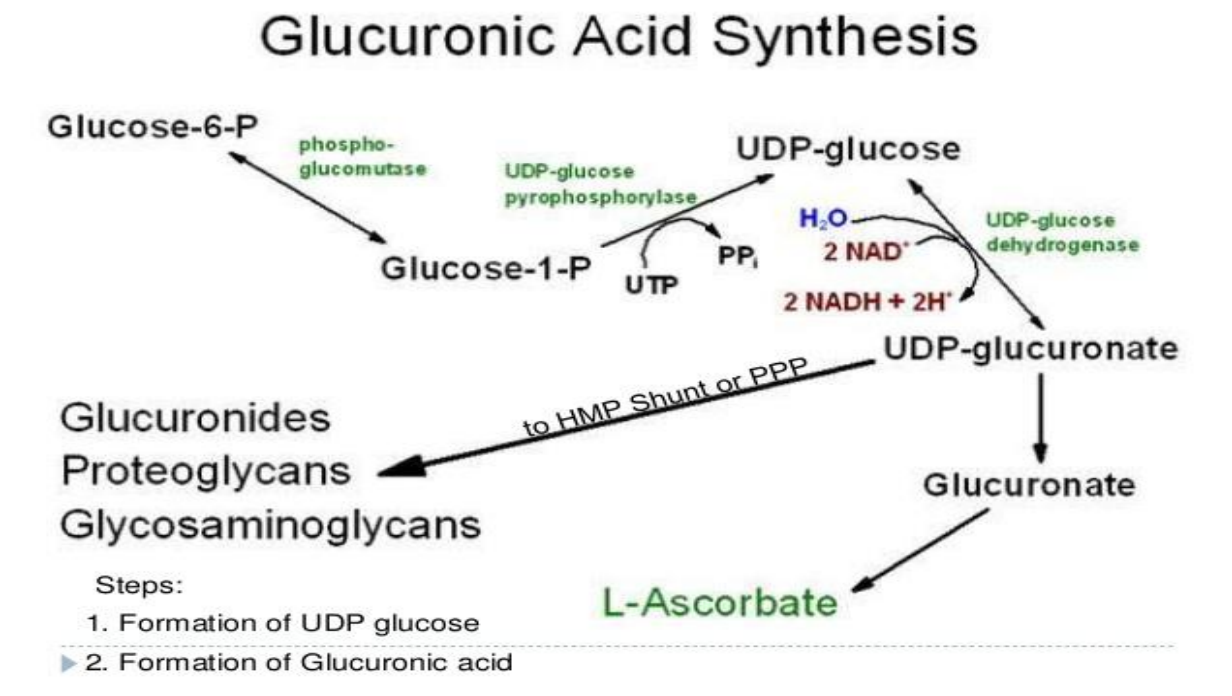
Glucuronic acid pathway

Biological role of glucuronic acid

- UDP-glucuronate participates in the detoxification of xenobiotics (phase II) by conjugation with the formation of glucuronides (detoxificative function of liver);

- UDP-glucuronate is used for the biosynthesis of glycosaminoglycans (GAG) which are part of the glycoconjugates of the intercellular matrix of connective tissue

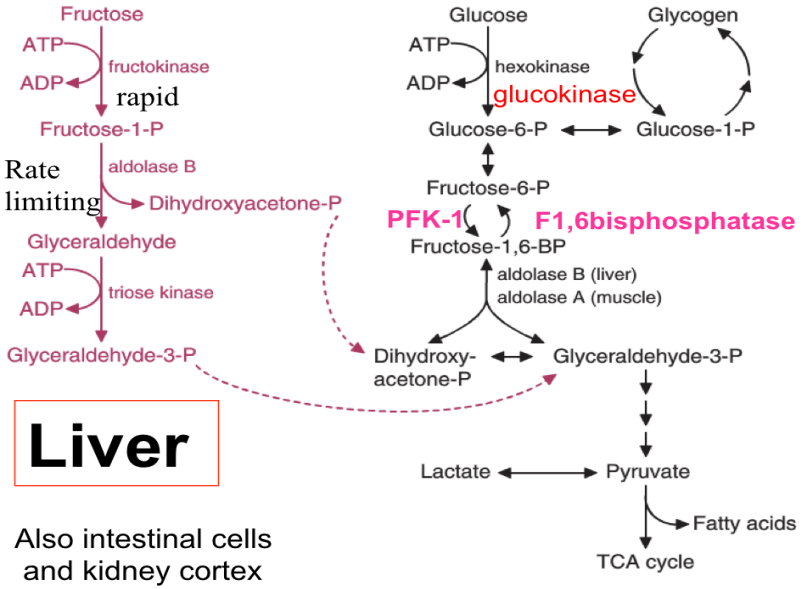




https://www.slideshare.net/DiwakarSharma71/ppp-glucuronate-pathway-lactose-synthesis

L-ascorbate is not synthesized in the human body!!!!!

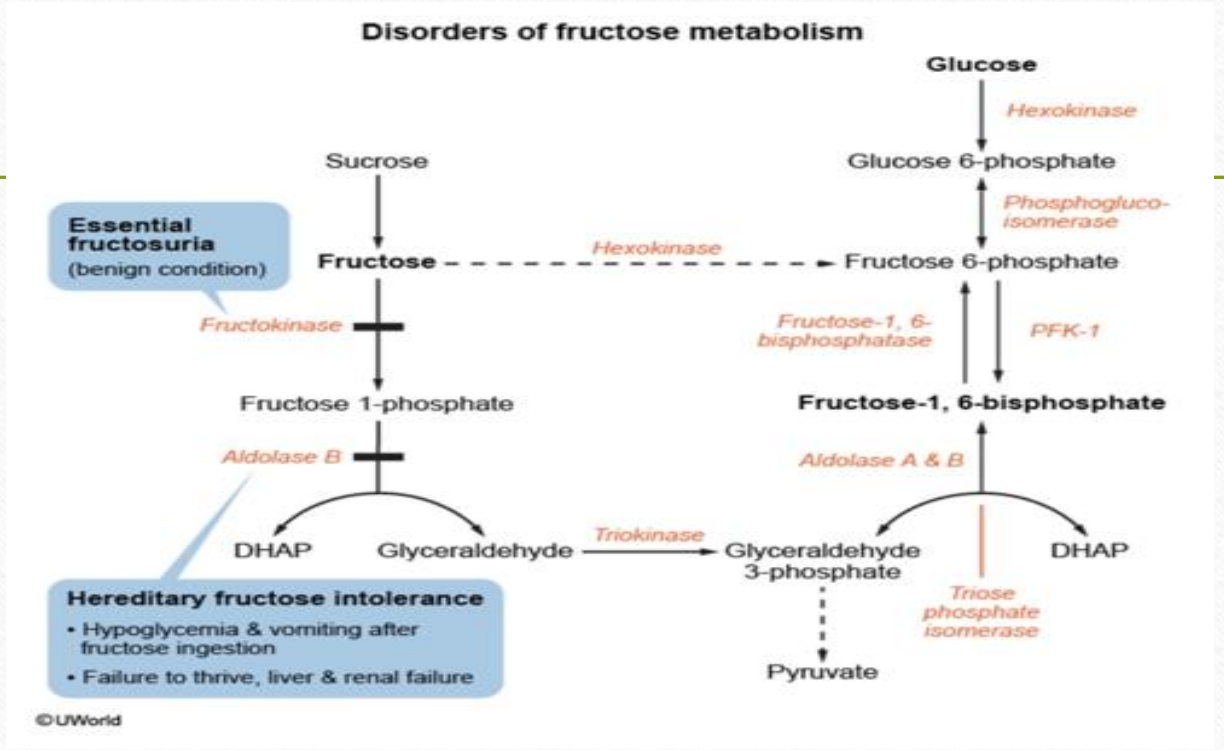
Metabolism of dietary fructose



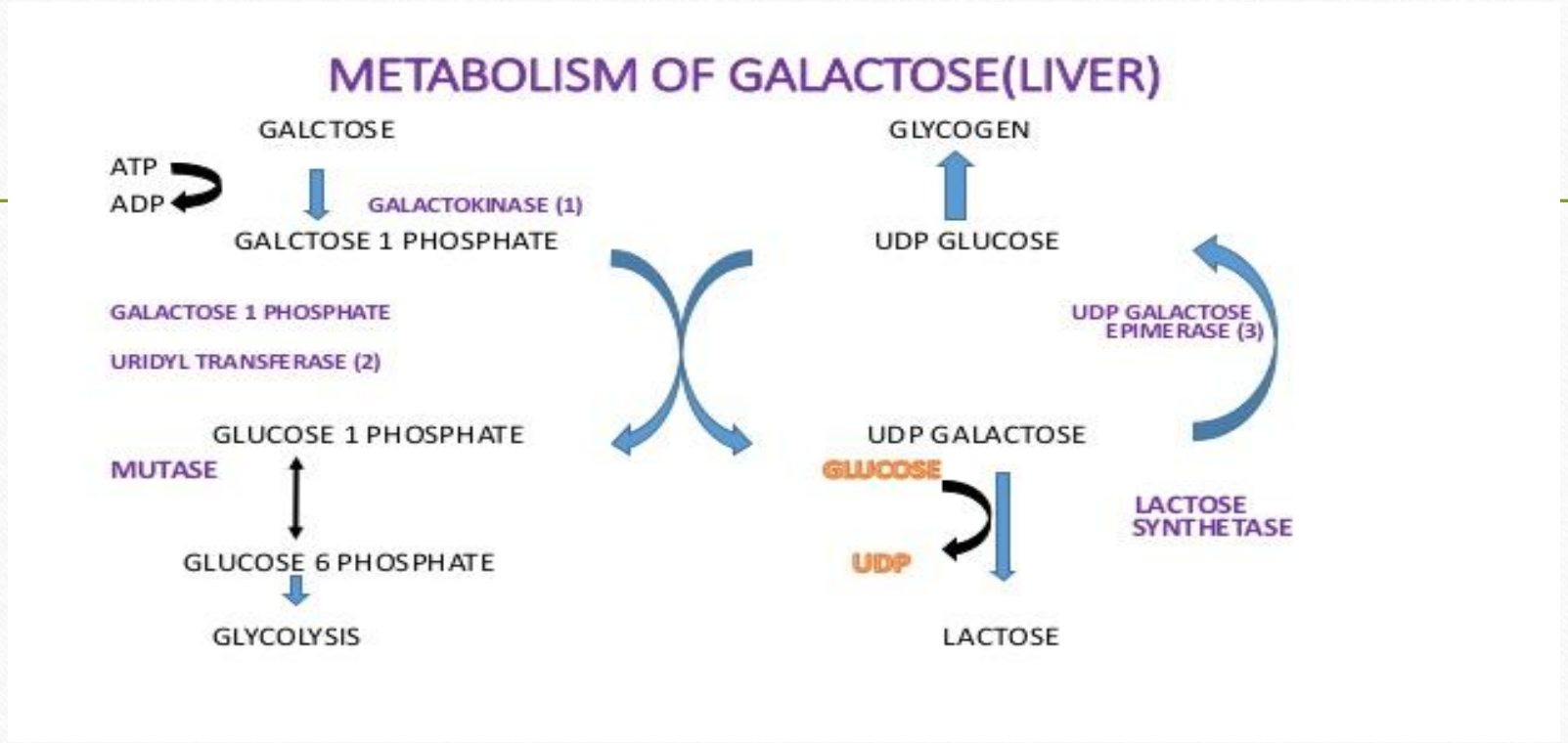
Liver

Also intestinal cells and kidney cortex

- Fructose metabolism occurs mainly in liver
- Fructokinase rapidly converts fructose to fructose-1-P
- Fructose 1-P is cleaved as glycolysis intermediates
- Aldolase B is the rate-limiting enzyme



https://quizlet.com/pl/303050811/fructose-and-galactose-metabolism-disorders-flash-cards/



https://microbenotes.com/galactose-metabolism/

Disorders of galactose metabolism

Galactosemia

	Enzyme	Name
Type I	Galactose-1-phosphate uridyl transferase	Classic galactosemia
Type II	Galactokinase	Galactokinase deficiency
Type II	UDP galactose epimerase	Galactose epimerase deficiency

- Autosomal recessive
- Caused by the deficiency in an enzyme responsible for adequate galactose metabolism
- Toxic levels of galactose-1-phosphate result in hepatomegaly, cirrhosis, brain damage, cataracts, renal failure, hypoglycemia.
- Without treatment, mortality in infants with galactosemia is about 75%.

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Sources of information

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