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|---|---|
| Name of Unit | Carbohydrate Metabolism and Biological Oxidation |
| Subject /Course name | Biochemistry |
| Subject/Course ID | BP203T |
| Class: B.Pharm. Semester | II |
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## Learning Outcome of Module 02

| LO | Learning Outcome | Course Outcome Code |
|---|---|---|
| LO1 | To learn about Properties of Carbohydrates. | BP203.1 |
| LO2 | To gain knowledge about the biological roles of Carbohydrates. | BP203.1 |
| LO3 | To Learn about metabolism of Carbohydrate. | BP203.2 |
| LO4 | To learn about Properties of Amino acid | BP203.1 |

## Content Table

| Topic |
| --- |
| • Carbohydrate Metabolism: Glycolysis and citric acid cycle- Pathway, energetics and significance.<br>• HMP Shunt and its significance; Glucose-6-Phosphate dehydrogenase deficiency.<br>• Glycogen metabolism pathways and glycogen storage diseases.<br>• Gluconeogenesis- Pathway and its significance.<br>• Hormonal regulation of blood glucose level and Diabetes mellitus.<br>• Biological Oxidation: Electron transport chain and its mechanism.<br>• Oxidative phosphorylation and its mechanism and substrate phosphorylation.<br>• Inhibitors ETC and oxidative phosphorylation/ uncouples. |

# CARBOHYDRATE METABOLISM

- Glucose is the major form of sugar moiety present in blood and other body fluids. The digestion of food carbohydrates, such as starch, sucrose, and lactose produces the monosaccharides glucose, fructose and galactose, which pass into the blood stream. The study of synthesis (Anabolism) and degradation (Catabolism) of biomolecules is biochemically termed as metabolism.

***Anabolism* + Catabolism = *Metabolism***

- Since glucose is the most important carbohydrate existing in physiological amounts in the body and is easily absorbed from the diet, the metabolism of carbohydrate resolves itself to the study of the metabolism of glucose and its main derivatives.
- The monosaccharides galactose and fructose are converted to glucose in the liver. All the monosaccharides are completely absorbed in the small intestine.
- The glucose in the circulating blood and tissue fluids is drawn upon by all the cells of the body and used for the production of energy. Normally carbohydrate metabolism supplies more than half of the energy requirements of the body. In fact the brain largely depends upon carbohydrate metabolism as a source of energy and quickly ceases to function properly when the blood glucose level falls much below normal.
- The major function of carbohydrate in metabolism is to serve as fuel and get oxidised to provide energy for other metabolic processes. The metabolic intermediates are used for various biosynthetic reactions. For this purpose, carbohydrate is utilized by the cells mainly in the form of glucose.
- A major part of dietary glucose is converted to glycogen for storage in liver. Glucose is degraded in the cell by way of a series of phosphorylated intermediates mainly via two metabolic pathways.

  ***1. Glycolysis***

  ***2. Tricarboxylic acid cycle***

## GLYCOLYSIS

Oxidation of glucose to pyruvate is called *glycolysis*. It was first described by *Embden-Meyerhof* and *Parnas*. Hence it is also called as *Embden-Meyerh* of pathway. Glycolysis occurs virtually in all tissues. Erythrocytes and nervous tissues derive the energy mainly from glycolysis. This pathway is unique in the sense that it can proceed in both aerobic (presence

of $O_2$) and anaerobic (absence of $O_2$) conditions. Allthe enzymes of glycolysis are found in the extra mitochondrial soluble fraction of the cell, the cytosol.

The overall equation for glycolysis from glucose to lactate is as follows:

$$Glucose + 2ADP + 2P_i \rightarrow 2L(+)-Lactate + 2ATP + 2H_2O$$

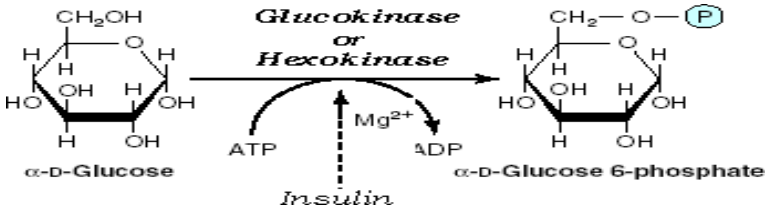
## Reactions of glycolytic pathway

Series of reactions of glycolytic pathway which degrades glucose to pyruvate are represented below. The sequence of reactions occurring in glycolysis may be considered under four stages.

***Stage I:*** This is a *preparatory phase*. Before the glucose molecule can be split, the rather asymmetric glucose molecule is converted to almost symmetrical form, fructose 1, 6-diphosphate by donation of two phosphate groups from ATP.

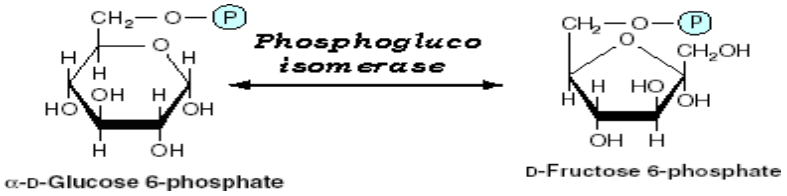
### 1. *Uptake of glucose by cells and its phosphorylation:*

Glucose is freely permeable to liver cells, intestinal mucosa and kidney tubules where glucose is taken up by 'active' transport. In other tissues ***insulin*** facilitates the uptake of glucose. Glucose is phosphorylated to form ***glucose 6-phosphate***. The enzyme involved in this reaction is *glucokinase or hexokinase*. This reaction is irreversible.

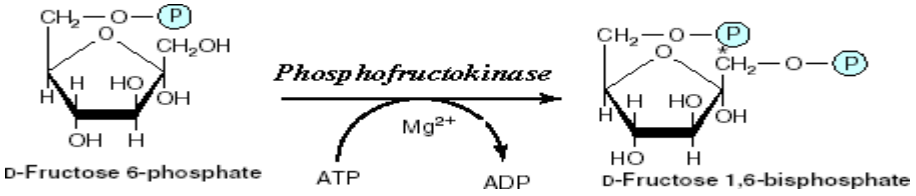



### 2. *Conversion of glucose 6-phosphate to fructose 6-phosphate:*

Glucose 6-phosphate is converted to fructose 6-phosphate by the enzyme *phosphogluco isomerase*.



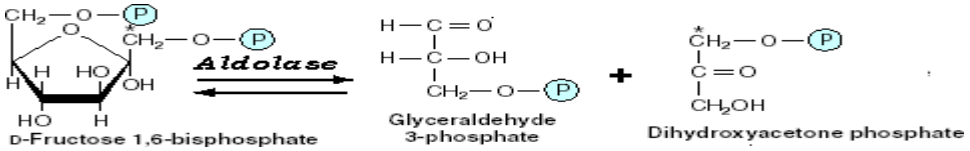

### 3. *Conversion of fructose 6-phosphate to fructose 1, 6 diphosphate:*



Fructose 6-phosphate is phosphorylated irreversibly at 1 position catalyzed by the enzyme *phosphofructokinase* to produce fructose1, 6-diphosphate.

***Stage II:***

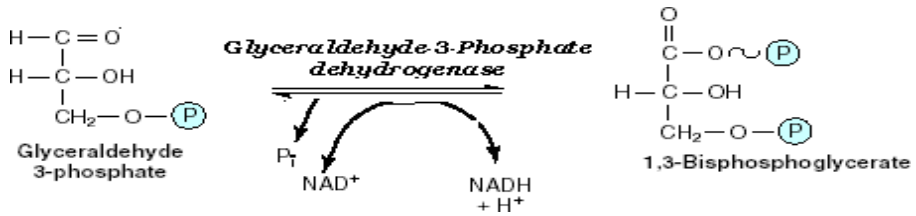
***Actual splitting of fructose 1, 6 diphosphate:*** Fructose 1, 6 diphosphate is split by the enzyme *aldolase* into two molecules of triose phosphates, an aldotriose-glyceraldehyde 3-phosphate and one *ketotriose*-dihydroxy acetone phosphate. The reaction is reversible. There is neither expenditure of energy nor formation of ATP.



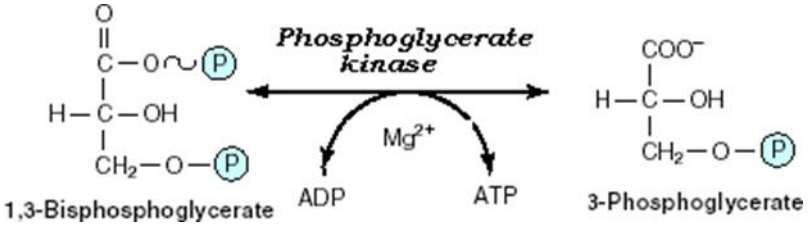

***Interconversion of triose phosphates:*** Both triose phosphates are interconvertible.


***Stage III:*** It is the energy yielding stage. Reactions of this type in which an aldehyde group is oxidised to an acid are accompanied by liberation of large amounts of potentially useful energy.

***Oxidation of glyceraldehyde 3-phosphate to 1, 3-bisphosphoglycerate:*** Glycolysis proceeds by the oxidation of glyceraldehyde 3-phosphate to form 1, 3−bisphosphoglycerate. The reaction is catalyzed by the enzyme *glyceraldehyde 3-phosphate dehydrogenas*.

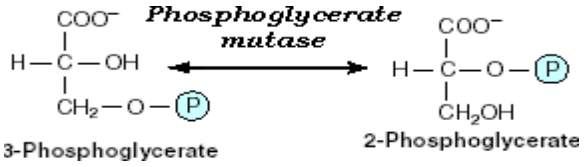



***Conversion of 1, 3-bisphosphoglycerate to 3-phosphoglycerate:*** The reaction is catalyzed by the enzyme *phosphoglycerate kinase*. The high energy phosphate bond at position-1 is transferred to ADP to form ATP molecule.

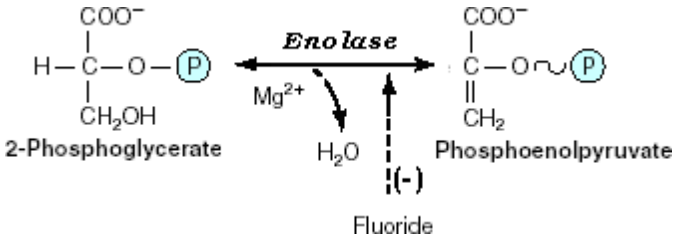


***Stage IV:*** It is the recovery of the phosphate group from *3-phosphoglycerate*. The two molecules of *3- phosphoglycerate*, the end product of the previous stage, still retains the phosphate group, originally derived from ATP in Stage I.

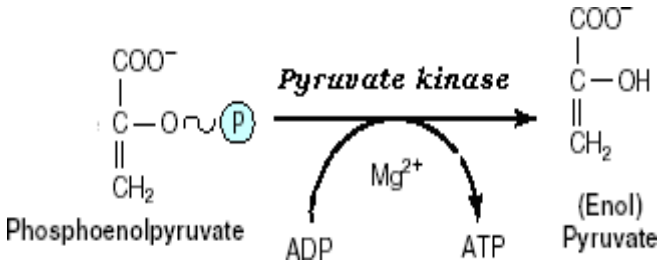
***Conversion of 3-phosphoglycerate to 2-phosphoglycerate:*** *3-phosphoglycerate* formed by the above reaction is converted to 2-phosphoglycerate, catalyzed by the enzyme *phosphoglycerate mutase*.




***Conversion of 2-phosphoglycerate to phosphoenol pyruvate:*** The reaction is catalyzed by the enzyme *enolase*, the enzyme requires the presence of either $Mg^{2+}$ or $Mn^{2+}$ ions for activity.




***Conversion of phosphoenol pyruvate to pyruvate:*** *Phosphoenol pyruvate* is converted to *pyruvate*, the reaction is catalysed by the enzyme *pyruvate kinase*. The high energy phosphate group of phosphoenol pyruvate is directly transferred to ADP, producing ATP. The reaction is irreversible.

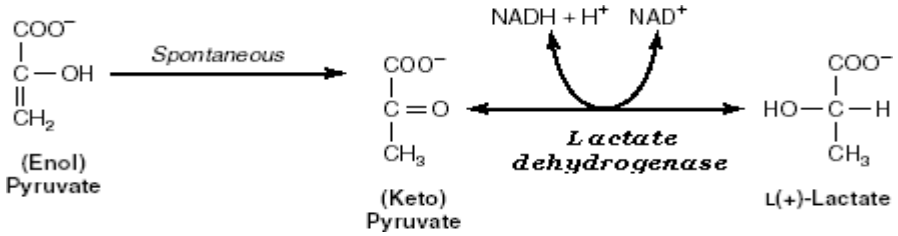



**Summary of glycolysis**

During glycolysis $NAD^+$ is reduced to NADH. At the same time, *glyceraldehyde 3-phosphate* is oxidized to *1, 3-bisphosphoglycerate*. To conserve the coenzyme $NAD^+$, NADH must be reoxidized. Under anaerobic conditions this is done when pyruvic acid is converted to lactic acid. In the presence of oxygen, NADH can be oxidized to $NAD^+$ with the help of the respiratory enzymes.

## ANAEROBIC PHASE

- In the absence of $O_2$, reoxidation of NADH at glyceraldehyde 3-phosphate dehydrogenase stage cannot take place in respiratory chain. But the cells have limited coenzyme. Hence to continue the glycolysis **NADH** must be reoxidized to **$NAD^+$**. This is achieved by reoxidation of NADH by conversionof pyruvate to lactate (without producing ATP).



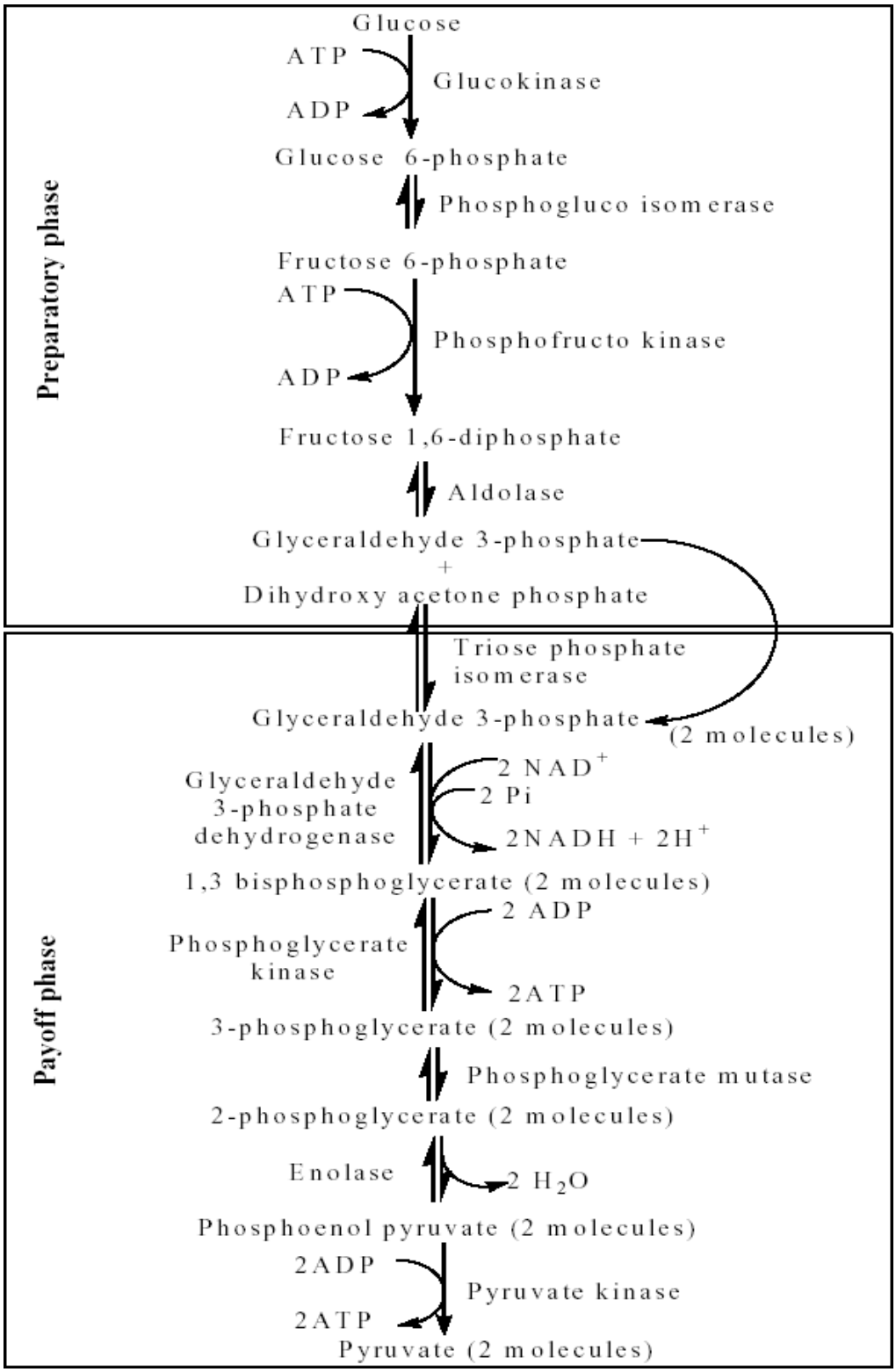

- It is to be noted that in the reaction catalyzed by *glyceraldehyde 3-phosphate dehydrogenase*, therefore,no ATP is produced.
- In the anaerobic phase oxidation of one glucose molecule produces 4 - 2 = 2 ATP.

## Energy yield per glucose molecule oxidation

During glycolysis ATP molecules are used and formed in the following reactions (aerobic phase).

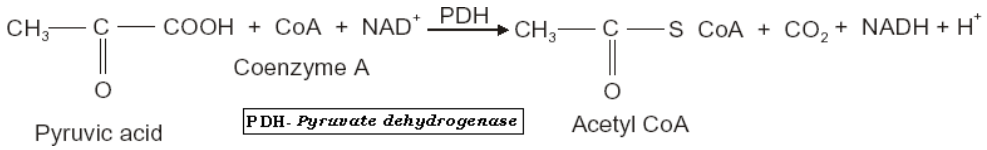
| ***Reactions Catalyzed*** | ***ATP used*** | ***ATP formed*** |
|---|---|---|
| ***Stage I:*** 1. Glucokinase (for phosphorylation) | 1 | |
| 2. Phosphofructokinase I (for phosphorylation) | 1 | |
| ***Stage II:*** 3. Glyceraldehyde 3-phosphate dehydrogenase (oxidation of 2 NADH in respiratory chain) | | 6 |
| 4. Phosphoglycerate kinase (substrate level phosphorylation) | | 2 |
| ***Stage IV:*** 5. Pyruvate kinase (substrate level phosphorylation) | | 2 |
| ***Total*** | **2** | **10** |
| ***Net gain*** | **08** | |

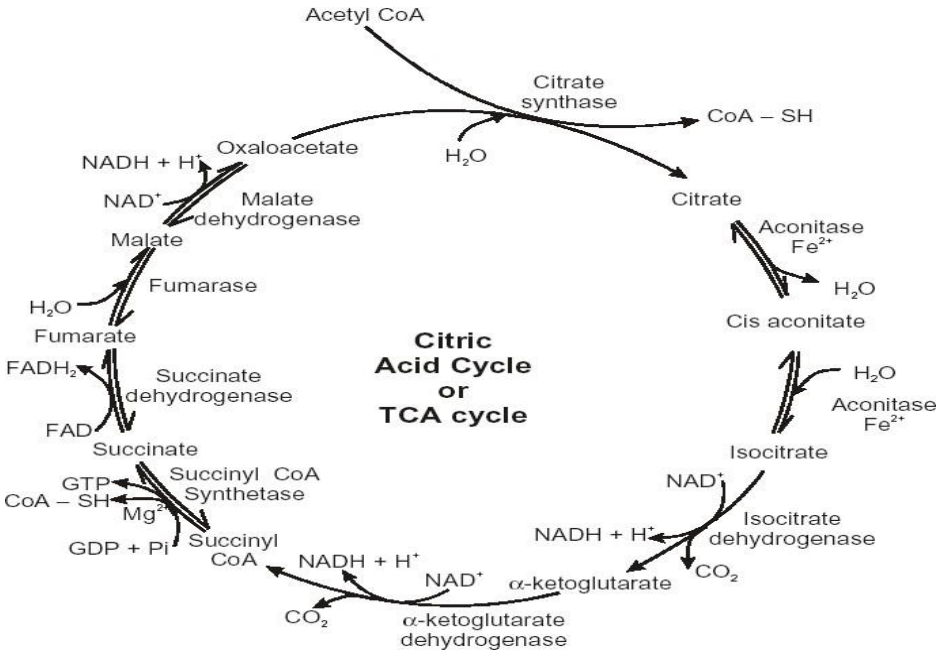
**Schematic diagram of glycolytic pathway**



## TRICARBOXYLIC ACID CYCLE (TCA CYCLE)

- This cycle is the aerobic phase of carbohydrate metabolism and follows the anaerobic pathway from the stage of pyruvate and is called as citric acid cycle or TCA cycle. The name citric acid cycle stems from citric acid which is formed in the first step of this cycle. This cycle is also named "Kerbs cycle" after H.A. Krebs, an English biochemist who worked on it.
- Under aerobic conditions, pyruvate is oxidatively decarboxylated to acetyl coenzyme A (active acetate) before entering the citric acid cycle. This occurs in the mitochondrial matrix and forms a link between glycolysis and TCA cycle.
- This reaction is catalysed by the multienzyme complex known as pyruvate dehydrogenase complex.

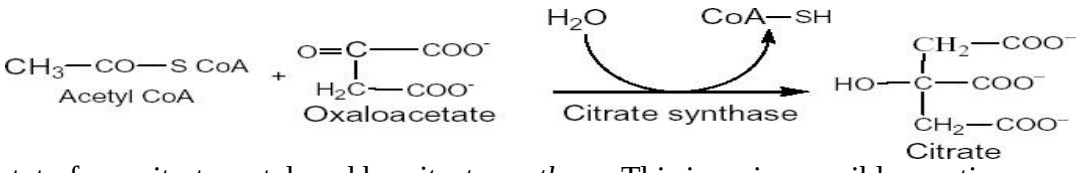



***Schematic Diagram of Krebs Cycle***

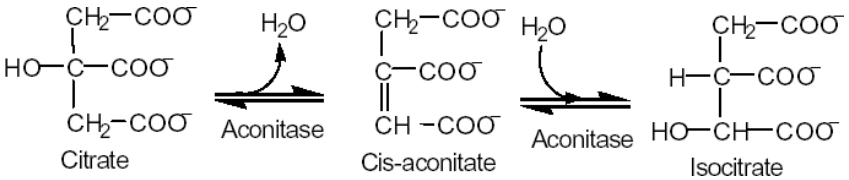
**Reactions of the citric acid cycle:** There are eight steps in the cycle and the reactions are as follows.

1. ***Formation of citrate:*** The first reaction of the cycle is the condensation of acetyl CoA with

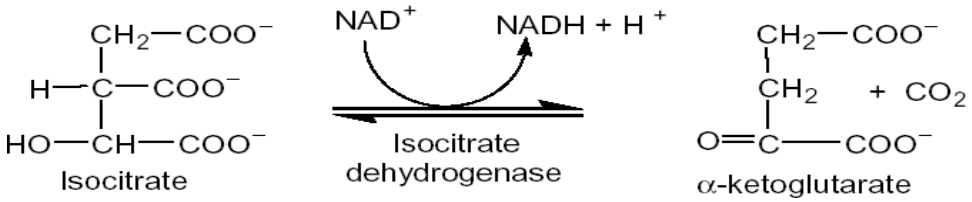



oxaloacetateto form citrate, catalyzed by *citrate synthase*. This is an irreversible reaction.

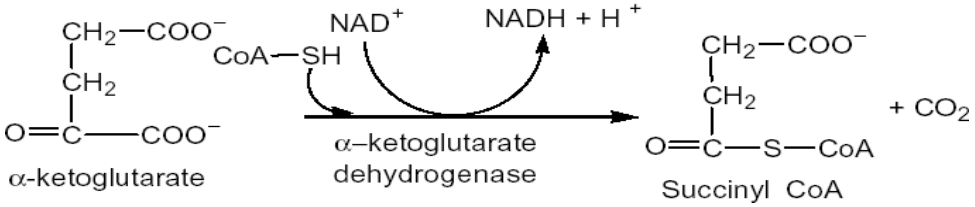
2. ***Formation of isocitrate via cis aconitate:*** The enzyme *aconitase* catalyzes the reversible transformation of citrate to isocitrate, through the intermediary formation of cis aconitate.



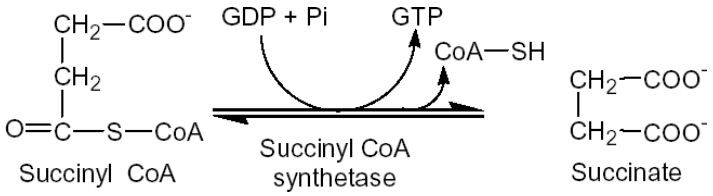

3. ***Oxidation of isocitrate to α-ketoglutarate and $CO_2$:*** In the next step, *isocitrate dehydrogenase* catalyzesoxidative decarboxylation of isocitrate to form α-ketoglutarate.



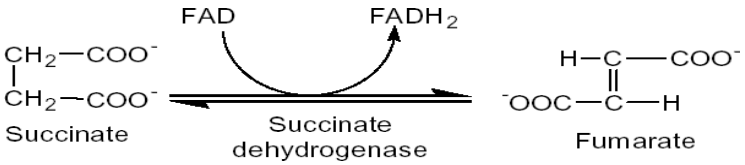

4. ***Oxidation of α-keto glutarate to succinyl CoA and $CO_2$:*** The next step is another oxidative decarboxylation, in which a-ketoglutarate is converted to succinyl CoA and CO2 by the action of the *α- ketoglutarate dehydrogenase* complex. The reaction is irreversible.



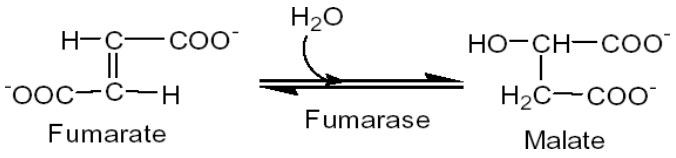

5. ***Conversion of succinyl CoA to succinate:*** The product of the preceding step, succinyl CoA is converted to succinate to continue the cycle. GTP is formed in this step (substrate level phosphorylation) and the enzyme that catalyzes this reversible reaction is called succinyl CoA synthetase or *succinic thiokinase*.



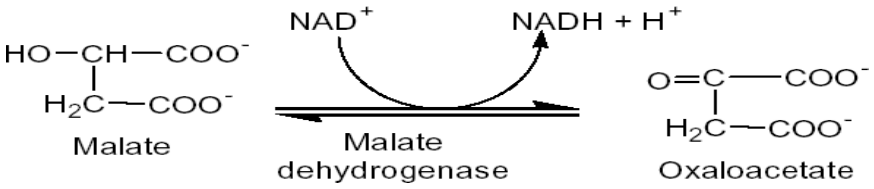

6. ***Oxidation of succinate to fumarate:*** The succinate formed from succinyl CoA is oxidized to fumarate by the enzyme *succinate dehydrogenase*.




7. ***Hydration of fumarate to malate:*** The reversible hydration of fumarate to malate is catalyzed by *fumarase*.




8. ***Oxidation of malate to oxaloacetate:*** The last reaction of the citric acid cycle is, NAD linked *malate- dehydrogenase* which catalyses the oxidation of malate to oxaloacetate.




**Energy yield from TCA cycle:** If one molecule of the substrate is oxidized through NADH in the electron transport chain three molecules of ATP will be formed and through FADH2, two ATP molecules will be generated. As one molecule of glucose gives rise to two molecules of pyruvate by glycolysis, intermediates of citric acid cycle also result as two molecules.

| Reactions | No.of ATP formed |
|---|---|
| 1. 2 isocitrate → 2 α-ketoglutarate (2 NADH + 2H⁺) (2 × 3) | 6 |
| 2. 2 α-ketoglutarate → 2 succinyl CoA (2 NADH + 2H⁺) (2 × 3) | 6 |
| 3. 2 succinyl CoA → 2 succinate (2 GTP = 2ATP) | 2 |
| 4. 2 succinate → 2 Fumarate ($2\ FADH_2$) (2 × 2) | 4 |
| 5. 2 malate → 2 oxaloacetate (2 NADH + 2H⁺) (2 × 3) | 6 |
| Total No.of ATP formed | 24 |

**Vitamins play key roles in the citric acid cycle:**

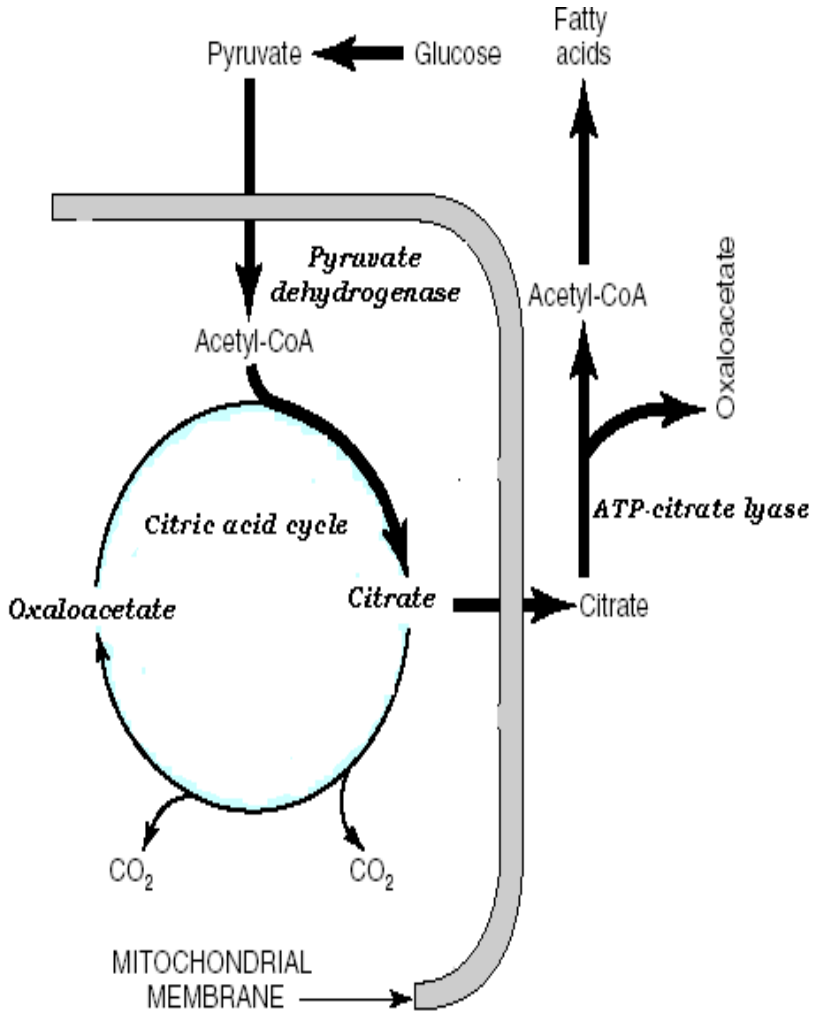
Four of the B vitamins are essential in the citric acid cycle and therefore in energy-yielding metabolism:

1. ***Riboflavin,*** in the form of flavin adenine dinucleotide (FAD), a cofactor in the α-ketoglutarate dehydrogenase complex and in succinate dehydrogenase.
2. ***Niacin,*** in the form of nicotinamide adenine dinucleotide (NAD), the coenzyme for three dehydrogenases in the cycle — isocitrate dehydrogenase, α-ketoglutarate

dehydrogenase and malate dehydrogenase.

3. ***Thiamin* (vitamin B1),** as thiamin diphosphate, the coenzyme for decarboxylation in the α-ketoglutarate dehydrogenase reaction.
4. ***Pantothenic acid*,** as part of coenzyme A, the cofactor attached to ―activel carboxylic acid residues such as acetyl-CoA and succinyl-CoA.

**The Citric Acid Cycle Takes Part in Fatty Acid Synthesis:** Acetyl-CoA, formed from pyruvate by the action of *pyruvate dehydrogenase*, is the major building block for long-chain fatty acid synthesis in nonruminants. (In ruminants, acetyl-CoA is derived directly from acetate.) *Pyruvate dehydrogenase* is a mitochondrial enzyme and fatty acid synthesis is a cytosolic pathway, but the mitochondrial membrane is impermeable to acetyl- CoA. Acetyl-CoA is made available in the cytosol from citrate synthesized in the mitochondrion, transported into the cytosol and cleaved in a reaction catalyzed by *ATP-citrate lyase*.




*Participation of the citric acid cycle in fatty acid synthesis from glucose*

## HMP shunt pathway

Although glycolysis and citric acid cycle are the common pathways by which animal tissues oxidise glucose to $CO_2$ and $H_2O$ with the liberation of energy in the form of ATP, a number of alternative pathways are also discovered. The most important one is Hexose Monophosphate Shunt Pathway (HMP shunt). The pathway occurs in the extra mitochondrial soluble portion of the cells.

- It has two major functions:

I. The formation of **NADPH** for synthesis of fatty acids and steroids

II. The synthesis of **ribose** for nucleotide and nucleic acid formation.

- The fundamental difference between NADPH and NADH (reduced nicotinamide adenine dinucleotide) is that NADH is oxidised by the respiratory chain to generate ATP whereas NADPH serves as a hydrogen and electron donor in reductive biosynthesis, for example in the biosynthesis of fatty acids and steroids.
- Glucose, fructose and galactose are the main hexoses absorbed from the gastrointestinal tract, derived principally from dietary starch, sucrose and lactose respectively. Fructose and galactose are converted to glucose, mainly in the liver.
- The overall equation of the hexose phosphate pathway is

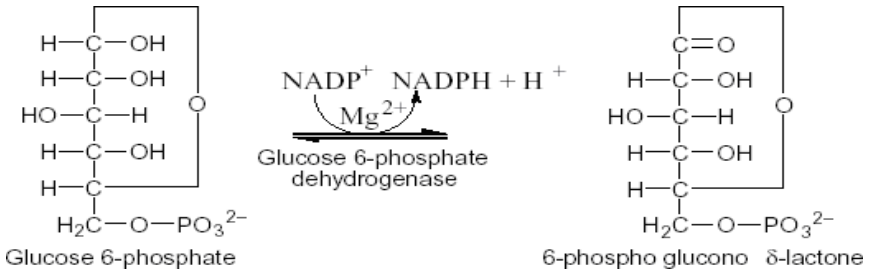
$$\text{Glucose 6-phosphate} + 2NADP^+ + H_2O \longrightarrow \text{Ribose 5-phosphate} + CO_2 + 2NADPH + 2H^+$$

and the net result is the production of NADPH, a reductant for biosynthetic reactions and ribose 5-phosphate, a precursor for nucleotide synthesis.

***Oxidative reactions of the hexose mono-phosphate pathway:***

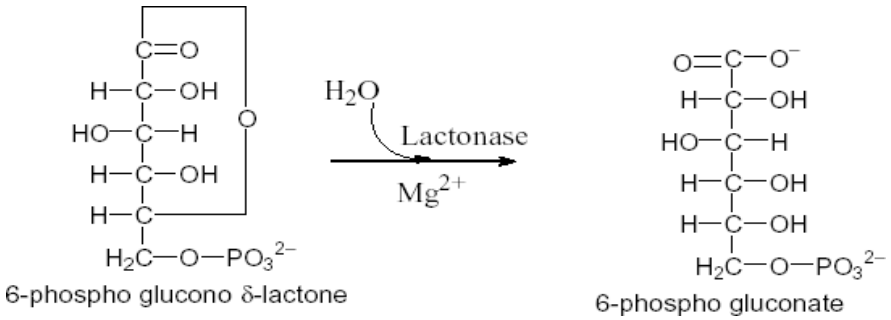
**Step 1:**

Glucose 6-phosphate in the presence of $NADP^+$ and the enzyme *glucose 6-phosphate dehydrogenase*, forms *6-phospho glucono-δ -lactone*. The first molecule of $NADPH^+$ is produced in this step.



**Step 2:**

The *6-phospho glucono- δ-lactone* is unstable and the ester spontaneously hydrolyses to 6- phosphogluconate. The enzyme that catalyses the reaction is *lactonase*.

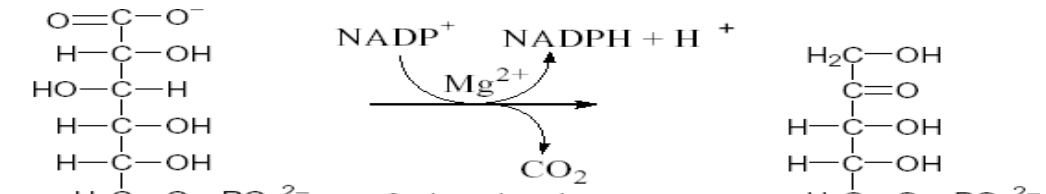



6-phospho glucono δ-lactone

6-phospho gluconate

**Step 3:**

6-phospho gluconate further undergoes dehydrogenation and decarboxylation by *6-phosphogluconate dehydrogenase* to form the ketopentose, D-ribulose 5-phosphate. This reaction generates the second molecule of NADPH.



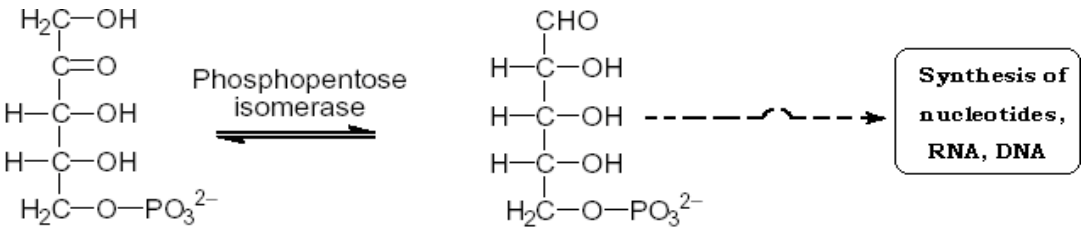

6-phospho gluconate

6-phospho gluconate dehydrogenase

D-Ribulose 5-phosphate

**Step 4:**

The enzyme *phosphopentose isomerase* converts ribulose 5-phosphate to its aldose isomer, D-ribose 5-phosphate.




D-Ribulose 5-phosphate

D-Ribose 5-phosphate

## GLUCONEOGENESIS

- The synthesis of glucose from non-carbohydrate precursors is known as gluconeogenesis.
- The major site of gluconeogenesis is liver.
- It usually occurs when the carbohydrate in the diet is insufficient to meet the demand in the body, with the intake of protein rich diet and at the time of starvation, when tissue proteins are broken down to amino acids.

## Substrates for Gluconeogenesis

Gluconeogenic precursors are molecules that can be used to produce a net synthesis of glucose. They include the intermediates of glycolysis and the citric acid cycle. Glycerol, lactate, and the α-keto acids obtained from the deamination of glucogenic amino acids are the most important gluconeogenic precursors.

1. ***Glycerol:*** Glycerol is released during the hydrolysis of triacylglycerols in adipose tissue and in the liver. Glycerol is phosphorylated by *glycerol kinase* to glycerol phosphate, which is oxidized by *glycerol phosphate dehydrogenase* to dihydroxyacetone phosphate as an intermediate of glycolysis.
2. ***Lactate:*** It is released into the blood by exercising skeletal muscle, and by cells that lack mitochondria, such as red blood cells. In the Cori cycle, glucose is converted by exercising muscle to lactate, which diffuses into the blood. This lactate is taken up by the liver and reconverted to glucose, which diffuse into the circulation.
3. ***Amino acids:*** Amino acids derived from hydrolysis of tissue proteins are the major sources of glucose during fasting.

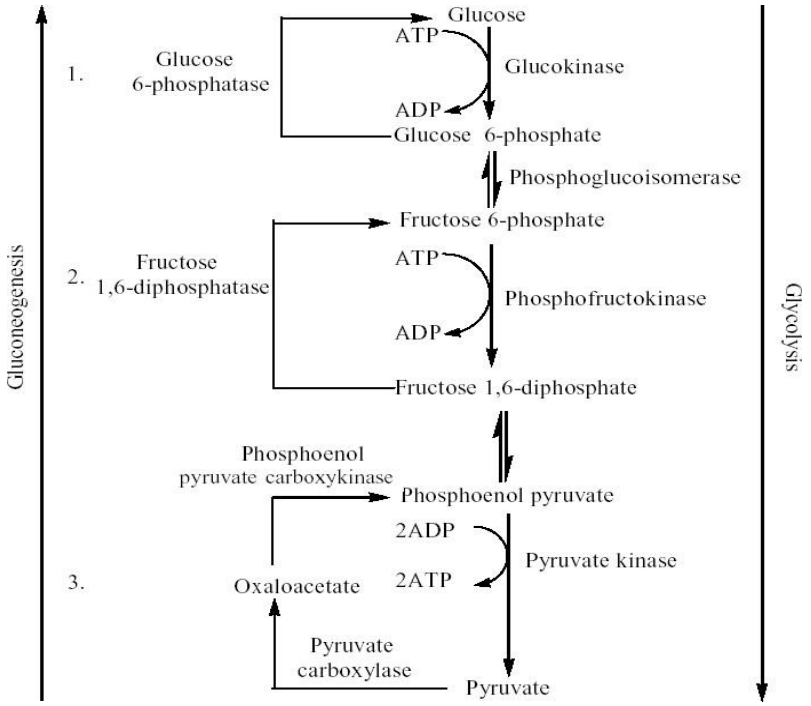
## Gluconeogenesis and glycolysis

Gluconeogenesis and glycolysis are opposing metabolic pathways and share a number of enzymes. In glycolysis, *glucose* is converted to *pyruvate* and in gluconeogenesis *pyruvate* is converted to *glucose*. However gluconeogenesis is not exact reversal of glycolysis.

There are three essentially irreversible steps in glycolysis which are

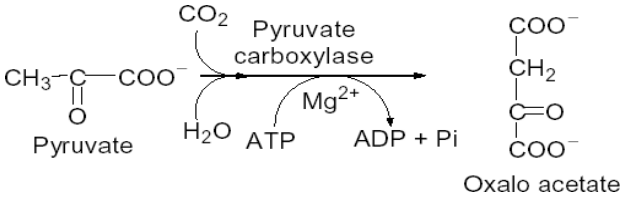
1. Glucose + ATP $\xrightarrow{\text{Glucokinase}}$ Glucose 6-phosphate + ADP
2. Fructose 6-phosphate + ATP $\xrightarrow{\text{Phosphofructo kinase}}$ Fructose 1,6-diphosphate + ADP
3. Phosphoenol pyruvate + ADP $\xrightarrow{\text{Pyruvate kinase}}$ Pyruvate + ATP

In gluconeogenesis these three reactions are bypassed or substituted by the following news ones.

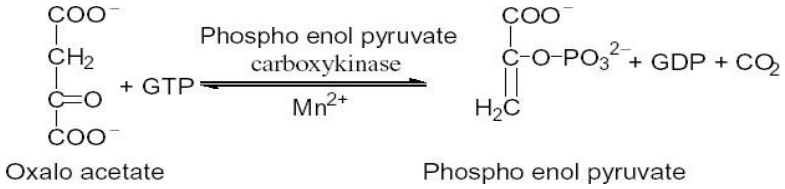



## Reactions of gluconeogenesis

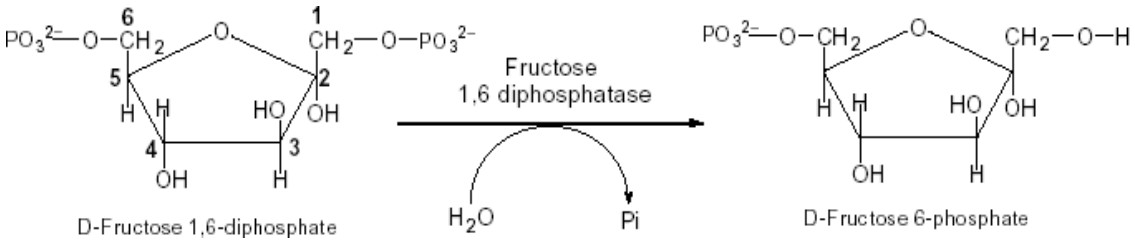
1. The formation of ***phosphoenol pyruvate*** begins with the carboxylation of ***pyruvate*** at the expense of ATPto form ***Oxalo acetate***.



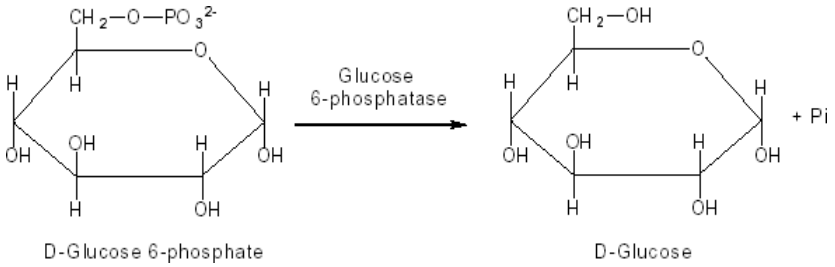

***Oxalo acetate*** is converted to ***phosphoenolpyruvate*** by phosphorylation with GTP, accompanied by asimultaneous *decarboxylation*.




2. ***Fructose 6-phosphate*** is formed from ***fructose 1, 6-diphosphate*** by hydrolysis and the enzyme *fructose 1,6 diphosphatase* catalyses this reaction.



3. ***Glucose*** is formed by hydrolysis of ***glucose 6-phosphate*** catalysed by *glucose 6-phosphatase*.




## Gluconeogenesis of amino acids

Amino acids which could be converted to glucose are called glucogenic amino acids. Most of the glucogenicamino acids are converted to the intermediates of citric acid cycle either by transamination or deamination.

## Gluconeogenesis of Propionate:

Propionate is a major source of glucose in ruminants, and enters the main gluconeogenic pathway via thecitric acid cycle after conversion to succinyl CoA.

## Gluconeogenesis of Glycerol

At the time of starvation glycerol can also undergo gluconeogenesis. When the

$$\text{Glycerol + ATP} \xrightarrow{\text{Glycerol kinase}} \text{Glycerol 3-phosphate + ADP}$$

triglycerides are hydrolysed in the adipose tissue, glycerol is released. Further metabolism of glycerol does not take place in the adipose tissue because of the lack of glycerol kinase necessary to phosphorylate it. Instead, glycerol passes to the liver where it is phosphorylated to glycerol 3-phosphate by the enzyme glycerol kinase.

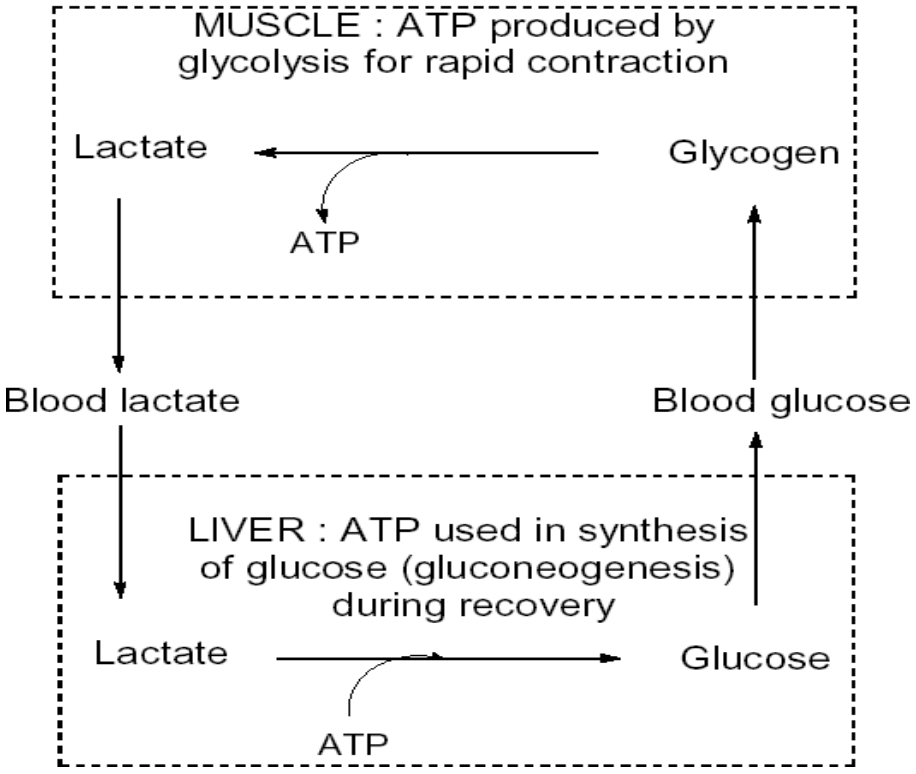
This pathway connects the triose phosphate stage of glycolysis, because glycerol 3-phosphate is oxidized to dihydroxy acetone phosphate in the presence of NAD+ and glycerol 3-phosphate dehydrogenase.

$$\text{Glycerol 3-phosphate + } NAD^+ \xrightarrow{\text{Glycerol 3-phosphate dehydrogenase}} \text{Dihydroxy acetone phosphate + NADH + } H^+$$

This dihydroxy acetone phosphate enters gluconeogenesis pathway and gets converted to glucose. Liver and kidney are able to convert glycerol to blood glucose by making use of the above enzymes.

## Gluconeogenesis of lactic acid (Cori cycle)

The liver and skeletal muscles exhibit a special metabolic cooperation as far as carbohydrates areconcerned by the way of a cycle of conversions known as Cori cycle.




*Cori cycle*

- In this cycle liver glycogen may be converted into muscle glycogen and vice versa and the major raw material of this cycle is lactate produced by the active skeletal muscles.
- At the time of heavy muscular work or strenuous exercise, $O_2$ supply is inadequate in active muscles but the muscles keep contracting to the maximum. Hence, glycogen stored up in the muscle is converted into lactic acid by glycogenolysis followed by anaerobic glycolysis and thus lactate gets accumulated in the muscle. Muscle tissue lacks the enzyme *glucose 6-phosphatase* hence it is incapable of synthesizing glucose from lactic acid and the conversion take place only in the liver.
- Lactate diffuses out of the muscle and enters the liver through blood. In the liver lactate is oxidised to pyruvate which undergoes the process of gluconeogenesis resulting in the resynthesis of glucose.
- The glycogen may be once again converted to glucose (glycogenolysis) and may be recycled to the muscle through the blood. The process of gluconeogenesis completes the cycle by converting glucoseonce again to muscle glycogen.

## DIABETES MELLITUS

- Diabetes mellitus is an important disorder of carbohydrate metabolism. However, fat and protein metabolism are also affected in diabetic condition. Diabetes means excretion of excessive volume of urine and mellitus means sweet. So the word diabetes mellitus refers to chronic excretion of large volume of urine containing glucose.
- Diabetes mellitus, caused by a deficiency in the secretion or action of insulin, is a relatively common disease. Insulin is an endocrine hormone which is secreted by β-cells of *islets of Langerhans* of pancreas. The abnormality in glucose metabolism is indicative of diabetes or a tendency towards the condition. Diabetes mellitus is really a group of diseases in which the regulatory activity of insulin is defective.
- There are two major clinical classes of the disease:

1. ***Type-I or insulin dependent diabetes mellitus (IDDM),*** this disease begins early in the life and quickly becomes severe.
2. ***Type - II or non-insulin dependent diabetes mellitus (NIDDM),*** this disease is slow to develop, milder and often goes unrecognized.

- Type one requires insulin therapy and careful, life-long control of the balance between glucose intake and insulin dose. The decreased or defective production of insulin is characterized by the following symptoms.

i. Decreased permeability of the cell membrane for glucose resulting in the accumulation of glucose in the blood. This condition is known as hyperglycemia. Glucose concentration increases as high as 500 mg/100 ml of blood.

ii. **Polyuria:** This means excretion of increased quantity of urine. This is to excrete the additional quantity of glucose in urine (glucosuria).

iii. **Polydipsia:** The excessive thirst which leads to increased consumption of water. This condition is known as polydipsia. This is to replace the volume of water excreted due to polyuria.

iv. **Polyphagia**: Excessive appetite leads to polyphagia and increased intake of food. This is to replace the lost nourishment. The diabetic has voracious appetite, but inspite of over eating; they lose weight and become lean and emaciated.

v. As glucose is not enough for energy production, increased mobilisation of fat from adipose tissue occurs. But the metabolism of fat is incomplete resulting in the production of large amounts of the intermediary products of fat metabolism namely ketone bodies (e.g. Acetoacetate and β-hydroxybutarate). This condition is known as 'ketosis' and excess ketone bodies cause severe acidosis, ultimately resulting in 'coma'.

***Deposition of lipids in the walls of the blood vessels resulting "atherosclerosis".***

- Biochemical measurements on the blood and urine are essential in the diagnosis and treatment of diabetes, which causes profound changes in metabolism. A sensitive diagnostic criterion is provided by the *glucose tolerance test* (GTT).

***Classification of antidiabetic drugs:*** Antidiabetic drugs can be classified into two categories:

1. ***Insulin injections*:** Mostly used on serious cases of diabetes.
2. *Oral hypoglycaemic agents*: These agents are the group of drugs that may be taken singly or in combination to lower the blood glucose in type 2 diabetes. Type 2 diabetes can be due to increased peripheral resistance to insulin or to reduced secretion of insulin. Oral hypoglycaemic should be used together with changes in diet and lifestyle to achieve good glycaemic control and it is customary to monitor such changes for three months before considering medication.

Oral hypoglycaemic agents are not usually used in type 1 diabetes, but *metformin* may be of use inoverweight type 1 diabetics.

The following groups of oral hypoglycaemics are currently available:

*Biguanides derivatives*: Metformin; *Sulphonylureas derivatives:* glimepiride;

*Postprandial glucose regulators*: Repaglinide and Nateglinide;

*Thiazolidinediones derivatives*: Pioglitazone and Rosiglitazone and *Acarbose*: *which* acts by inhibiting intestinal alpha glucosidases, which delays the absorption and digestion of sucrose and starch.

## Glucose Tolerance Test (GTT) or Oral Glucose Tolerance Test (OGTT)

A Glucose Tolerance Test in medical practice is the administration of glucose to determine how quickly it is cleared from the blood. The test is usually used to test for diabetes, insulin resistance, and sometimes reactive hypoglycemia. The glucose is most often given orally so technically is terms as an oral glucose tolerance test (OGTT)

## Preparation and cautions

The patient is instructed not to restrict carbohydrate intake in the days or weeks before the test. The test should not be done during an illness, as results may not reflect the patient's glucose metabolism when healthy. A full adult dose should not be given to a

person weighing less than 43 kg (94 lb).

**Procedure for OGTT**

- The patient should have been fasting for the previous 8-14 hours (water is allowed).
- Then the patient has given a glucose solution to drink about 1.75 grams per kilogram of body weight, to amaximum dose of 75 g. It should be drunk within 5 minutes.
- Blood is drawn at intervals for measurement of glucose (blood sugar), the intervals and number ofsamples varies according to the purpose of the test.
- If renal glycosuria (sugar excreted in the urine despite normal levels in the blood) is suspected, urine samples may also be collected for testing along with the fasting and 2 hour blood tests.

**Interpretation of OGTT results**

- Fasting plasma glucose should be below 6.1 mmol/l (110 mg/dl). Fasting levels between 6.1 and 7.0 mmol/l (110 and 126 mg/dl) are borderline ("impaired fasting glycaemia"), and fasting levels repeatedly ator above 7.0 mmol/l (126 mg/dl) are diagnostic of diabetes.
- The 2 hour glucose level should be below 7.8 mmol/l (140 mg/dl). Levels between this and 11.1 mmol/l (200 mg/dl) indicate, "Impaired Glucose Tolerance." A glucose level above 11.1 mmol/l (200 mg/dl) at 2 hours confirms a diagnosis of diabetes.

**REGULATION OF GLUCONEOGENESIS**

The regulation of gluconeogenesis is determined primarily by the circulating level of ***glucagon***, and by theavailability of gluconeogenic substrates.

**A. Glucagon**

This pancreatic islet hormone stimulates gluconeogenesis by three mechanisms.

- **Changes in allosteric effectors:** Glucagon lowers the level of fructose 2, 6-bisphosphate, resulting in activation of *fructose 1, 6- bisphosphatase* and inhibition of *phosphofructokinase* , thus favoring gluconeogenesis over glycolysis.
- **Covalent modification of enzyme activity:** Glucagon, via an elevation in cyclic AMP (cAMP) level and *c AMP- dependent protein kinase* activity, stimulates the conversion of *pyruvate kinase* to its inactive (phosphorylated) form. This decreases the conversion of PEP to pyruvate, which has the effect of diverting PEP to the synthesis of glucose.
- **Induction of enzyme synthesis:** Glucagon increases the transcription of the *PEP- carboxykinase* gene, thereby increasing the availability of this enzyme's

activity as levels of its substrate rise during fasting.

[***Note by:*** Insulin causes decreased transcription of the mRNA for this enzyme.]

**B. Substrate availability**

The availability of gluconeogenic precursors, particularly glucogenic amino acids, significantly influences the rate of hepatic glucose synthesis. Decreased levels of insulin favor mobilization of amino acids from muscle protein and provide the carbon skeletons for gluconeogenesis.

**C. Allosteric activation by acetyl CoA**

Allosteric activation of hepatic *pyruvate carboxylase* by acetyl CoA occurs during fasting. As a result of excessive lipolysis in adipose tissue, the liver is flooded with fatty acids. The rate of formation of acetyl CoA by β-oxidation of these fatty acids exceeds the capacity of the liver to oxidize it to $CO_2$ and $H_2O$. As a result, acetyl CoA accumulates and leads to activation of *pyruvate carboxylase*.

[**Note:** Acetyl CoA inhibits *pyruvate dehydrogenase*. Thus, this single compound can divert pyruvate toward gluconeogenesis and away from the TCA cycle]

## GLYCOGEN METABOLISM

- Glycogen is a branched polymer of **α-D-glucose**.
- The main stores of glycogen in the body are found in skeletal muscle and liver, although most other cells store small amounts of glycogen for their own use.
- The function of muscle glycogen is to serve as a fuel reserve for the synthesis of adenosine triphosphate (ATP) during muscle contraction. That of liver glycogen is to maintain the blood glucose concentration, particularly during the early stages of a fast.
- Approximately 400 g of glycogen make up one to two percent of the fresh weight of resting muscle, and approximately 100 g of glycogen make up to ten percent of the fresh weight of a well-fed adult liver.

***Structure of glycogen:*** Glycogen is a branched-chain homopolysaccharide made exclusively from α-D- glucose. The primary glycosidic bond is an **α(1→4)** linkage. After an average of eight–ten glucosyl residues, there is a branch containing an **α(1→6)** linkage. A single molecule of glycogen can have a molecular mass of up to $10^8$ daltons.

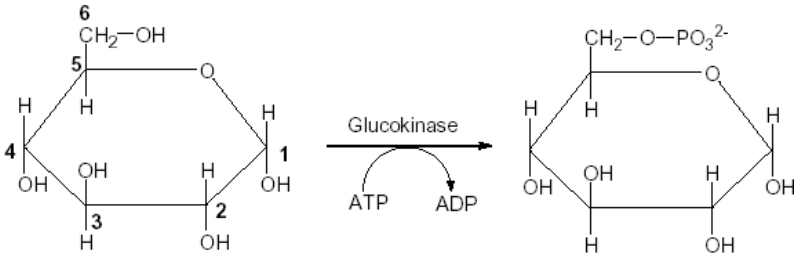
- Liver glycogen stores increase during the well-fed state, and are depleted during a fast. Muscle glycogen is not affected by short periods of fasting (a few days) and is only moderately decreased in prolonged fasting (weeks).

**Glycogen biosynthesis**:

- The process of biosynthesis of glycogen from glucose is known as glycogenesis.
- The process occurs in the cytosol, and requires energy supplied by ATP for the phosphorylation of glucose and uridine triphosphate (UTP).
- Glycogenesis is a very essential process since the excess of glucose is converted and stored up as glycogen which could be utilised at the time of requirement. In the absence of this process the tissues are exposed to excess of glucose immediately after a meal and they are starved of it at other times.
- The following are the various reactions of glycogenesis are as follows:

### Step 1

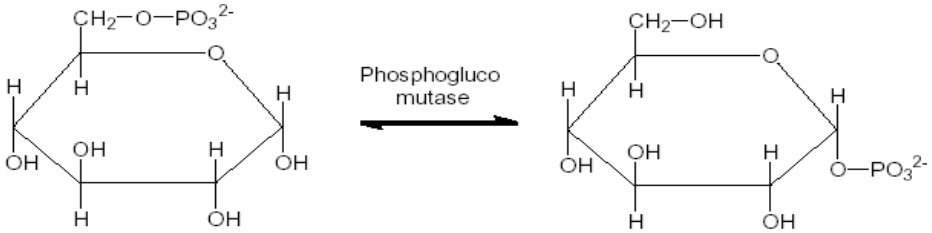
**Glucose** is phosphorylated to **glucose 6-phosphate**, a reaction that is common to the first reaction in the pathway of glycolysis from glucose. This reaction is catalysed by ***hexokinase*** in muscle and ***glucokinase*** in liver in the presence of ATP.




Glucose Glucose 6-phosphate

### Step 2

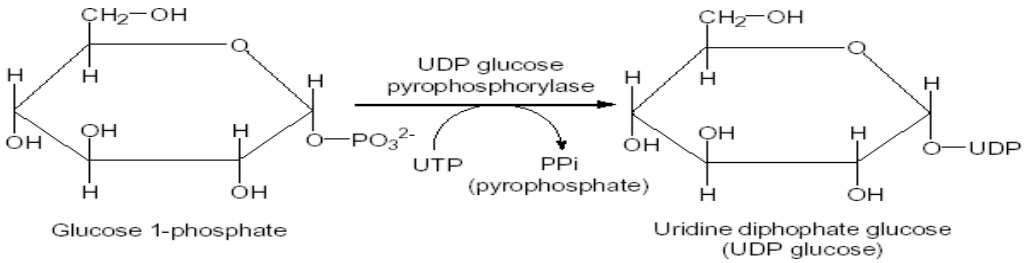
**Glucose 6-phosphate** is then reversibly converted to **glucose 1-phosphate** in a reaction catalysed by enzyme ***phosphogluco mutase***. This process requires $Mg^{2+}$ and a small amount of **glucose 1, 6-diphosphate** as coenzyme.




Glucose 6-phosphate Glucose 1-phosphate

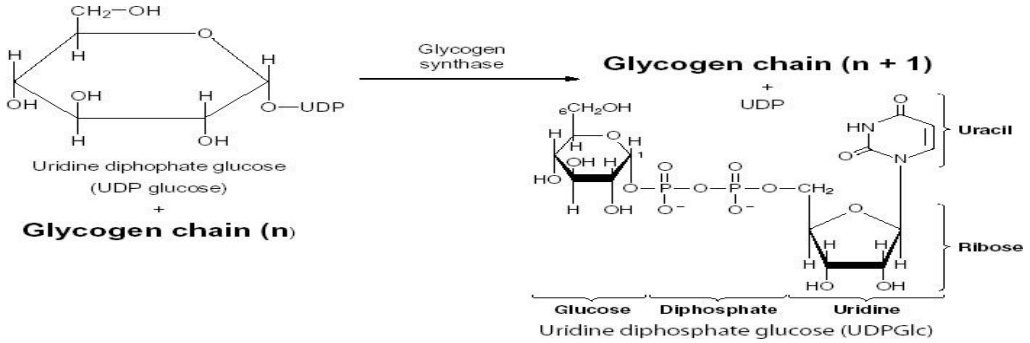
### Step 3

The **glucose 1-phosphate** is then activated by the energy produced by the hydrolysis of **uridine triphosphate** (UTP) in the presence of ***uridine diphosphate glucose pyrophophosrylase***. This is a key reaction in glycogen biosynthesis.




## Step 4

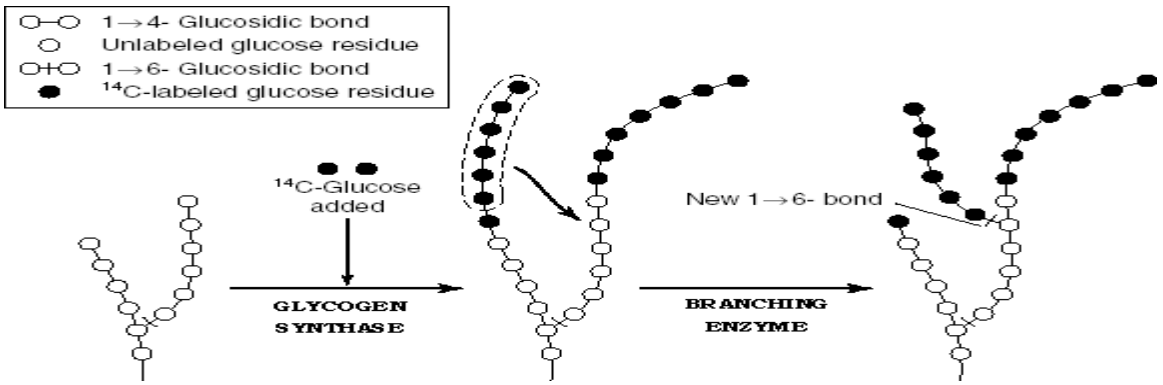
**UDP-glucose** is the immediate donor of glucose residues in the reaction catalyzed by ***glycogen synthase***, which promotes the transfer of the glucose residue from UDP-glucose to a non-reducing end of a branched glycogen chain.




## Step 5

When the chain has become long with more than 8 glucose units, a second enzyme, namely branching enzyme ***amylo 1-4 to 1-6 transglycosylase*** acts on the glycogen. Glycogenthus formed may be stored in liver, muscles and tissues.

If no other synthetic enzyme acted on the chain, the resulting structure would be a linear (unbranched) molecule of glucosyl residues attached by α(1→4) linkages. Such a compound is found in plant tissues, and is called **amylose**. In contrast, glycogen has branches located, on average, eight glucosyl residues apart, resultingin a highly branched, tree-like structure that is far more soluble than the unbranched amylose. Branching also increases the number of nonreducing ends to which new glucosyl residues can be added.



## DEGRADATION OF GLYCOGEN (GLYCOGENOLYSIS)

- When the blood sugar level falls (Hypoglycemia), glycogen stored in the tissues specially glycogen of liver and muscles may be broken down and this process of breakdown of glycogen is called glycogenolysis.
- When glycogen is degraded, the primary product is **glucose 1-phosphate**, obtained by breaking **α (1→4)** glycosidic bond. In addition, **free glucose** is released from each **α (1→6)**-linked glucosyl residue.

### *A.* Shortening of chains

Glycogen phosphorylase sequentially cleaves the α(1→4) glycosidic bonds between the glucosyl residues at the nonreducing ends of the glycogen chains by simple phosphorolysis until four glucosyl units remain on each chain before a branch point. The resulting structure is called a limit dextrin, and phosphorylase cannot degrade it any further.

### *B.* Removal of branches

Branches are removed by the two enzymic activities of a single bifunctional protein, the debranching enzyme. First, ***oligo-α (1→4)→α(1→4)-glucan transferase*** removes the outer three of the four glucosyl residues attached at a branch. It next transfers them to the nonreducing end of another chain, lengthening it accordingly. Thus, an α(1→4) bond is broken and an **α(1→4)** bond is made, and the enzyme functionsas a ***4:4 transferase***.

Then the remaining single glucose residue attached in an **α(1→6)** linkage is removed hydrolytically by ***amylo-α(1→6)-glucosidase*** activity, releasing **free glucose**.

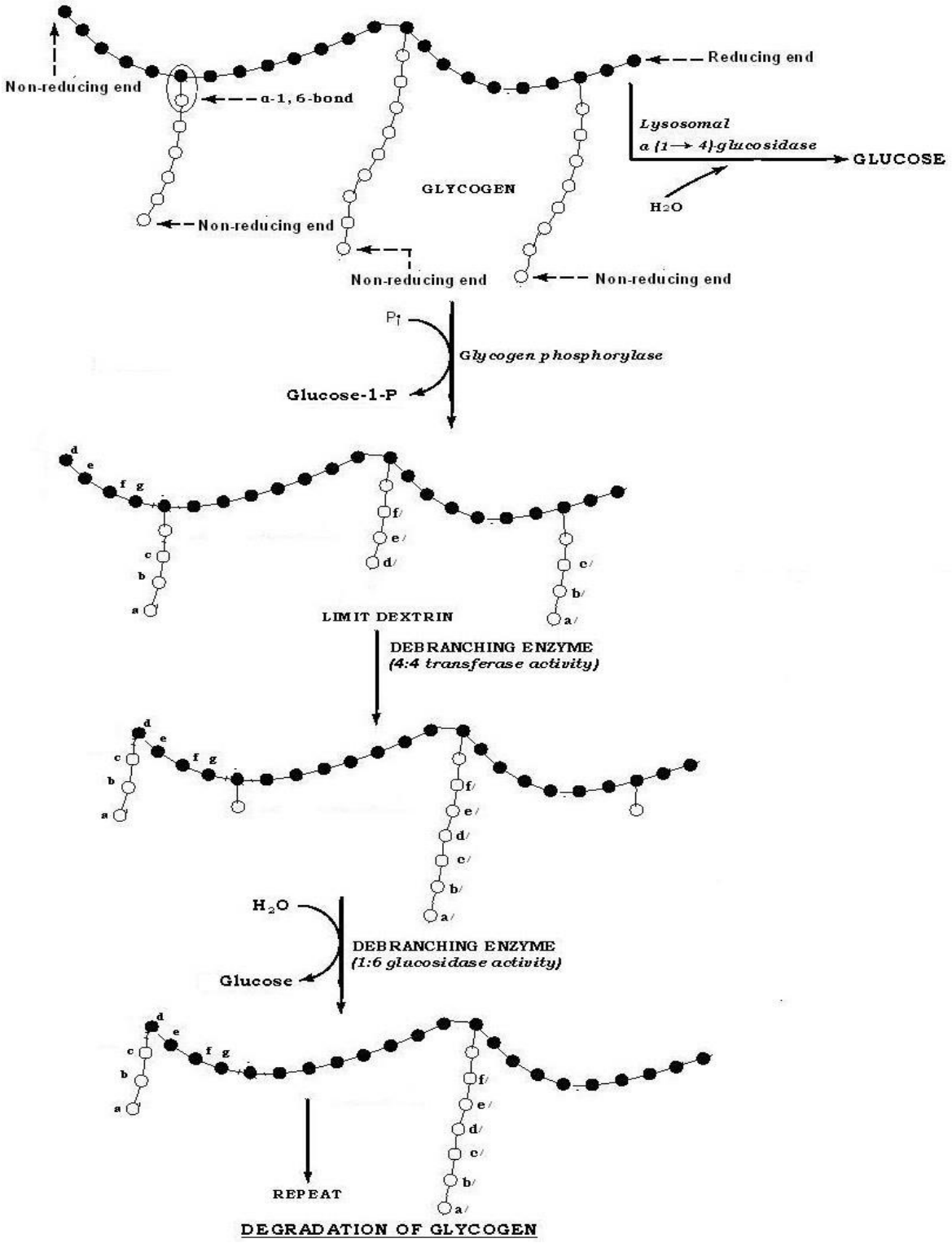
The glucosyl chain is now available again for degradation by glycogen phosphorylase until four glucosyl units from the next branch are reached.

### *C.* Conversion of glucose 1-phosphate to glucose 6-phosphate

Glucose 1-phosphate, produced by ***glycogen phosphorylase***, is converted in the cytosol to glucose 6- phosphate by ***phosphoglucomutase***.

In the liver, glucose 6-phosphate is translocated into the endoplasmic reticulum (ER) by ***glucose 6- phosphate translocase***. There it is converted to glucose by glucose 6-phosphatase—the same enzyme used in the last step of gluconeogenesis.

In the muscle, glucose 6-phosphate cannot be dephosphorylated because of a lack of ***glucose 6- phosphatase***. Instead, it enters glycolysis, providing energy needed for muscle contraction.




### *D.* Lysosomal degradation of glycogen

A small amount (one–three percent) of glycogen is continuously degraded by the lysosomal enzyme, ***α(1→4)-glucosidase*** (***acid maltase***). The purpose of this pathway is unknown. However, a deficiency of this enzyme causes accumulation of glycogen in vacuoles in the lysosomes, resulting in the serious glycogen storage disease type II (**Pompe disease**).

## GLYCOGEN STORAGE DISEASES (GSD)

- Glycogen storage disease (GSD, also glycogenosis and dextrinosis) Glycogen storage disease (GSD, also glycogenosis and dextrinosis) is the result of defects in the processing of glycogen synthesis or breakdown within muscles, liver, and other cell types
- Glycogen is a major source of energy for the body. It is stored in the form of glycogen in both the liverand muscles and later released with the help of enzymes. Persons affected by GSD have an inheriteddefect in one of the enzymes responsible for forming or releasing glycogen as it is needed by the body during exercise and/or between meals.

### Types of Glycogen Storage Disease

#### 1. Type 0 - glycogen synthase deficiency:

The enzyme *glycogen synthase* is needed for the body to make glycogen. A deficiency results in very low amounts of glycogen stored in the liver. A person between meals can develop very low blood sugar levels, known as hypoglycemia.

#### 2. Type I - Von Gierke Disease:

- It is also known as *glucose-6-phosphatase* deficiency, in which the body cannot break down glycogen for energy.
- Gycogen is stored in the liver and muscles and is normally broken down into glucose when you do not eat. It occurs when the body lacks the protein (enzyme) that releases glucose from glycogen. This causes abnormal amounts of glycogen to build up in certain tissues. When glycogen is not broken down properly,it leads to low blood sugar.
- *Von Gierke* disease is inherited, which means it is passed down through families. If both parents carry the defective gene related to this condition, each of their children has a 25% chance of developing the disease.

#### 3. Glycogen Storage Disease Type II:

- It is also known as Pompe disease or acid maltase deficiency.
- It is an inherited disorder caused by the buildup of a complex sugar called glycogen in the body's cells. The accumulation of glycogen in certain organs and tissues, especially muscles, impairs their ability to function normally.
- Three types of Pompe disease,

i. The classic form of infantile-onset Pompe disease begins within a few months of birth. Infants with this disorder typically experience muscle weakness (myopathy), poor muscle tone (hypotonia), an enlarged liver (hepatomegaly), and heart defects. Affected

infants may also fail to gain weight and grow at the expected rate (failure to thrive) and have breathing problems. If untreated, this form of Pompe disease leads to death from heart failure in the first year of life.

ii. The non-classic form of infantile-onset Pompe disease usually appears by age 1. It is characterized by delayed motor skills (such as rolling over and sitting) and progressive muscle weakness. The heart may be abnormally large (cardiomegaly), but affected individuals usually do not experience heart failure. The muscle weakness in this disorder leads to serious breathing problems, and most children with non-classic infantile-onset Pompe disease live only into early childhood.

iii. The late-onset type of Pompe disease may not become apparent until later in childhood, adolescence, or adulthood. Late-onset Pompe disease is usually milder than the infantile-onset forms of this disorder and is less likely to involve the heart. As the disorder progresses, breathing problems can lead to respiratory failure take place.

### *4.* Glycogen Storage Disease Type IV:

- It is also known as *Andersen disease* or *brancher enzyme* deficiency.
- Deficient activity of the glycogen-branching enzyme is the cause of GSD Type IV. It results in accumulation of abnormal glycogen in the liver, muscle and other tissues.

### *5.* Glycogen Storage Disease Type V:

- It is also known as *McArdle Disease.*
- It cause due to *myophosphorylase* deficiency.
- It is a rare metabolic disorder which causes muscle pain in everyday activities and exercise. If activity is prolonged despite the pain then muscle damage ensues with the risk of muscle breakdown and kidney failure.

### *6.* Glycogen Storage Disease Type VI:

- It is also known as *Hers* disease.
- It cause due to *liver phosphorylase* deficiency.

### *7.* Glycogen Storage Disease Type VII:

- It is also known as *Tarui* disease.
- It cause due to *muscle phosphofructokinase* deficiency.
- The phosphofructokinase enzyme which is needed to facilitate the breakdown of glycogen into energy in muscle. This results in reduced amount of energy available to muscles during exercise.

- The body breaks down muscle when trying to attain energy, which causes symptoms such as muscle pain, cramping, fatigue and tenderness. With the breakdown of muscle and the release of the red protein myoglobin, red-brown urine may be seen.
- The enzyme deficiency is due to abnormalities in the muscle phosphofructokinase gene. GSD VII is inherited as an autosomal recessive genetic disorder.

### 8. Glycogen Storage Disease Type IX:

- It cause due to *liver glycogen phosphorylase kinase* deficiency.
- In most individuals apart from liver enlargement there are few other problems. There is usually no tendency to low blood sugar, the liver becomes smaller with age and children grow normally.

| Glycogenosis | Name | Cause of Disorder | Characteristics |
|---|---|---|---|
| Type I | Von Gierke's disease | Deficiency of glucose-6-phosphatase | Liver cells and renal tubule cells loaded with glycogen. Hypoglycemia, lactic-acidemia, ketosis, hyperlipemia. |
| Type II | Pompe's disease | Deficiency of lysosomal α-1→4- and 1→6-glucosidase (acid maltase) | Fatal, accumulation of glycogen in lysosomes, heart failure. |
| Type III | Limit dextrinosis, Forbes' or Cori's disease | Absence of debranching enzyme | Accumulation of a characteristic branched polysaccharide. |
| Type IV | Amylopectinosis, Andersen's disease | Absence of branching enzyme | Accumulation of a polysaccharide having few branch points. Death due to cardiac or liver failure in first year of life. |
| Type V | Myophosphorylase deficiency, McArdle's syndrome | Absence of muscle phosphorylase | Diminished exercise tolerance; muscles have abnormally high glycogen content (2.5–4.1%). Little or no lactate in blood after exercise. |
| Type VI | Hers' disease | Deficiency of liver phosphorylase | High glycogen content in liver, tendency toward hypoglycemia. |
| Type VII | Tarui's disease | Deficiency of phosphofructokinase in muscle and erythrocytes | As for type V but also possibility of hemolytic anemia. |
| Type VIII | | Deficiency of liver phosphorylase kinase | As for type VI. |

***Summary of Glycogen storage disease (GSD, also glycogenosis and dextrinos***

# HORMONE CONTROL OF CARBOHYDRATE METABOLISM

## Introduction:

The metabolism of carbohydrates is regulated by a variety of hormones and other molecules. Some of these have already been mentioned in previous sections. The proper functions of the body are dependent on precise control of the glucose concentration in the blood. The normal fasting level of glucose in the blood is 70-90 mg/100 ml.

If the concentration of glucose in blood is too high (above 120 mg/100 mL) a condition known as **hyperglycemia** results. Hyperglycemia may temporarily exist as a result of eating a meal rich in carbohydrates.

If the concentration of glucose is too low (below 70 mg/100 ml) a condition of hypoglycemia exists. Hypoglycemia is characterized by general weakness, trembling, drowsiness, headache, profuse perspiration, rapid heart beat, and possible loss of consciousness.

## INSULIN:

**Insulin**, a polypeptide, is secreted from the pancreas in response to a hyperglycemia condition which usually results shortly after ingesting a meal. The major effect of insulin is to promote the transport of sugar across the cell membrane of fat and muscle cells. In addition, insulin promotes anabolic processes such as increasing the rate of synthesis for glycogen (glycogenesis), fatty acids, and proteins. Insulin inhibits the catabolic processes such as the breakdown of glycogen and fat.

A deficiency of insulin (hypoinsulinism) results in a permanent hyperglycemic condition known as diabetes mellitus. If little or no insulin is present, glucose cannot be utilized properly by the cells and accumulates in the blood. Fatty acid metabolism is also upset. For this reason, a detailed study of diabetes mellitus must wait until the next chapter.

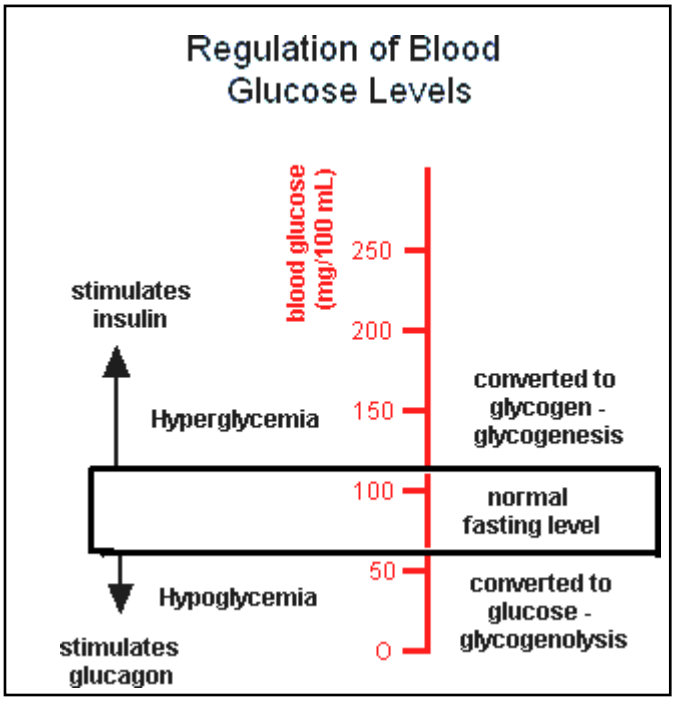
Hyperinsulinism (too much insulin) leads to the hypoglycemic condition. Excessive amounts of glucose are removed from the blood. Severe hypoglycemia may result when a diabetic injects too much insulin. A severe insulin shock may result in a coma since glucose does not reach the brain. A diabetic usually carries a glucose rich food, such as candy, to provide a quick supply of glucose to replenish depleted glucose levels caused by too much insulin.

A functional type of hypoglycemia results in some individuals from an over stimulation of insulin. The causes of hypoglycemia are not completely understood, but it occurs in some people after eating heavily sugared food such as heavily sugared cereal and/or coffee and sweet rolls. The initial high glucose levels over stimulates the pancreas to produce too much insulin

## GLUCAGON:

If one hormone, insulin, controls the excess of glucose in the blood by stimulating synthesis of glycogen, then other hormones must respond to low levels of glucose. The liver is more responsive to **glucagon**, a peptide also secreted by the pancreas.

Glucagon increases glucose levels in the blood by stimulating the breakdown of glycogen (glycogenolysis) in the liver into glucose which leaves the liver cells and enters the blood stream. The method of hormone stimulation is a complex cascade effect. The exact sequence has been worked out in the most detail for epinephrine (adrenalin) although glucagon works in a similar fashion.




## BIOLOGICAL OXIDATION

### Biological oxidation-reduction reaction

Oxidation-reduction (or "redox") reactions are a very large class of chemical reactions in which both oxidation and reduction necessarily occur.

An **oxidation** is defined as loss of electrons in the course of a chemical reaction. If a species gains electrons, it is undergoing a **reduction**. Since electrons are "conserved" in a chemical reaction (they are not created or destroyed), one chemical species' loss is another's gain. Thus, a reduction cannot occur with a corresponding oxidation, and vice-versa. The term "redox" also nicely encapsulates how inextricably tied together oxidation and reduction are in reality.

Other terminology used in discussing redox chemistry: A chemical species that gets reduced is acting as an **oxidizing agent**, or **oxidant**, while the species undergoing oxidation is acting as the **reducing agent**, or **reductant**.

**Oxidation state** (or **oxidation number**) is a bookkeeping device employed by chemists to help them classify and understand chemical reactions. The simplest way to interpret oxidation number is to think of it as the number of electrons lost or gained by an atom (compared to its neutral, uncombined form) when it reacts to form ions or molecules. Consider first the case of ions. For monatomic ions, such as $Na^+$ or $Cl^-$, the oxidation number is the same as the charge, +1 and −1, for the sodium cation and chloride anion, respectively. In molecules and polyatomic ions, oxidation states for atoms are calculated by comparing the number of valence electrons in the neutral atom with a count of the surrounding bonding and nonbonding electrons in the Lewis structure. In this respect, it is similar to determining formal charge of an atom in a Lewis structure. A general rule in determining an oxidation number of an atom in a complete Lewis structure, is any differences in **electronegativity** between covalently bondedatoms is treated as if the bond is actually ionic. That means both electrons are counted as belonging to the more electronegative atom.

In computing formal charge, electrons in covalent bonds are treated as equally shared, despite differences in electro negativity. But like formal charge, the sum of the oxidation numbers for each atom in a formula or Lewis structure for a molecular or ionic species must sum to the net charge of that formula or Lewis structure (zero for a molecule). We will soon see that assignment of oxidation numbers and following how they change in a chemical reaction allows us to recognize redox reactions and determine the stoichiometry of the electron transfer occurring.

It is easy to recognize any reaction featuring an uncombined, neutral element as a redox reaction. examples are

$$CH_4(g) + 2\,O_2(g) \rightarrow CO_2(g) + 2\,H_2O(l)$$

$$Fe(s) + O_2(g) \rightarrow Fe_2O_3(s)$$

$$2\,Na(s) + Cl_2(g) \rightarrow 2\,NaCl$$

# ELECTRON TRANSPORT CHAIN

## Definition

The electron transport chain is a cluster of proteins that transfer electrons through a membrane within mitochondria to form a gradient of protons that drives the creation of adenosine triphosphate (ATP). ATP is used by the cell as the energy for metabolic processes for cellular functions.

During the process, a proton gradient is created when the protons are pumped from the mitochondrial matrix into the intermembrane space of the cell, which also helps in driving ATP production. Often, the use of a proton gradient is referred to as the chemiosmotic mechanism that drives ATP synthesis since it relies on a higher concentration of protons to generate "proton motive force". The amount of ATP created is directly proportional to the number of protons that are pumped across the inner mitochondrial membrane.

The electron transport chain involves a series of redox reactions that relies on protein complexes to transfer electrons from a donor molecule to an acceptor molecule. As a result of these reactions, the proton gradient is produced, enabling mechanical work to be converted into chemical energy, allowing ATP synthesis. The complexes are embedded in the inner mitochondrial membrane called the cristae in eukaryotes. Enclosed by the inner mitochondrial membrane is the matrix, which is where necessary enzymes such as pyruvate dehydrogenase and pyruvate carboxylase are located. The process can also be found in photosynthetic eukaryotes in the thylakoid membrane of chloroplasts and in prokaryotes, but with modifications.

By-products from other cycles and processes, like the citric acid cycle, amino acid oxidation, and fatty acid oxidation, are used in the electron transport chain. As seen in the overall redox reaction, energy is released in an exothermic reaction when electrons are passed through the complexes; three molecules of ATP are created.

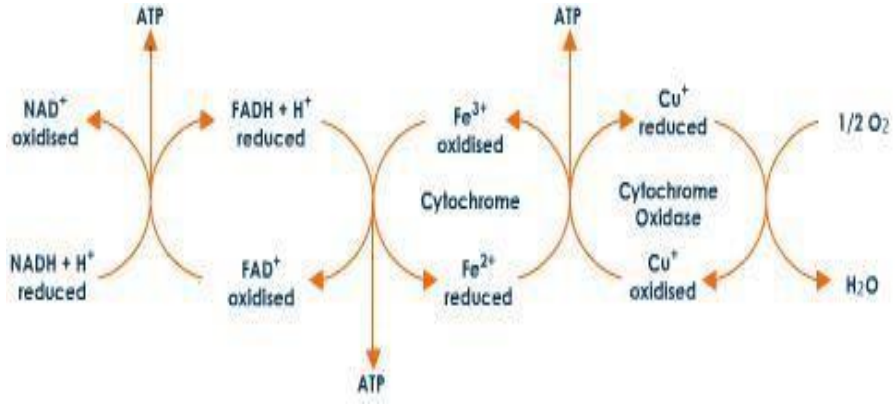
$$2\,H^{+} + 2\,E^{+} + \tfrac{1}{2}\,O_2 \rightarrow H_2O + ENERGY$$

Phosphate located in the matrix is imported via the proton gradient, which is used to create more ATP. The process of generating more ATP via the phosphorylation of ADP is referred to oxidative phosphorylation since the energy of hydrogen oxygenation is used throughout the electron transport chain. The ATP generated from this reaction go on to power most cellular reactions necessary for life.

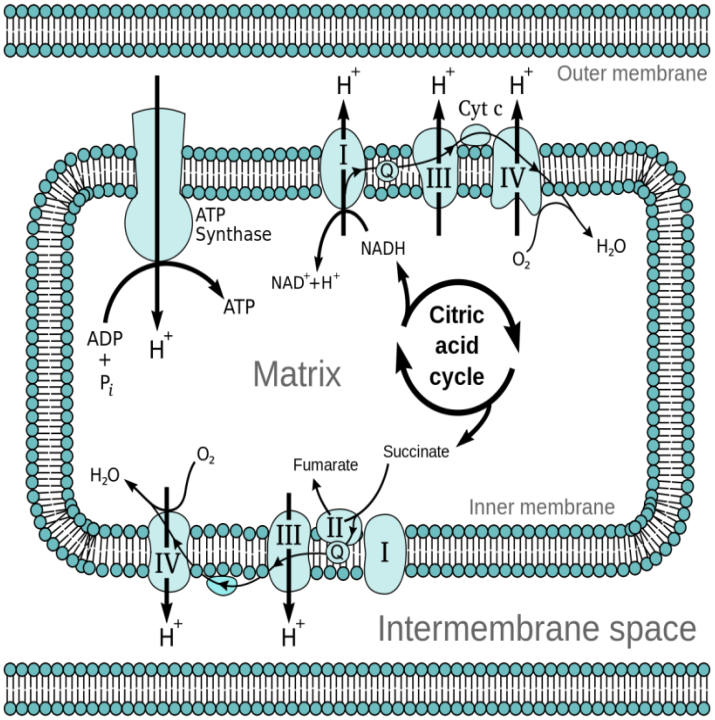
## Mechanism of ETC

In the electron transfer chain, electrons move along a series of proteins to generate an expulsion type force to move hydrogen ions, or protons, across the mitochondrial membrane. The electrons begin their reactions in Complex I, continuing onto Complex II, traversed to Complex III and cytochrome c via coenzyme Q, and then finally to Complex IV. The complexes themselves are complex-structured proteins embedded in the phospholipid membrane. Theyare combined with a metal ion, such as iron, to help with proton expulsion into the intermembrane space as well as other functions. The complexes also undergo conformational changes to allow openings for the transmembrane movement of protons.

These four complexes actively transfer electrons from an organic metabolite, such as glucose. When the metabolite breaks down, two electrons and a hydrogen ion are released and then picked up by the coenzyme $NAD^+$ to become NADH, releasing a hydrogen ion into the cytosol.




The NADH now has two electrons passing them onto a more mobile molecule, ubiquinone (Q), in the first protein complex (Complex I). Complex I, also known as NADH dehydrogenase, pumps four hydrogen ions from the matrix into the intermembrane space, establishing the proton gradient. In the next protein, Complex II or succinate dehydrogenase, another electron carrier and coenzyme, succinate is oxidized into fumarate, causing FAD (flavin-adenine dinucleotide) to be reduced to $FADH_2$. The transport molecule, $FADH_2$ is then reoxidized, donating electrons to Q (becoming $QH_2$), while releasing another hydrogen ion into the cytosol. While Complex II does not directly contribute to the proton gradient, it serves as another source for electrons.




Complex III, or cytochrome c reductase, is where the Q cycle takes place. Thereis an interaction between Q and cytochromes, which are molecules composed of iron, to continue the transfer of electrons. During the Q cycle, the ubiquinol ($QH_2$) previously produced donates electrons to ISP and cytochrome b becoming ubiquinone. ISP and cytochrome b are proteins that are located in the matrix that then transfers the electron it received from ubiquinol to cytochrome c1. Cytochrome c1 then transfers it to cytochrome c, which moves the electrons to the last complex. (Note: Unlike ubiquinone (Q), cytochrome c can only carry one electron at a time). Ubiquinone then gets reduced again to $QH_2$, restarting the cycle. In the process, another hydrogen ion is released into the cytosol to further create the proton gradient.

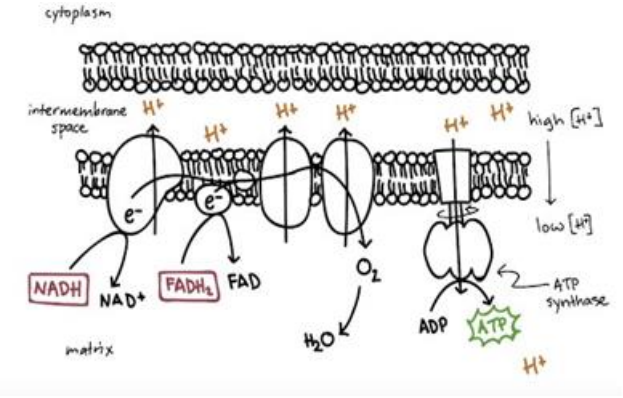
The cytochromes then extend into Complex IV, or cytochrome c oxidase. Electrons are transferred one at a time into the complex from cytochrome c. Theelectrons, in addition to hydrogen and oxygen, then react to form water in an irreversible reaction. This is the last complex that translocates four protons across the membrane to create the proton gradient that develops ATP at the end. As the proton gradient is established, $F_1F_0$ ATP synthase, sometimes referred to as Complex V, generates the ATP. The complex is composed of several subunits that bind to the protons released in prior reactions. As the protein rotates, protons are brought back into the mitochondrial matrix, allowing ADPto bind to free phosphate to produce ATP. For every full turn of the protein, three ATP is produced, concluding the electron transport chain.

## Oxidative phosphorylation

### Definition

Oxidative phosphorylation (UK or electron transport-linked phosphorylation) is the metabolic pathway in which cells use enzymes to oxidize nutrients, thereby releasing the chemical energy of molecular oxygen, which is used to produce adenosine triphosphate (ATP). In most eukaryotes, this takes place inside mitochondria. Almost all aerobic organisms carry out oxidative phosphorylation. This pathway is so pervasive because the energy of the double bond of oxygen is so much higher than the energy of the double bond in carbondioxide or in pairs of single bonds in organic molecules observed in alternative fermentation processes such as anaerobic glycolysis.

### Overview:




The electron transport chain is a series of proteins embedded in the inner mitochondrial membrane. In the matrix, NADH and FADH2 deposit their electrons in the chain (at the first and second complexes of the chain, respectively). The energetically "downhill" movement of electrons through the chain causes pumping of protons into the intermembrane space by the first, third, and fourth complexes.

Finally, the electrons are passed to oxygen, which accepts them along with protons to form water.

The ***electron transport chain*** is a series of proteins and organic molecules found in the inner membrane of the mitochondria. Electrons are passed from one member of the transport chain to another in a series of redox reactions. Energy released in these reactions is captured as a proton gradient, which is then used to make ATP in a process called *chemiosmosis.* Together, the electron transport chain and chemiosmosis make up ***oxidative phosphorylation***.

## Substrate-level phosphorylation

***Substrate-level phosphorylation*** is a metabolic reaction that results in the formation of ATP or GTP by conversion of a higher energy substrate (whether phosphate group attached or not) into lower energy product and a using some of the released chemical energy, the Gibbs free energy, to transfera phosphoryl ($PO_3$) group to ADP or GDP from another phosphorylated compound. Unlike oxidative phosphorylation, oxidation and phosphorylation are not coupled in the process of substrate-level phosphorylation, and reactive intermediates are most often gained in the course of oxidation processes in catabolism. Most ATP is generated by oxidative phosphorylation in aerobic or anaerobic respiration while substrate-level phosphorylation provides a quicker, less efficient source of ATP, independent of external electron acceptors. This is the case in human erythrocytes, which have no mitochondria, and in oxygen-depleted muscle.

Adenosine triphosphate is a major "energy currency" of the cell. The high energy bonds between the phosphate groups can be broken the power a variety of reactions used in all aspects of cell function. Substrate-level phosphorylation occurs in the cytoplasm of cells during glycolysis and in mitochondria either during the Krebs cycle or by MTHFD1L , an enzyme interconverting ADP + phosphate + 10- formyltetrahydrofolate to ATP + formate + tetrahydrofolate (reversibly), under both aerobic and anaerobic conditions. In the pay-off phase of glycolysis, a net of 2 ATP are produced by substrate-level phosphorylation.

## GLYCOLYSIS

The first substrate-level phosphorylation occurs after the conversion of 3-phosphoglyceraldehyde and Pi and NAD+ to 1,3-bisphosphoglycerate via glyceraldehyde 3-phosphate dehydrogenase. 1,3-bisphosphoglycerate is then dephosphorylated via phosphoglycerate kinase, producing 3-phosphoglycerate and ATP through a substrate-level phosphorylation.

The second substrate-level phosphorylation occurs by dephosphorylating phosphoenolpyruvate, catalyzed by pyruvate kinase, producing pyruvate and ATP.

During the preparatory phase, each 6-carbon glucose molecule is broken into two 3-carbon

molecules. Thus, in glycolysis dephosphorylation results in the production of 4 ATP. However, the prior preparatory phase consumes 2 ATP, so the net yield in glycolysis is 2 ATP. 2 molecules of NADH are also produced and can be used in oxidative phosphorylation to generate more ATP.

## Mitochondria

ATP can be generated by substrate-level phosphorylation in mitochondria in a pathway that is independent from the proton motive force. In the matrix thereare three reactions capable of substrate-level phosphorylation, utilizing either phosphoenolpyruvate carboxykinase or succinate-CoA ligase,or monofunctional C1-tetrahydrofolate synthase.

### *Phosphoenolpyruvate carboxykinase*

Mitochondrial phosphoenolpyruvate carboxykinase is thought to participate in the transfer of the phosphorylation potential from the matrix to the cytosol and vice versa. However, it is strongly favored towards GTP hydrolysis, thus it is not really considered as an important source of intra-mitochondrial substrate- level phosphorylation.

### *Succinate-CoA ligase*

Succinate-CoA ligase is a heterodimer composed of an invariant α-subunit and asubstrate-specific ß-subunit, encoded by either SUCLA2 or SUCLG2. This combination results in either an ADP-forming succinate-CoA ligase or a GDP- forming succinate-CoA ligase. The ADP-forming succinate-CoA ligase is potentially the only matrix enzyme generating ATP in the absence of a proton motive force, capable of maintaining matrix ATP levels under energy-limited conditions, such as transient hypoxia.

### *Monofunctional C1-tetrahydrofolate synthase*

This enzyme is encoded by MTHFD1L and reversibly interconverts ADP + phosphate + 10-formyltetrahydrofolate to ATP + formate + tetrahydrofolate.

## Other mechanisms

In working skeletal muscles and the brain, Phosphocreatine is stored as a readilyavailable high-energy phosphate supply, and the enzyme creatine phosphokinase transfers a phosphate from phosphocreatine to ADP to produce ATP. Then the ATP releases giving chemical energy. This is sometimes erroneously considered to be substrate-level phosphorylation, although it isa transphosphorylation.

## Importance of substrate-level phosphorylation in anoxia

During anoxia, provision of ATP by substrate-level phosphorylation in the matrix is important not only as a mere means of energy, but also to prevent mitochondria from straining glycolytic ATP reserves by maintaining the adenine nucleotide translocator in 'forward mode' carrying ATP towards thecytosol.

## Oxidative phosphorylation

An alternative method used to create ATP is through oxidative phosphorylation, which takes place during cellular respiration. This process utilizes the oxidation of NADH to $NAD^+$, yielding 3 ATP, and of $FADH_2$ to FAD, yielding 2 ATP. The potential energy stored as an electrochemical gradient of protons ($H^+$) across the inner mitochondrial membrane is required to generate ATP fromADP and $P_i$ (inorganic phosphate molecule), a key difference from substrate- level phosphorylation. This gradient is exploited by ATP synthase acting as a pore, allowing $H^+$ from the mitochondrial intermembrane space to move down its electrochemical gradient into the matrix and coupling the release of free energy to ATP synthesis. Conversely, electron transfer provides the energy required to actively pump $H^+$ out of the matrix.

## Uncouplers of oxidative phosphorylation

Uncouplers of oxidative phosphorylation in mitochondria inhibit the coupling between the electron transport and phosphorylation reactions and thus inhibit ATP synthesis without affecting the respiratory chain and ATP synthase. Uncouplers inhibit ATP synthesis by preventing this coupling reaction in such a fashion that the energy produced by redox reactions cannot be used for phosphorylation. Uncouplers include DNP, valinomycin, and CCCP. Most of them are hydrophobic weak acids that act by protonophoric action and activities.

Uncouplers of oxidative phosphorylation in mitochondria inhibit the coupling between the electron transport and phosphorylation reactions and thus inhibit ATP synthesis without affecting the respiratory chain and ATP synthase (H(+)- ATPase). Miscellaneous compounds are known to be uncouplers, but weakly acidic uncouplers are representative because they show very potent activities. The most potent uncouplers discovered so far are

the hindered phenol SF 6847, and hydrophobic salicylanilide S-13, which are active in vitro at concentrations in the 10 nM range. For induction of uncoupling, an acid dissociable group, bulky hydrophobic moiety and strong electron-withdrawing group are required. Weakly acidic uncouplers are considered to produce uncoupling by their protonophoric action in the H(+)-impermeable mitochondrial membrane. For exerting these effects, the stability of the respective uncoupler anions in the hydrophobic membrane is very important. High stability is achieved by delocalization of the polar ionic charge through uncoupler (chemical)-specific mechanisms. Such an action of weakly acidic uncouplers is characteristic of the highly efficient membrane targeting action of a nonsite-specific type of bioactive compound.

One example of an 'uncoupler' of oxidative phosphorylation is DNP (2,4-dinitrophenol).

2,4-Dinitrophenol (DNP), $C_6H_4N_2O_5$, is a cellular metabolic poison. It uncouples oxidative phosphorylation by carrying protons across the mitochondrial membrane, leading to a rapid consumption of energy without generation of ATP.

In living cells, DNP acts as a proton ionophore, an agent that can shuttle protons(hydrogen ions) across biological membranes. It defeats the proton gradient across mitochondrial membrane, collapsing the proton motive force that the celluses to produce most of its ATP chemical energy. Instead of producing ATP, the energy of the proton gradient is lost as heat.

DNP is often used in biochemistry research to help explore the bioenergetics ofchemiosmotic and other membrane transport processes.

The HMP shunt represents an alternative pathway for the breakdown of glucose. Briefly describe the main products produced by this pathway and it's biological significance.

The main product are Ribose-5-P, NADPH and Intermediates of the glycolytic pathway. HMP shunt represents an alternate degradative pathway for the breakdown of glucose, and it provides a link between glycolysis and nucleotide metabolism and fatty acid.

Biological significance of Ribose-5-P is that serves as the precursor to various nucleotides (ATP, NAD, NADP, coenzyme A) and nucleic acids (DNA) within our cells.

Biological significance of NADPH: represents the major source of reducing power for biosynthetic reactions within cells, particularly the synthesis of fatty acids. It follows that the

HMP shunt is active in tissues specialized for the synthesis of fatty acids or steroids.

Biological significance of Intermediates of the glycolytic pathway: the demand for NADPH in the cell is usually far greater than the demand for ribose-5-P, thus the second phase of this pathway is devoted to recycling the 5-carbon skeletons into intermediates of the glycolytic pathway so that the cell can harness the energy that is present in these molecules.

## Very Short Questions (2marks)

1) Define Glucose Tolerance Tests (GTT).
2) What is Galactosemia?
3) What are Glycogen storage diseases?
4) Define glycogenesis.
5) Define glycogenolysis.

## Short Questions (5marks)

1) Describe Cori's Cycle along with its significance.
2) Explain the amphibolic role of TCA cycle.
3) Explain the pyruvate dehydrogenase complex.
4) Name the irreversible enzymes of glycolysis and key enzymes of gluconeogenesis.
5) Write the Significance of HMP shunt pathway.
6) Write a short note on Electron Transport chain.

## Long Questions (10marks)

1) Describe in detail EM Pathway along with its energetics and regulation.
2) Describe TCA cycle along with regulation and it's energetic. Add a note on its Amphibolic role.
3) Explain the HMP shunt pathway and its significance.
4) Describe glycogen metabolism along with its regulation.
5) Explain the digestion and absorption of carbohydrates.
6) Describe various mechanisms for regulation of blood glucose.
7) Enumerate the gluconeogenic substrates and describe the reactions of gluconeogene