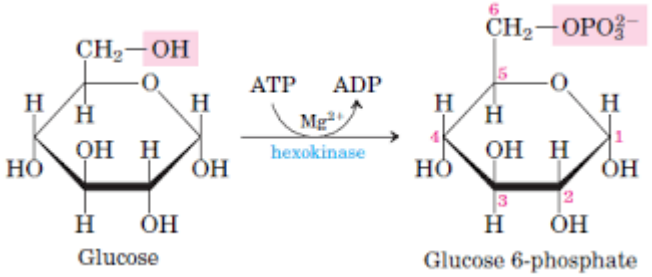
Mighty T

Steps in glycolysis

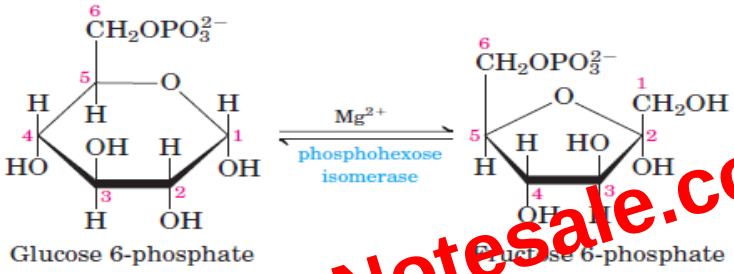
- ***Preparatory phase***

1. **Phosphorylation of Glucose**: In the first step of glycolysis, glucose is activated for subsequent reactions at C-6 to yield glucose-6-phosphate, with ATP as the phosphoryl donor.



This irreversible reaction is catalyzed by **hexokinase**. Note that kinases are enzymes that catalyze the transfer of the terminal phosphoryl group from ATP to an acceptor nucleophile. The type of hexokinase present in hepatocytes(liver cells) is called **hexokinase IV** or **glucokinase.**

2. **Conversion of Glucose-6-phosphate to fructose-6-phosphate:Phosphohexose isomerase (phosphoglucose isomerase)** catalyzes the reversible isomerization of glucose-6-phosphate (aldose) to fructose-6-phosphate (a ketose)



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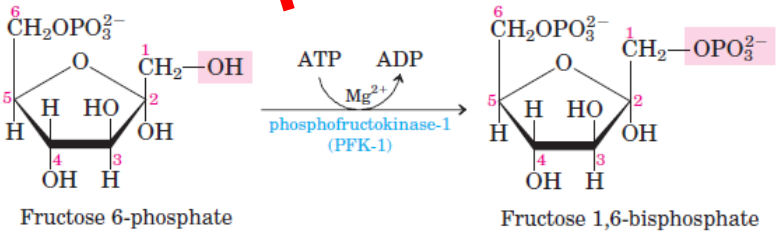
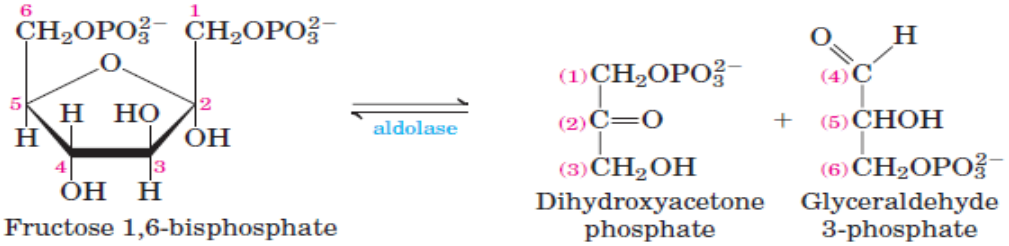
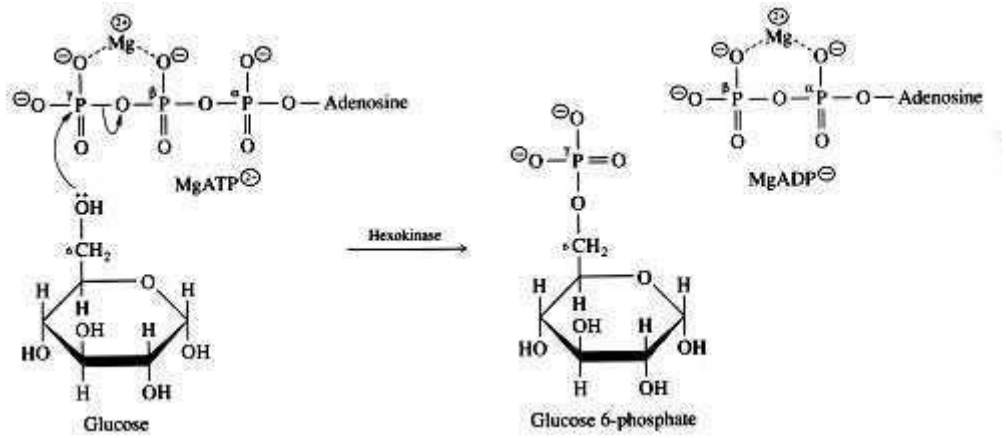
3. **Phosphorylation of fructose-6-phosphate to fructose 1,6 Bisphosphate:** This is the second priming reaction catalyzed **by phosphofructokinase-1(PFK-1).** This enzyme catalyzes the transfer of a phosphoryl group from ATP to fructose-6-phosphate to fructose 1,6- bisphosphate. The reaction catalyzed by this enzyme is the first committed step in the glycolytic pathway.



4. **Cleavage of fructose 1,6- Bisphosphate:** The enzyme **fructose 1,6-bisphosphate aldolase,** often called simply **aldolase**, catalyzes a reversible aldol condensation. Fructose 1,6- bisphosphate is cleaved to yield two different triose phosphates, **glyceraldehyde 3-phosphate,** an aldose, and **dihydroxyacetone phosphate,** a ketose.





Regulation of Glycolytic pathway

The regulation of glycolysis is through the action of the enzymes catalyzing the irreversible steps;

- **Glucokinase** has a higher Km for glucose than hexokinase. Because of this low affinity, glucokinase can only act when there is plenty supply of glucose. Thus, when the supply of glucose is limited, glucose-6-phosphate will inhibit hexokinase.

- **Phosphofructokinase(PFK-1)** is the most important rate limiting enzyme for glycolysis. ATP and Citrate are the most important allosteric inhibitors. AMP acts as an allosteric activator. **Fructose-2,6-bisphosphate** increases the activity of PFK. F-2,6-BP is formed from fructose-6-phosphate by the action of PFK-2.

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- **Pyruvate kinase** catalyses an irreversible step and is a regulatory enzyme of glycolysis. When energy is plenty in cell, glycolysis is inhibited. Insulin increases its activity whereas glucagon inhibits. Pyruvate kinase is inactive in the phosphorylated state.

- **Hormonal regulation**
  Insulin favours glycolysis by activating the above three key glycolytic enzymes
  Glucagon inhibit glycolysis and favours gluconeogenesis.

Past questions on glycolysis

- Discuss the fate of glucose in Embden-meyerhoff pathway.
- Explain how and why NAD+ is being regenerated in glycolysis
- Give the ten enzymatic reactions in Embden-meyerhoff pathway
- The irreversibility of pyruvate dehydrogenase reaction prelude the formation of pyruvate from acetyl-CoA, discuss! Explain why glucose cannot be formed from acetyl-CoA.
- Name the inorganic substrate level phosphorylation in glycolysis.
- Name the coenzymes and prosthetic groups of pyruvate dehydrogenase.
- State the number of ATP generated in glycolysis
- Discuss briefly the fates of pyruvate.

Muscle and brain lack this enzyme and so cannot carry out gluconeogenesis . Glucose produced by gluconeogenesis in liver or kidney or ingested in the diet is delivered to muscle and brain through the. bloodstream.

Regulation of gluconeogenesis

1. **Pyruvate Carboxylase**
   It is an allosteric enzyme. **Acetyl CoA** is an activator of pyruvate carboxylase so that generation of oxaloacetate is favoured when the acetyl CoA level is sufficiently high.
2. **Hormonal Regulation of Gluconeogenesis**
   The hormones **glucagon** and **glucocorticoids** and **insulin** regulate gluconeogenesis .
   Glucagon favours gluconeogenesis by the production of glucose from Its stored form(**glycogen)** which prevents blood sugar from falling and thus favours gluconeogenesis and inhibit glycogen Synthesis (glycogenesis) and glycolysis
   Glucocorticoids induce the synthesis of hepatic amino transferases thereby providing substrate for gluconeogenesis.
   Insulin inhibits the process of gluconeogenesis; by reducing the blood sugar(glucose);glucose synthesized in gluconeogenesis is reduced which favours glycogenesis and inhibits gluconeogenesis.
3. **ATP availability**
   Increased ATP(adenosine triphosphate) favours gluconeogenesis and vice-versa.
4. **Fructose 1,6-bisphosphatase**
   The availability of the enzyme **Fructose 1,6-bisphosphatase** that catalyzes the conversion of fructose 1,6 phosphate to fructose 6-phosphate regulate gluconeogenesis. Increased in the enzyme favours gluconeogenesis and vice versa.
   Fructose 2,6-bisphosphateand **AMP**reduces the action of ***fructose 1,6-bisphosphate*** and increases the activity of ***phosphofructokinase***(glycolytic enzyme)

Relationship between Glycolysis and gluconeogenesis

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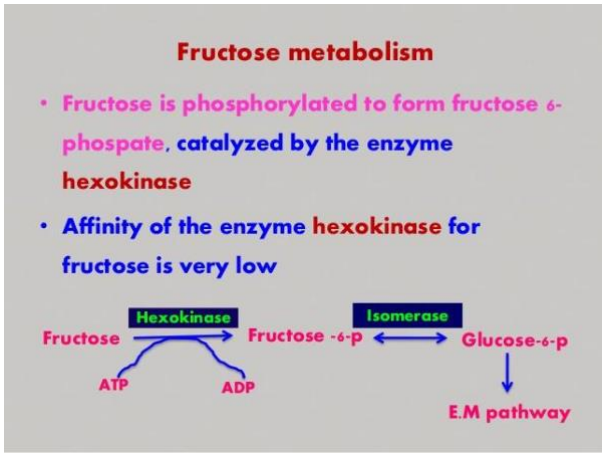
1. Kidney and muscle

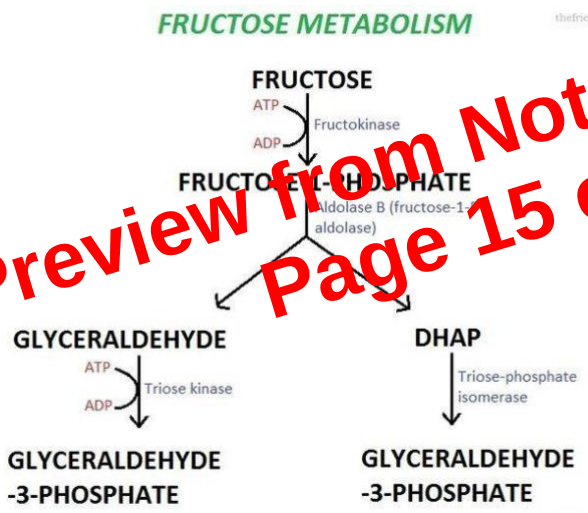
D-fructose(ketohexose) present in free form in many fruits and formed by hydrolysis of sucrose in the small intestine of vertebrates is phosphorylated by ***hexokinase.***



The **fructose-6-phosphate** formed is converted to its isomer **glucose-6-phosphate** which later continue the **Emden Meyerhof pathway.**

2. Liver

The liver enzyme **fructokinase** catalyzes the phosphorylation of fructose at C-1 rather than C-6 to form **fructose-1-phosphate.**



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The **fructose-1-phosphate** is then cleaved to glyceraldehyde and dihydroxyacetone phosphate by ***fructose-1-phosphate aldolase(aldolase B).***
Glyceraldehyde is phosphorylated by ATP and ***triose kinase*** to **glyceraldehyde-3-phosphate.** **Dihydroxyacetone phosphate** is converted to **glyceraldehyde-3-phosphate** by the glycolytic enzyme ***triose phosphate isomerase.***

Defects of fructose metabolism

- **Fructosuria**
  This is a metabolic defect due to the deficiency of the enzyme ***hepatic fructokinase.***

- Discuss the biosynthesis of a named bile acid from two carbon compound.

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De novo synthesis of fatty acids

The process of fatty acid synthesis was studied by Feodor Lyen(Nobel prize, 1964). This pathway is also referred to as Lynen's spiral. The process occurs in ***liver, adipose tissue, kidney, brain and mammary glands.***

Site of reaction: The biosynthesis of fatty acid takes place in the cytoplasm.

The starting material for *denovo synthesis of fatty acid is* ***acetyl-CoA.***

Note: Acetyl CoA is formed inside the mitochondria from pyruvate and therefore needs to be transported.

Transport of Acetyl CoA from mitochondria to cytoplasm

Pyruvate is converted to acetyl CoA in the mitochondria. The inner membrane is not freely permeable to acetyl CoA. Hence, the **acetyl CoA units is first converted to citrate and transported from the mitochondria into the cytoplasm through a *tricarboxylic transporter.*** In the cytoplasm, the **citrate** is then cleaved to **oxaloacetate** and **acetylCoA,** catalysed by the action of ***ATP citrate lyase.*** The oxaloacetate then return to the mitochondria as malate or pyruvate.

Stages of fatty acid biosynthesis

This biosynthetic system is catalysed by the action of a multi-enzyme complex known as ***Fatty acid synthase(FAS) complex.*** The enzymes are grouped into three units;

- **Condensing unit :** It is the initial substrate binding site. The enzymes involved are ***acetyl transacylase(AT), malonyl transacylase and beta-keto acyl synthase or condensing enzyme(CE)*** catalysing reaction 2 and 3 respectively.
- **Reduction unit:** it contains ***keto acyl reductase, dehydratase, enoyl reductase*** and ***acyl carrier protein(ACP)*** catalysing step 4,5,6 respectively
- **Releasing unit:** it is involved in the release of the palmitate synthesised. It contains ***thio-esterase(TE) or deacylase,*** catalysing the last reaction.

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Step 1: Carboxylation to acetyl CoA

The first step in the fatty acid synthesis is the carboxylation of **acetyl CoA** to form **malonyl CoA** catalysed by ***acetyl CoA carboxylase*** which requires a coenzyme **biotin*(carrier of activated carboxyl group).*** This enzyme is not a part of the multi-enzyme complex. But it is the **rate-limiting enzyme.**

Step 2: Binding of a molecule of acetyl CoA and malonyl CoA to a multi-enzyme complex

a. The ***acetyl transacylase(AT)*** catalyses the transfer of the acetyl group to the cysteinyl SH group of the **condensing enzyme(CE)**
b. The **Malonyl transacylase(MT)** transfers the malonyl group to the SH group of the **Acyl carrier protein(ACP).**

Step 3: Condensation

The acetyl(2c) and malonyl(3c) units are condensed to form **beta-keto acyl ACP(acetoacetyl ACP).** During this process, one carbon is lost as Co2. The enzyme is called ***condensing enzyme(keto acyl synthase)***

Step 4: Reduction

The acetoacetyl ACP is reduced by **NADPH** dependent beta-keto acyl **reductase(KR)** to form **beta-hydroxyl fatty acyl ACP.**

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