BIOCHEMISTRY **1st Year, 2nd Semester**

Prof. Catalin MARIAN, MD PhD Dr. Habil.
Email: cmarian@umft.ro

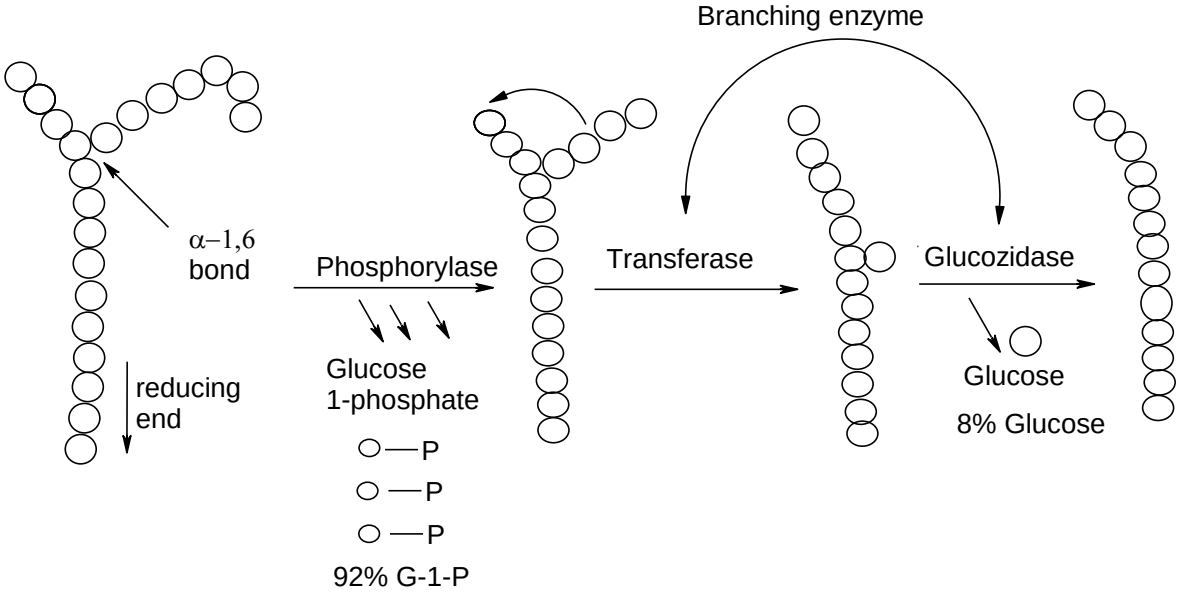
Lecture 5

II.2.5.2. Glycogenolysis

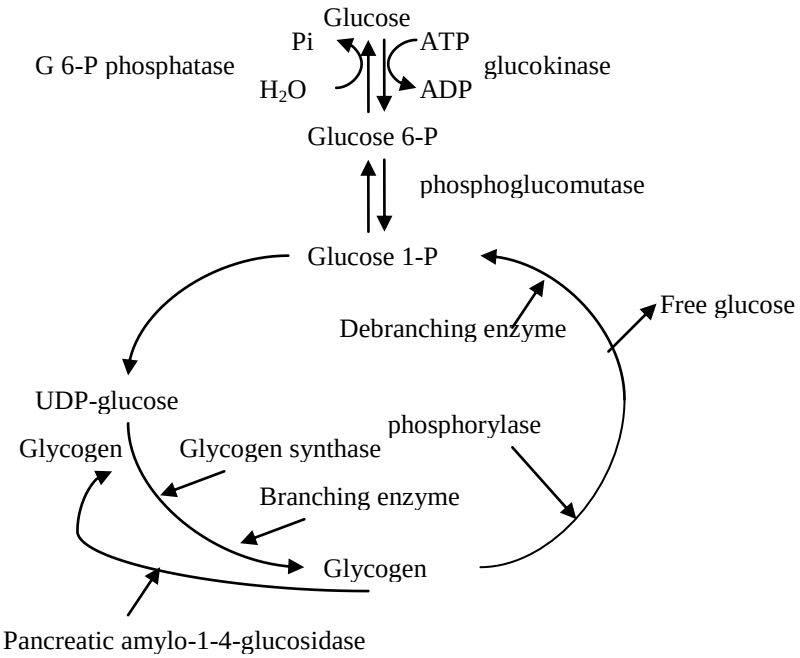
This is the metabolic pathway that mobilizes glucose from glycogen. The end product of this pathway is different according to tissue, as follows:

- Glucose is the end product in the liver, being released in the blood stream and used by tissues, especially insulin dependent tissues.
- In the muscle, the end product is glucose-6-phosphate which is being used for own energy needs of the muscle, this tissue lacking the glucose-6-phosphatase enzyme.

Glycogenolysis is not the reverse pathway of glycogen synthesis. The key enzyme of this process is glycogen **phosphorylase**, which breaks the alpha 1-4 glycosidic bonds at the non-reducing ends and phosphorylates glucose into glucose-1-phosphate. This action continues until it reaches the fourth glucose residue from a branch, when another enzyme called the debranching enzyme will act on the alpha 1-6 glycosidic bonds. Then glucose-1-P → glucose-6-P → glucose which is released in the blood stream.

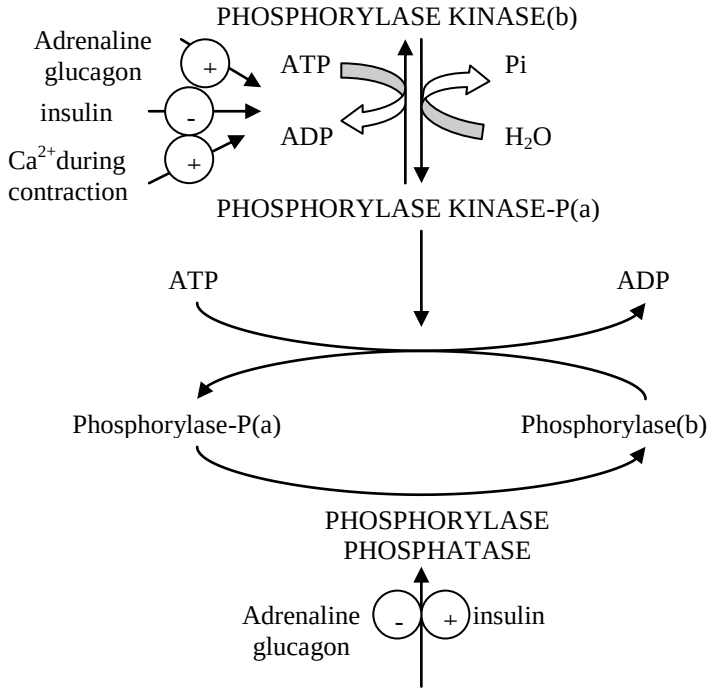


These reactions take place in the liver, kidney and secondarily in the intestine, the only tissues having the enzyme glucose-6-phosphate phosphatase. Because skeletal muscles lack this enzyme, the end product is glucose-6-phospate which cannot pass through the cell membrane and will remain in the muscle where it will be used for energy production.



Amylo-alpha-1-4-glucosidase from the pancreas will act after long periods of starvation, when rapid and sustained glycogen restoring is needed after a meal. This enzyme will cut the remaining glycogen into smaller dextrin molecules that will act as primers for synthesis of more glycogen in order to rapidly increase the glycogen deposits.

Glycogen phosphorylase is active in a phosphorylated form, realized by a phosphorylase kinase, which is in turn regulated by phosphorylation-dephosphorylation, its active form being phosphorylated.



The two sides of glycogen metabolism are interconnected, acting in a coordinated fashion. Glycogen synthesis increases glycogen deposits by using excess glucose from food (liver) or from lactate (muscle). Glycogenolysis mobilizes the glucose from the glycogen deposits, releasing it in the blood stream from the liver, and in the muscle it is being used as glucose-6-phosphate for energy production. In this case, there is no need to consume an ATP to activate glucose for glycolysis, because it is already in the form of glucose-6-phosphate.

II.2.5.3. Regulation of glycogen metabolism

1. Substrate regulation

The precursor glucose-6-phosphate will stimulate gluconeogenesis, while the end product glucose will inhibit glycogenolysis. In practical terms, glycemia is controlling the glycogen metabolism in the liver. In the muscle, the increase of calcium concentration during contraction will stimulate glycogenolysis, thus synchronizing the intensity of glycogen mobilization with the muscle contraction.

2. Enzymatic regulation

The key enzymes of glycogen metabolism are glycogen synthase and glycogen phosphorylase. Both are regulated by phosphorylation-dephosphorylation, and the enzymes controlling these transformations (kinases and phosphorylases) are also regulated by phosphorylation-dephosphorylation. The phosphorylation-dephosphorylation cascades are initiated by hormonal actions or calcium. While glycogen synthase is active in a dephosphorylated state, glycogen phosphorylase is active in a phosphorylated state, and thus the action of the kinase that will phosphorylated both these enzymes will inactivate the synthase (blocking glycogen synthesis) and activate the phosphorylase (activate glycogenolysis). This process will have a coordinated effect, the mobilization of glycogen.

3. Hormonal regulation

Glycogen metabolism is influenced by the hypo- and hyper-glycemic hormones. These hormones will bind to specific cellular receptors and initiate cascades of phosphorylation-dephosphorylation which will influence the activity of key enzymes of glycogen metabolism. Through this mechanism, insulin stimulates glycogen synthesis and inhibits glycogenolysis, therefore lowering blood glucose concentrations and increasing glycogen deposits. The hyperglycemic hormones have an opposite effect, which specific actions on liver and myocardium (glucagon) or skeletal muscles and liver (adrenalin).

II.2.5.4. Pathology related to glycogen metabolism

The pathological conditions are due to genetic enzymatic defects, affecting the proper function of the enzymes involved in various phases of glycogen metabolism. The pathological manifestations are due to glycogen accumulation in tissues, abnormal forms of glycogen or impaired use of glycogen.

According to the affected enzyme there are several types of pathological conditions, generically called **glycogenoses** (Table 5).

Table 5. Glycogen metabolism pathology

Glycogenosis type	Name of disease	Affected enzyme	Pathologic manifestations
1	V.Gierke	Glucose-6-phosphate phosphatase	Hypoglycemia, ketosis
II	Pompe	Amylo-1,4-glucosidase	Generalized glicogenosis
III	Forbes - Cori	Branching enzyme	Hepatosplenomegaly, extra-branched glycogen
IV	Anderson	Branching enzyme	Unbranched glycogen
V	Mc. Ardle	Muscle phosphirylase	Muscle glycogen of 4% Blocking muscle effort
VI	Hers	Liver phosphorylase	Hepatic glicogenosis

II.2.6. Pentose phosphates pathway

Carbohydrates are not only substrates for energy, they also participate in the synthesis of nucleotides, glycolipids and glycoproteins.

The pentose phosphates pathway occurs in the cytosol and uses as precursor glucose-6-phosphate to produce ribose-5-phosphate and $NADPH,H^+$. These two products are extremely important; ribose-5-phosphate is used in the synthesis of nucleotides and $NADPH,H^+$ is the source of hydrogen for synthesis reactions.

Fatty acids and cholesterol synthesis needs $NADPH,H^+$ and occurs in the liver, suprarenal glands, adipose tissue, sexual glands, mammary gland during lactation, these being also where the pentose phosphate pathway is taking place. $NADPH,H^+$ is also involved in antioxidant reactions and therefore the tissues exposed to high oxygen concentrations such as erythrocytes and cornea also intensely use the pentose phosphates pathway.

This pathway has two phases: **oxidative** and **non-oxidative** (redeeming phase). The end products ribose-5-phosphate and $NADPH,H^+$ are produced during the oxidative phase. Excepting during cell division, the cell's needs for $NADPH,H^+$ generally exceeds that of ribose-5-phosphate, and therefore the cell redeems the excess ribose-5-phosphate by converting it into glucose-6-phosphate during the non-oxidative phase.

Regulation of the pathway

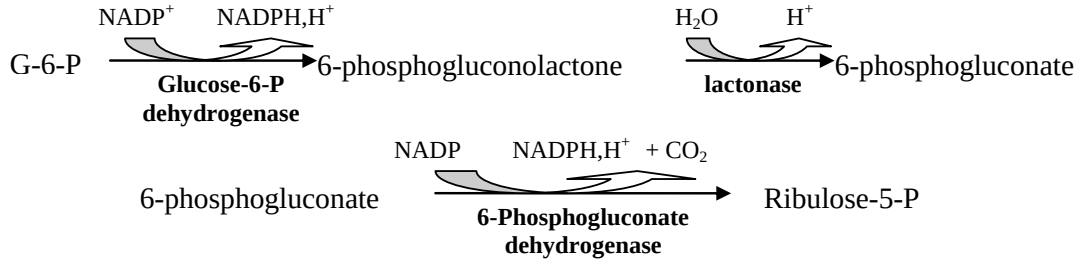
The pentose phosphates pathway is active during food intake due to the glucose excess. The key enzymes of this pathway are glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase. These are activated by NADP and insulin, and are inactivated by acyl-CoA and hyperglycemic hormones.

Related pathology.

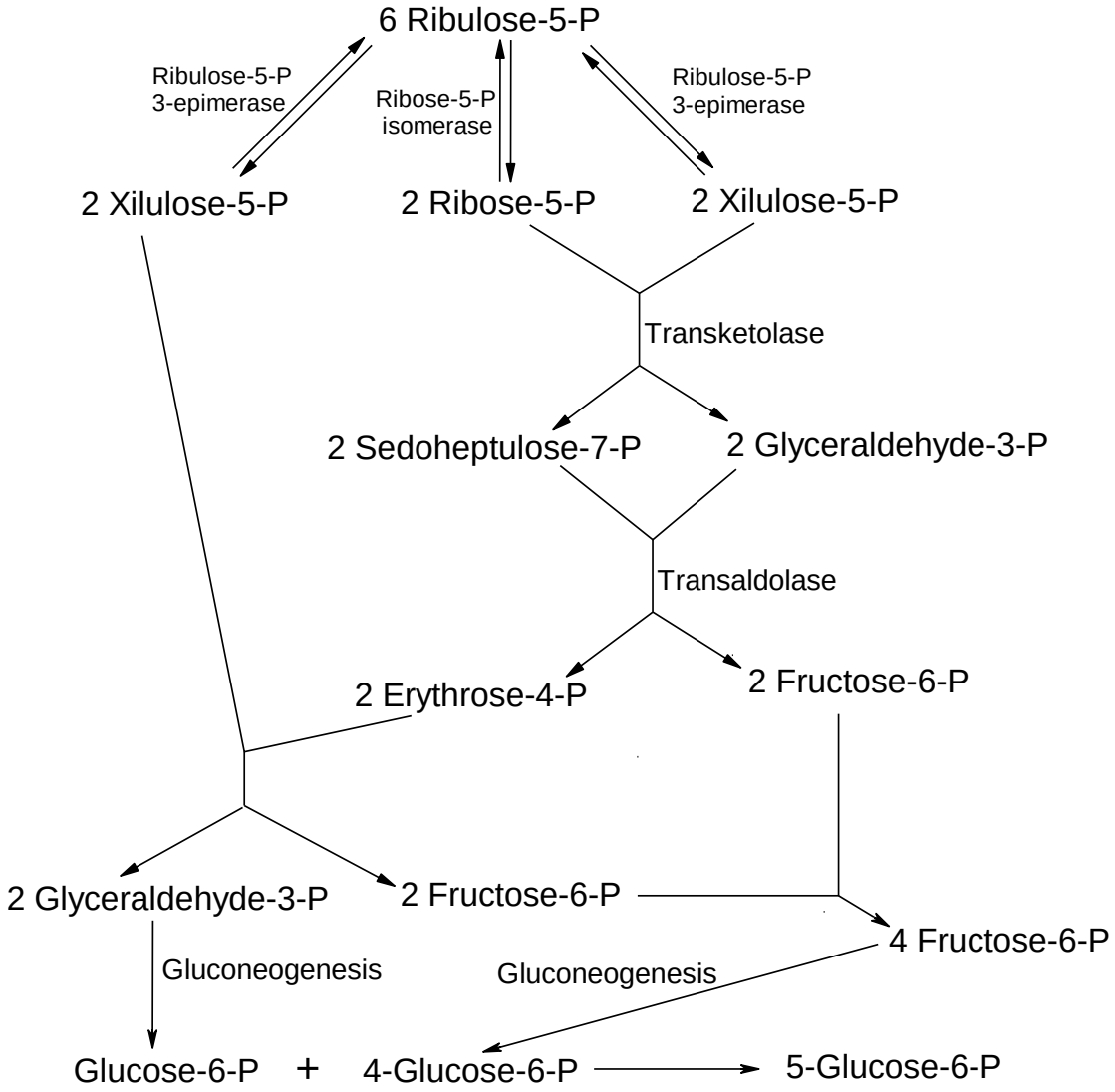
The deficiency in glucose-6-phosphate dehydrogenase is caused by a mutation in the gene encoding this enzyme. Although the mutation is present in all cells, the most affected are the erythrocytes because they have no other source of $NADPH+H^+$. This deficiency is manifest especially when red blood cells are exposed to oxidative stress produced by infections, medications (antimalarial – primaquine, sulfonamides - sulfamethoxazole, nalidixic acid, etc.) or drugs, when hemolysis and hemoglobinuria

occurs. The deficiency is rare in Europe, but quite frequent in Africa, India, South-East Asia.

1. Oxidative phase



2. Non-oxidative phase



Glucoza - completely oxidased to CO_2 and NADPH, H^+

$6\ G\text{-}6\text{-}P + 12\ NADP^+ + 6\ H_2O \longrightarrow 6$ Ribulozo-5-P $+ 12\ NADPH, H^+ + 6\ CO_2$

$5\ G\text{-}6\text{-}P$

$G\text{-}6\text{-}P + 12\ NADP^+ + 6\ H_2O \longrightarrow 6\ CO_2 + 12\ NADPH, H^+ + P_i$

II.3. Fructose metabolism

Fructose is found in honey and most fruits; however, the main source of fructose in food is sugar. Although fructose is absorbed slower than glucose in the intestine, it is metabolized faster than glucose.

Fructose metabolism is somewhat tissue specific, occurring in different manners in different tissues. In the kidney and muscles, fructose is phosphorylated by **hexokinase** into **fructose 6-phosphate** which will enter the glycolysis pathway. In the liver, fructose is phosphorylated at the C1 carbon by **fructokinase** into **fructose 1-phosphate**. This compound will then be split into **dihydroxyacetone phosphate** and **glyceraldehyde** under the action of **fructose 1-phosphate aldolase (aldolase B)**. Dihydroxyacetone phosphate is then converted into **glyceraldehyde 3-phosphate** by the enzyme **triose-phosphate isomerase**. Glyceraldehyde is in turn phosphorylated by a **triose kinase** and **ATP** into glyceraldehyde 3-phosphate. Therefore, fructose 1-phosphate will enter the glycolysis pathway as glyceraldehyde 3-phosphate.

In the retina, kidneys and peripheral nerves, which are tissues where glucose enters independent of insulin, there is another pathway in use, called the **polyol pathway** which involves the transformation of **glucose** into **sorbitol** by **aldose reductase**, an enzyme using NADPH as cofactor. Sorbitol is then converted to **fructose** by **sorbitol dehydrogenase** which uses NAD^+ as enzymatic cofactor and is expressed mainly in the seminal vesicles, liver and kidneys. Fructose is then phosphorylated by hexokinase or fructokinase and enters the glycolysis pathway.

The polyol pathway has a low level of activity in normal circumstances; however in diabetic patients where glycemia levels are high, this pathway will have a more intense activity producing a larger quantity of sorbitol which will accumulate in the cells due to its inability to pass through cell membranes. This will determine an osmotic effect causing large quantities of water to enter the cells and eventually producing cellular damage. In addition, NADPH storage levels will be reduced, thus lowering glutathione and NO synthesis and increasing **oxidative stress** which can also cause cellular damage. All these effects associated with intense sorbitol synthesis in diabetics will contribute to the development of complications specific to this disease such as cataracts and retinopathy (sorbitol accumulation in the crystalline lens), neuropathy, nephropathy, microagiopathy.

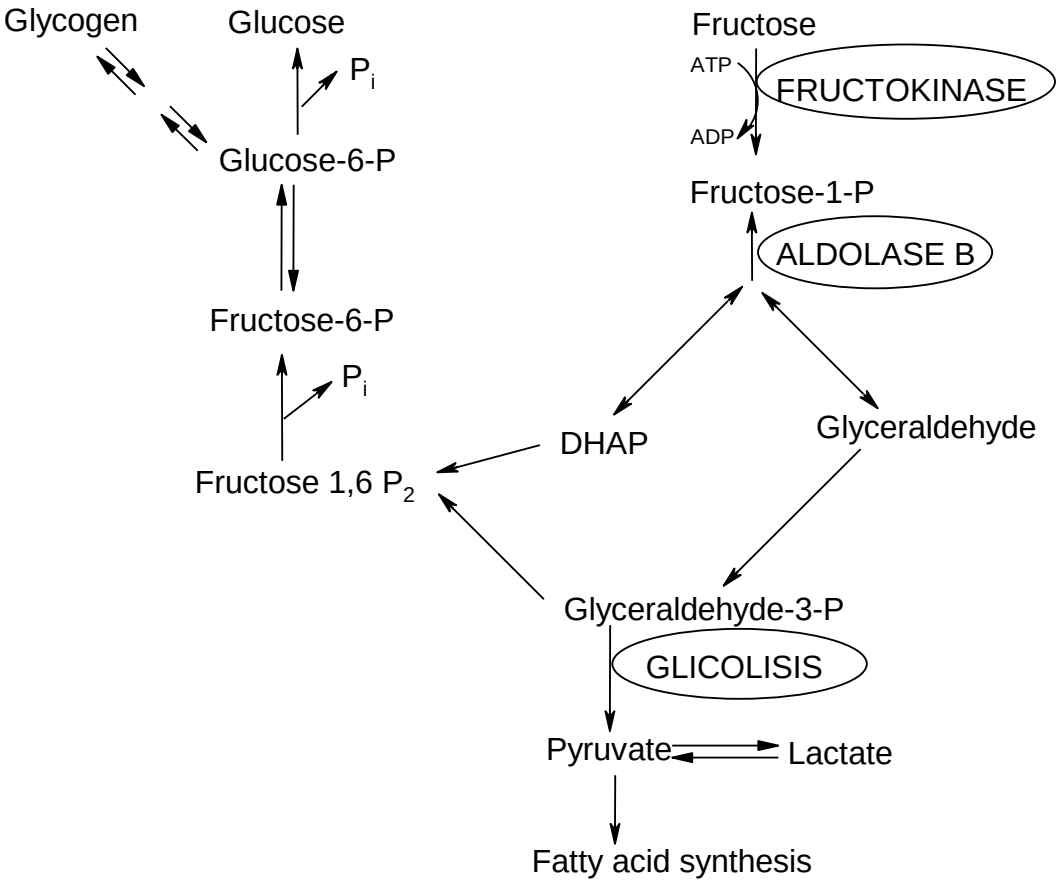
Fructose has a concentration of about 200mg% in the seminal fluid, being the main monosaccharide and the principal source of energy for the sperm cells. The evolutionary advantage of using fructose as an energy source for sperm cells is that bacteria that could compete for energy prefer glucose.

Associated pathology

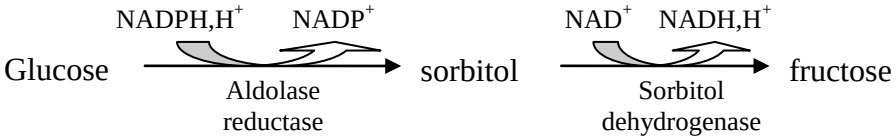
It is due to genetic defects of the enzymes involved in fructose metabolism, causing several diseases:

1. **Essential fructosuria** – caused by a deficiency in **hepatic fructokinase**, and the unmetabolized fructose is eliminated through urine. This phenomenon is especially intense after meals rich in fructose.
2. **Hereditary fructose intolerance** – caused by a deficiency in **aldolase B**. As a consequence, fructose-1-phosphate is accumulated which will inhibit the **fructose-1,6-bisphosphate aldolase** (**aldolase A**, catalyzing the conversion of dihydroxyacetone phosphate and glyceraldehyde 3-phosphate into fructose-1,6-bisphosphate), causing severe hypoglycemia. In addition, blocking the cell's

phosphate as fructose-1-phospate could lead to inhibited protein synthesis, disturbance of liver and kidney functions, cirrhosis, and in severe cases even death. The treatment consists of a **diet free of fructose, sucrose or sorbitol**, which is easy to follow especially because afflicted children develop a spontaneous aversion towards sweets.



Fructose can also be obtained from glucose through the polyol pathway in the seminal vesicles, retina, kidneys, and peripheral nerves:

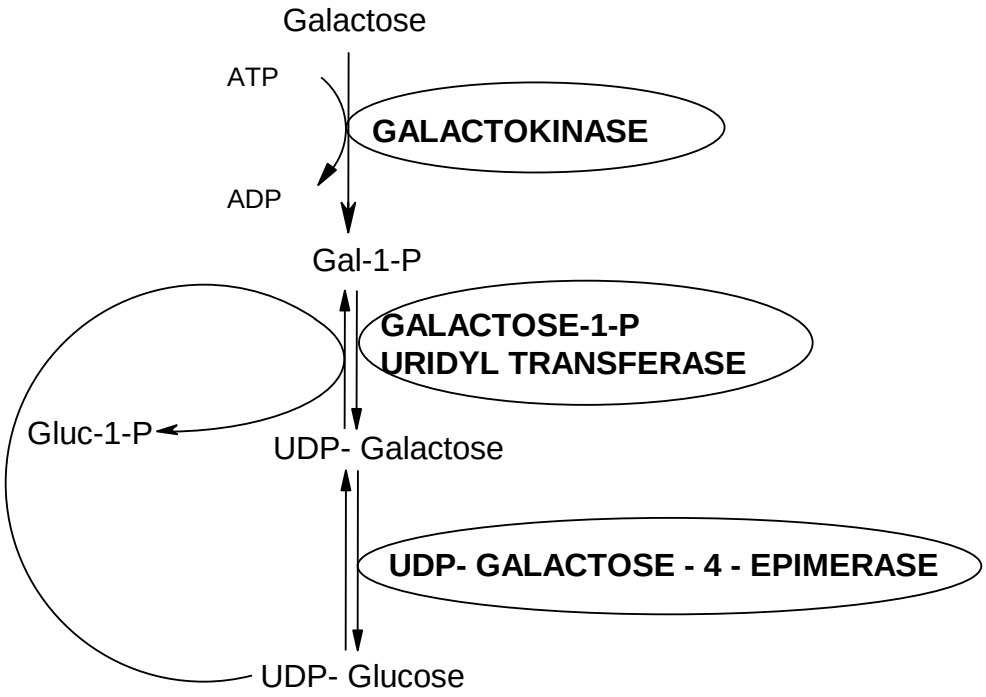


Fructose therapy

Intravenously administered fructose was proposed as a glucose substitute in diabetics. However, it has been shown that the fructose excess leads to increased lactate concentrations, hypertriglyceridemia and hyperuricemia. Moreover, through triose phosphates, fructose can be converted into glucose via gluconeogenesis, generating hyperglycemia. Therefore, this treatment was rapidly abandoned from clinical practice.

II.4. Galactose metabolism

The food sources of galactose are lactose from milk and dairy products, glycolipids and glycoproteins. After digestion and absorption, galactose is preferentially metabolized in the liver.



Global reaction

Galactose + ATP → Glucose – 1 – phosphate + ADP

Galactose plays a role in the synthesis of lactose in the mammary gland, of galactolipids, galactoproteins and mucopolysaccharides (glycosaminoglycans).

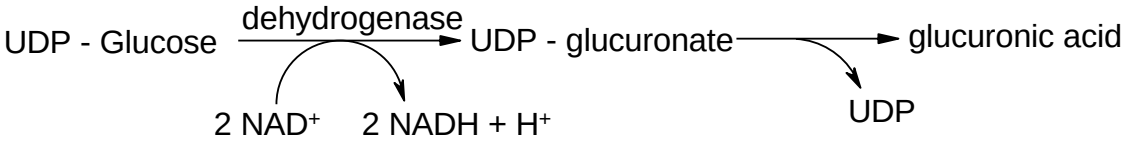
Pathology

The galactose loading test is one of the tests that can be used for exploring the liver function, galactose being found in urine in case of poor liver function.

Congenital galactosemia is an autosomal recessive disorder with an incidence of 1:40000 caused by a deficiency in galactose 1-phosphate uridyl transferase. This will cause accumulation of galactose and galactose-1-phosphate after consumption of food containing galactose. The symptoms of the disease include hepatic dysfunctions manifested as jaundice, vomiting, lethargy, mental deficiency and cataractss. Cataracts is caused by the transformation of excess galactose into galactitol by the reductase enzyme, which will accumulate in the crystalline lens. The treatments consists in adopting a dairy free diet as early as possible.

II.5. Metabolism of glucuronic acid

Glucuronic acid is obtained by oxidation of the hydroxyl group of C6 carbon atom of glucose, into a carboxyl group. The synthesis takes place mainly in the liver, kidneys and intestines.



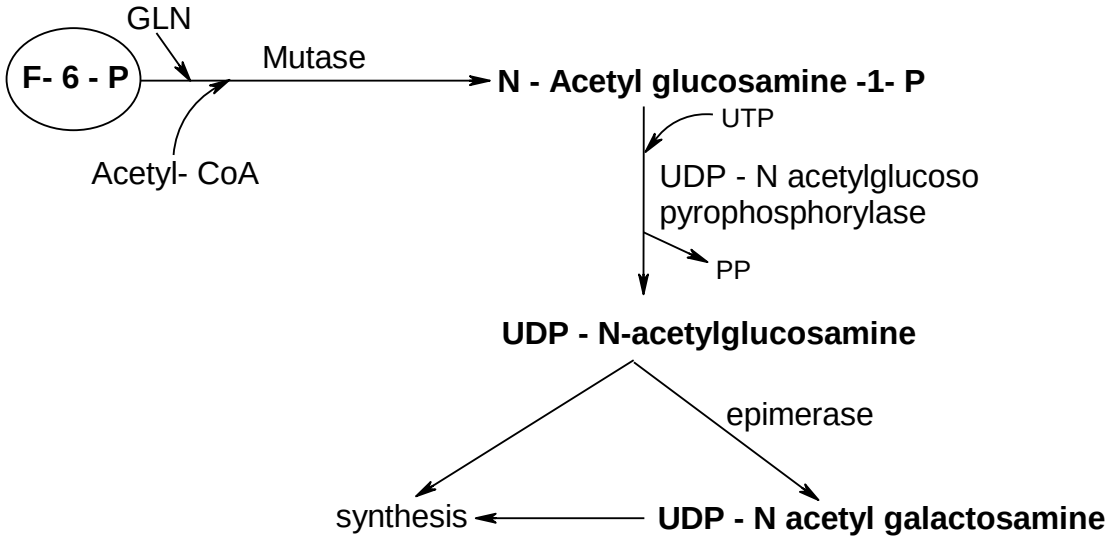
Glucuronic acid plays a role in the detoxifying and synthesis processes. During the detoxifying processes, glucuronic acid is linked to the compound that needs to be eliminated forming glucuronide conjugates (glucuronides), lowering its toxicity and increasing its solubility and thus making it easier to eliminate from the body. This is done through a β glycosidic bond between the glycosidic –OH of glucuronic acid and organic functions (hydroxyl, carboxyl, amino) of certain endogenous (hormones, bilirubin) or exogenous (drugs or toxic substances) compounds. For example the insoluble pre-hepatic bilirubin is conjugated with glucuronic acid producing bilirubin diglucuronide, a more soluble compound which will be eliminated through bile.

As a role in synthesis reactions, it is worth mentioning the role of glucuronic acid in the synthesis of glycosaminoglycans, hyaluronic acid, heparin, gulonic acid. In the case of gulonic acid there is pathology called essential pentosuria, which is due to the genetic deficiency of the enzyme that converts xylitol into D-xylulose, and thus L-xylulose will be massively eliminated through urine.

Glucuronic Acid ⟶ Gulonic Acid ⟶ L-xylulose ⟶ Xylitol ⟶
D-xylulose ⟶ Pentose phosphate pathway

II.6. Metabolism of hexosamines

The most important hexosamines are glucosamine (1-amino-2-deoxy glucose) and galactosamine (2-amino-2-deoxy galactose). These are constituent parts of glycosaminoglycans, sialic acid and glycoproteins, usually in the N-substituted form. They are synthesized from fructose-6-phosphate (F-6P).



Sialic acid = N-acetyl-glucosamine-6-phosphate + pyruvic acid + CTP

II.7. Glycemia and its regulation

Normal glucose concentration in blood is between 65 and 120 mg/dL. The concentration is higher after a meal to 120-140 mg/dL and decreases during starvation to 60-70 mg/dL. A hypoglycemic coma can happen at values lower than 40 mg/dL, for example when a too high insulin dose is administered, and values higher than 126 mg/dL are characteristic for diabetes (measured on an empty stomach). Other types of coma, keto-diabetic (diabetic keto-acidosis) and hyperosmolar (hyperosmolar hyperglycemic syndrome), can take place at glycemia values between 250-1500 mg/dL. Maintaining constant clycemia values is called **glucidic homeostasis**. This process ensures the glucose needs for glucose dependent tissues as well as its storage through physiological and biochemical mechanisms. Among the physiological mechanisms, worth mentioning are the hunger feeling during hypoglycemia and glycosuria during hyperglycemia higher than 180 mg/dL. Regarding the biochemical mechanisms involved in glycemia regulation, these are dependent on the physiological state according to the food intake, early postprandial and late postprandial.

Early postprandial, the glucose excess from food needs to be used and stored as glycogen or lipids. This step is controlled by insulin, which increases the cell membrane permeability for glucose and stimulates glucose metabolism through pathways such as glycolysis, glycogen synthesis, pentose phosphate pathway, fatty acids and triglycerides synthesis. Insulin will also inhibit the metabolic pathways that produce or mobilize glucose from deposits such as gluconeogenesis, glycogenolysis, lipolysis. Insulin acts through regulating the activity (phosphorylation-dephosphorylation) or synthesis of key enzymes involved in these metabolic pathways.

Late postprandial (postabsorbtive state, fasting), there is a glucose shortage because of lack of food intake, and therefore there is a need to synthesize glucose and use alternative energy sources. This step is characterized by stopping the insulin secretion and the action of hyperglycemic hormones. These hormones have opposite effects to that of insulin, stimulating metabolic pathways that produce glucose such as gluconeogenesis in the liver and kidneys, glycogenolysis in the liver and muscles, as well as those that mobilize alternative energy sources such as lipolysis in adipose tissue, fatty acids catabolism and proteolysis in muscles. Concomitantly, the metabolic pathways that are using glucose are inhibited. The most important hyperglycemic hormones are: glucagon – acting mainly in the liver, catecholamines – acting in the muscles, glucocorticoids, thyroxin, and growth hormone.

Related pathology

The main disease caused by pathologic alterations of glucose metabolism is diabetes mellitus, which affects over 10% of the population with continuously increasing incidence. There are two main types of diabetes mellitus:

- **Type I, insulin-dependent or juvenile diabetes**, which is caused by the progressive autoimmune destruction of β pancreatic cells, leading to absolute deficiency of insulin secretion. Represents about 5-10% of all diabetes cases and it mostly appears during childhood or in young adults, but it can also rarely

be found at older ages (even in 80-90 year olds). It is treated exclusively by insulin administration.

- **Type II, insulin independent or mature diabetes**, is characterized by the increased tissue resistance to insulin action as well as the dysfunction of β pancreatic cells. During incipient stages, there is an increased insulin secretion, trying to compensate the increased tissue resistance to its action, followed later on by diminished insulin secretion and hyperglycemia. Type II diabetes is often associated with central or visceral obesity, as well as other cardiovascular risk factors such as hypertension, dyslipidemia with high triglyceride and low HDL-cholesterol values in blood.

During both types of diabetes there is hyperglycemia which can evolve to glycosuria, ketonuria, acidosis, diabetic coma.

Other diabetes types are: gestational diabetes, congenital diabetes – caused by genetic mutations leading to β pancreatic cells' dysfunction (mitochondrial DNA point mutations, mutations in the gene encoding for the ATP sensitive potassium channel – K_{ATP} involved in insulin secretion regulation), secondary diabetes as a consequence of other pathologies affecting the pancreas (pancreatitis, cystic fybrosis).

Methods of exploration of glycemic homeostasis

Investigation methods for the glucose metabolism equilibrium in diabetes includes: determination of glycemia on an empty stomach (à jeun), determining the presence of glucosuria, glucose tolerance test, determination of glycated hemoglobin and serum proteins, determination of serum insulin levels, and the levels of the C peptide.