METABOLISM OF CARBOHYDRATES -1

TEJASVI NAVADHITAMASTU

"Let our (the teacher and the taught) learning be radiant"

Let our efforts at learning be luminous and filled with joy, and endowed with the force of purpose

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Major pathways of carbohydrate metabolism

The important pathways of carbohydrate metabolism are listed

1. **Glycolysis** (Embden-Meyerhof pathway) **:** The oxidation of glucose to pyruvate and lactate.

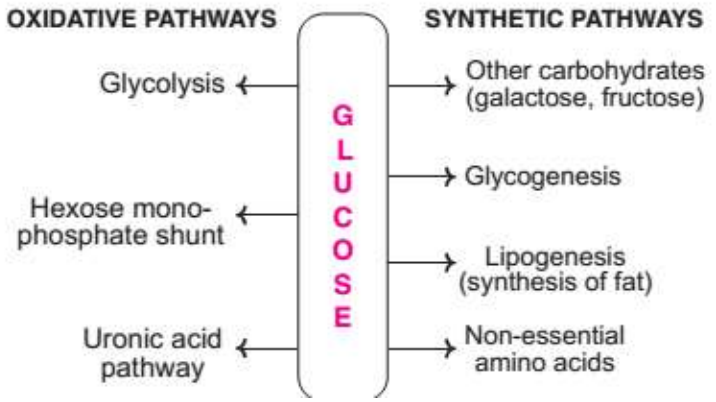
2. **Citric acid cycle** (Krebs cycle or tricarboxylic acid cycle) **:** The oxidation of acetyl CoA to CO_2. Krebs cycle is the final common oxidative pathway for carbohydrates, fats or amino acids, through acetyl CoA.

3. **Gluconeogenesis :** The synthesis of glucose from non-carbohydrate precursors (e.g. amino acids, glycerol etc.).

4. **Glycogenesis :** The formation of glycogen from glucose.

5. **Glycogenolysis :** The breakdown of glycogen to glucose.

6. **Hexose monophosphate shunt** (pentose phosphate pathway or direct oxidative pathway) **:** This pathway is an alternative to glycolysis and TCA cycle for the oxidation of glucose (directly to carbon dioxide and water).



7. **Uronic acid pathway :** Glucose is converted to glucuronic acid, pentoses and, in some animals, to ascorbic acid (not in man). This pathway is also an alternative oxidative pathway for glucose.

8. **Galactose metabolism :** The pathways concerned with the conversion of galactose to glucose and the synthesis of lactose.

9. **Fructose metabolism :** The oxidation of fructose to pyruvate and the relation between fructose and glucose metabolism.

10. **Amino sugar and mucopolysaccharide metabolism :** The synthesis of amino sugars and other sugars for the formation of mucopoly-saccharides and glycoproteins.

Entry of glucose into cells

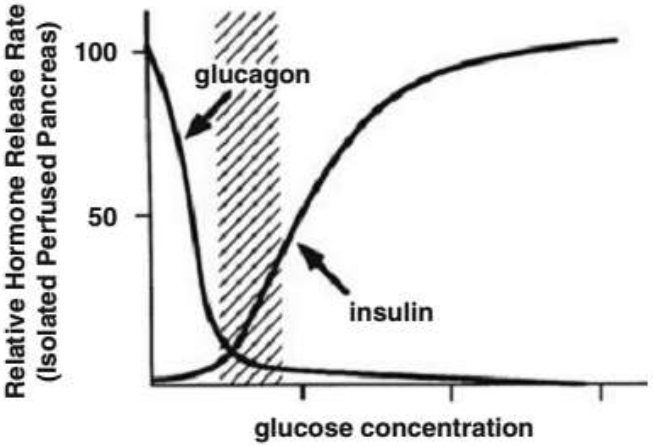
Glucose concentration is very low in the cells compared to plasma (for humans < 100 mg/dl). However, glucose does not enter the cells by simple diffusion. Two specific transport systems are recognized for the entry of glucose into the cells

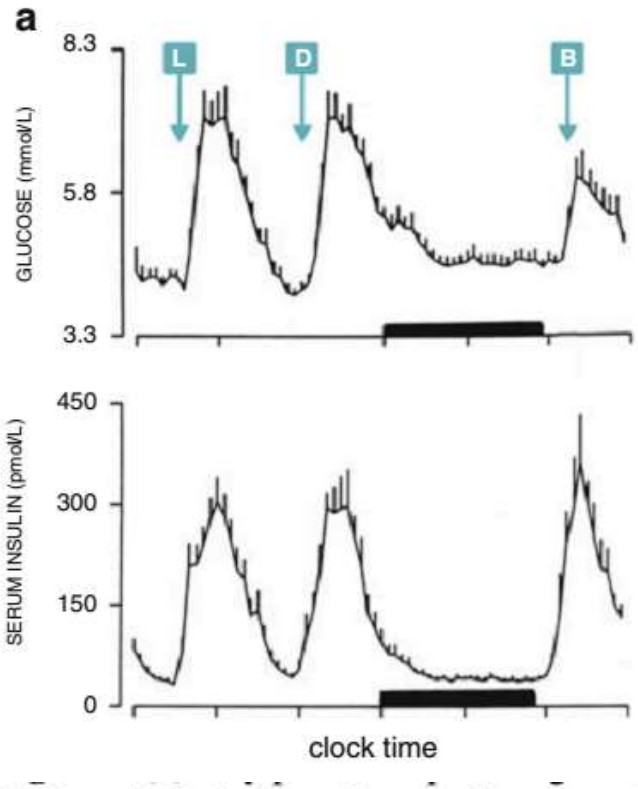
1. **Insulin-independent transport system of glucose :** This is a carrier mediated uptake of glucose which is not dependent on the hormone insulin. This is operative in hepatocytes, erythrocytes and brain.

2. **Insulin-dependent transport system :** This occurs in muscle and adipose tissue.

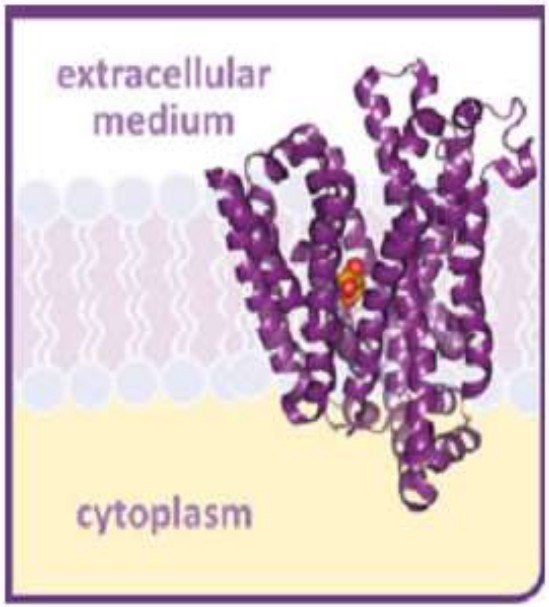
Glucose transporters : In recent years, at least six glucose transporters (GLUT-1 to GLUT-5 and GLUT-7) in the cell membranes have been identified. They exhibit tissue specificity. For instance, GLUT-1 is abundant in erythrocytes whereas GLUT-4 is abundant in skeletal muscle and adipose tissue.

Insulin increases the number and promotes the activity of GLUT-4 in skeletal muscle and adipose tissue. In type 2 diabetes mellitus, insulin resistance is observed in these tissues. This is due to the reduction in the quantity of GLUT-4 in insulin deficiency.

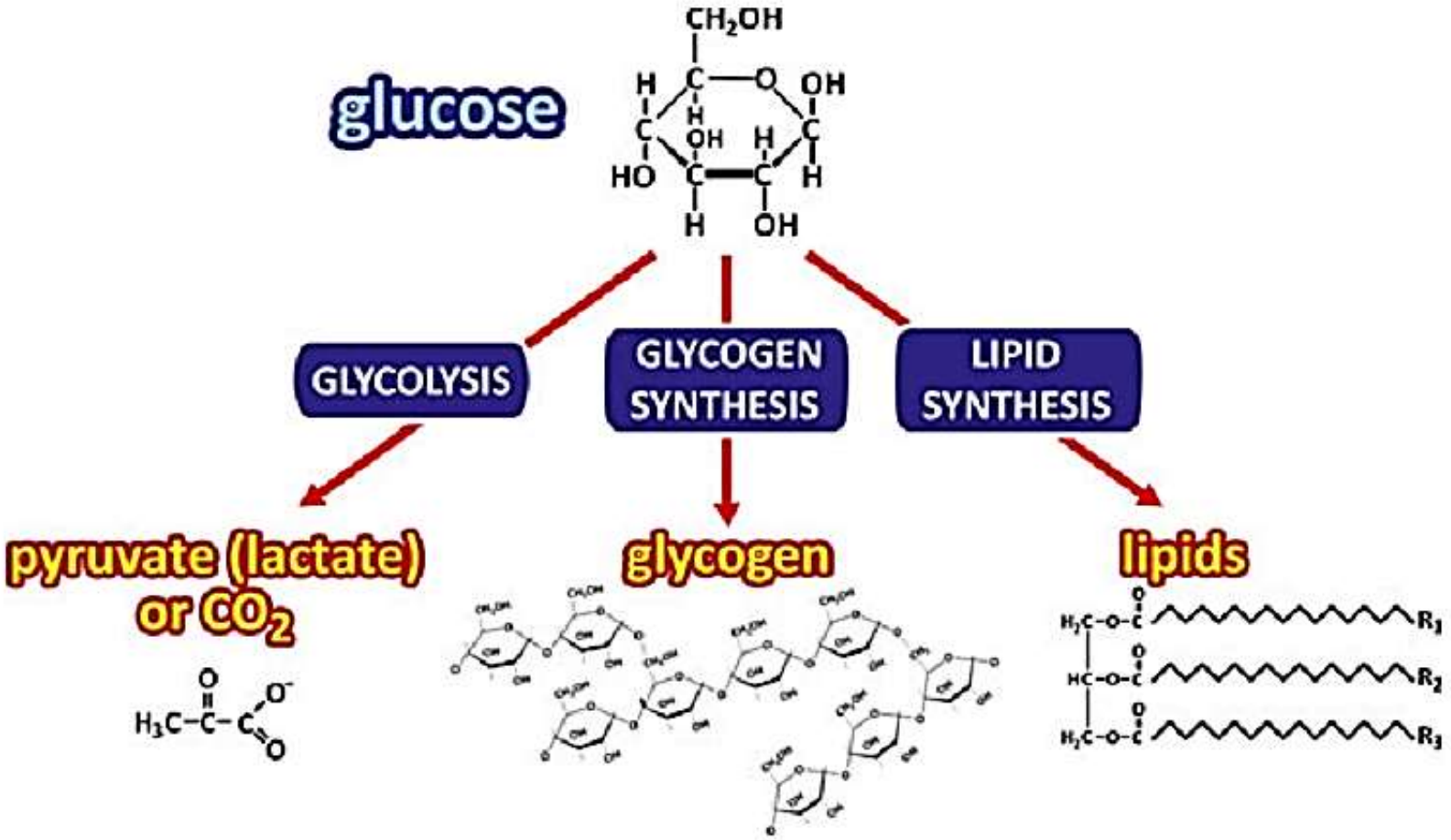




L, D, and B indicate lunch, dinner, and breakfast

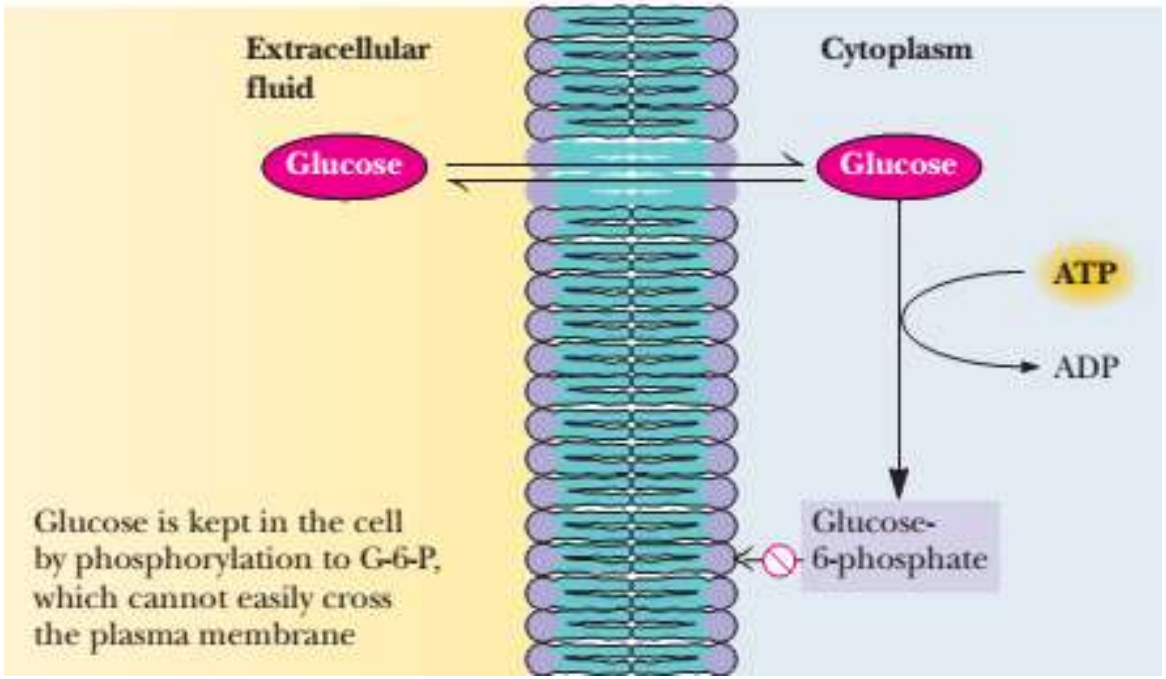
Transporter	K_M (mM)	Distribution	Features	Putative Structure[a]
GLUT-1	1–2	Ubiqitous, erythrocytes	Constitutive glucose transporter	
GLUT-2	20	Liver, β-cells, intestine, kidney	Low affinity, high capacity transporter	
GLUT-3	1	Neurons, Placenta	Low affinity transporter	
GLUT-4	5	Adipose tissue, skeletal muscle heart	Insulin-dependent transporter	

[a]The structures of mammalian GLUTs have not been determined yet, so the figure shows the structure of the *E. coli* homologue of GLUTs, the xylose-H^+ symporter (XylE), complexed with xylose (*orange*) (PDB 4GBY). It contains 12 segments that cross the plasma membrane, forming a "pore" through which the sugar is transported



Possible fates of glucose after its uptake by the cells. The metabolic pathway s that may be followed by glucose are shown in the blue boxes

The Cellular Advantages of Phosphorylating Glucose The incorporation of a phosphate into glucose in this energetically favorable reaction is important for several reasons. First, phosphorylation keeps the substrate in the cell. Glucose is a neutral molecule and could diffuse across the cell membrane, but phosphorylation confers a negative charge on glucose and the plasma membrane is essentially impermeable to glucose-6-phosphate (Figure 18.4). Moreover, rapid conversion of glucose to glucose-6-phosphate keeps the *intracellular* concentration of glucose low, favoring faciliated diffusion of glucose *into* the cell. In addition, because regulatory control can be imposed only on reactions not at equilibrium, the favorable thermodynamics of this first reaction makes it an important site for regulation.



The Isozymes of Hexokinase In most animal, plant, and microbial cells, the enzyme that phosphorylates glucose is **hexokinase.** Magnesium ion (Mg^{2+}) is required for this reaction, as for the other kinase enzymes in the glycolytic pathway. The true substrate for the hexokinase reaction is $MgATP^{2-}$. There are four isozymes of hexokinase in most animal tissues. Hexokinase I is the principal form in the brain. Hexokinase in skeletal muscle is a mixture of types I (70% to 75%) and II (25% to 30%). The K_m for glucose is 0.03 m*M* for type I and 0.3 m*M* for type II; thus, hexokinase operates efficiently at normal blood glucose levels of 4 m*M* or so. The animal isozymes are allosterically inhibited by the product, glucose-6-phosphate. High levels of glucose-6-phosphate inhibit hexokinase activity until consumption by glycolysis lowers its concentration. The hexokinase reaction is one of three points in the glycolysis pathway that are *regulated*. As the generic name implies, hexokinase can phosphorylate a variety of hexose sugars, including glucose, mannose, and fructose.

The type IV isozyme of hexokinase, called **glucokinase,** is found predominantly in the liver and pancreas. Type IV is highly specific for D-glucose, has a much higher K_m for glucose (approximately 10 m*M*), and is not product inhibited. With such a high K_m for glucose, glucokinase becomes important metabolically only when liver glucose levels are high (for example, when the individual has consumed large amounts of carbohydrates). When glucose levels are low, hexokinase is primarily responsible for phosphorylating glucose. However, when glucose levels are high, glucose is converted by glucokinase to glucose-6-phosphate and is eventually stored in the liver as glycogen. Glucokinase is an *inducible* enzyme—the amount present in the liver is controlled by *insulin* (secreted by the pancreas). (Patients with **diabetes mellitus** produce insufficient insulin. They have low levels of glucokinase, cannot tolerate high levels of blood glucose, and produce little liver glycogen.) Because glucose-6-phosphate is common to several metabolic pathways (Figure 18.5), it occupies a branch point in glucose metabolism.

Hexokinase Binds Glucose and ATP with an Induced Fit In most organisms, hexokinase occurs in a single form: a two-lobed 50-kD monomer that resembles a clamp, with a large groove in one side (Figure 18.6; see also Figure 13.24). Daniel Koshland predicted, years before structures were available, that hexokinase would undergo an induced fit (see Chapter 13), closing around the substrates ATP and glucose when they were bound. Koshland's prediction was confirmed when structures of the yeast enzyme were determined in the absence and presence of glucose (Figure 18.6).

The human hexokinase isozymes I, II, and III are twice as big as those of lower organisms. They are composed of two separate domains, each similar to the yeast enzyme, and connected head to tail by a long α-helix (Figure 18.7). The sequence and structure similarity apparently arose from the duplication and fusion of a primordial hexokinase gene. Interestingly, both halves of hexokinase II support catalysis, but only the C-terminal half of isozymes I and III performs phosphorylation of glucose. The N-terminal half, on the other hand, has apparently evolved into a form that allosterically regulates the activity of the C-terminal half! Type IV hexokinase (glucokinase) is similar in structure to the yeast enzyme, with a single clamp domain, a single active site, and a mass of 50 kD (Figure 18.7).

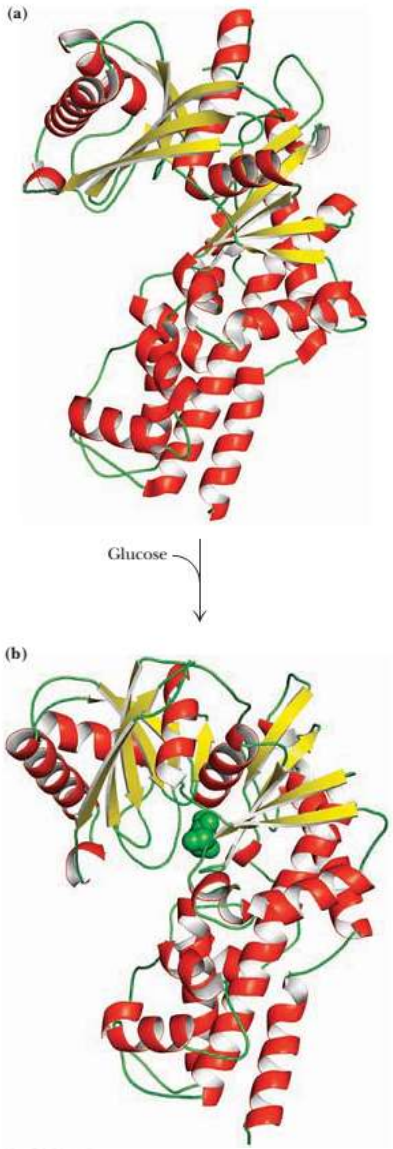


FIGURE 18.6 The **(a)** open and **(b)** closed states of yeast hexokinase. Binding of glucose (green) induces a conformation change that closes the active site, as predicted by Koshland (a: pdb id = 1IG8; b: pdb id = 1BDG).

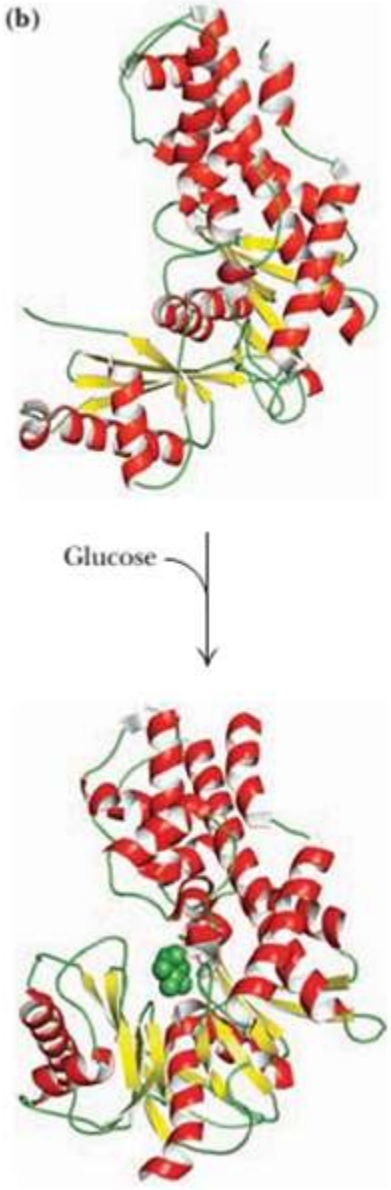
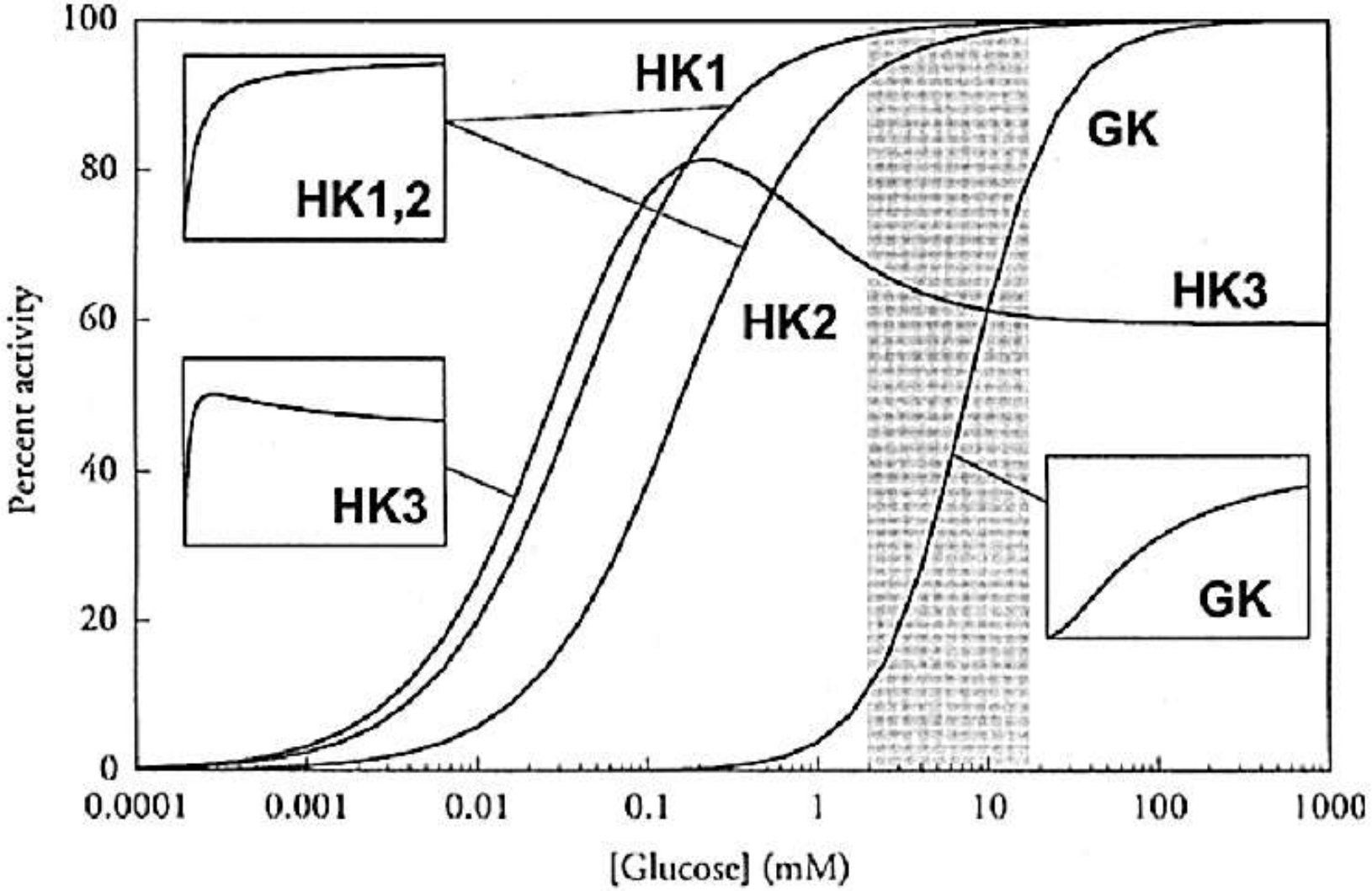


FIGURE 18.7 **(a)** Mammalian hexokinase I contains an N-terminal domain (top) and a C-terminal domain (bottom) joined by a long α-helix. Each of these domains is similar in sequence and structure to yeast hexokinase (pdb id = 1CZA). **(b)** Human glucokinase undergoes an induced fit upon binding glucose (green). (Top: pdb id = 1V4T; bottom: pdb id = 1V4S).



Comparison of the kinetic properties of HK isoforms. The physiological range of blood glucose concentration is indicated by the gray section. The insets show the curves redrawn with a linear scale of glucose concentration to better illustrate the kinetic profile

GLYCOLYSIS

Glycolysis is derived from the *Greek* words (glycose—sweet or sugar; lysis—dissolution). It is a universal pathway in the living cells. The complete pathway of glycolysis was elucidated in 1940. This pathway is often referred to as ***Embden-Meyerhof pathway (E.M. pathway)*** in honour of the two biochemists who made a major contribution to the knowledge of glycolysis.

Glycolysis is defined as the sequence of reactions converting glucose (or glycogen) to pyruvate or lactate, with the production of ATP.

Reactions of glycolysis

The sequence of reactions of glycolysis is given in ***Fig.13.2***. The pathway can be divided into three distinct phases

A. Energy investment phase or priming stage

B. Splitting phase

C. Energy generation phase.

Salient features

1. Glycolysis takes place in all cells of the body. The ***enzymes*** of this pathway are present in the ***cytosomal fraction*** of the cell.

2. Glycolysis occurs in the absence of oxygen (anaerobic) or in the presence of oxygen (aerobic). Lactate is the end product under anaerobic condition. In the aerobic condition, pyruvate is formed, which is then oxidized to CO_2 and H_2O.

3. Glycolysis is a major pathway for ATP synthesis in tissues lacking mitochondria, e.g. erythrocytes, cornea, lens etc.

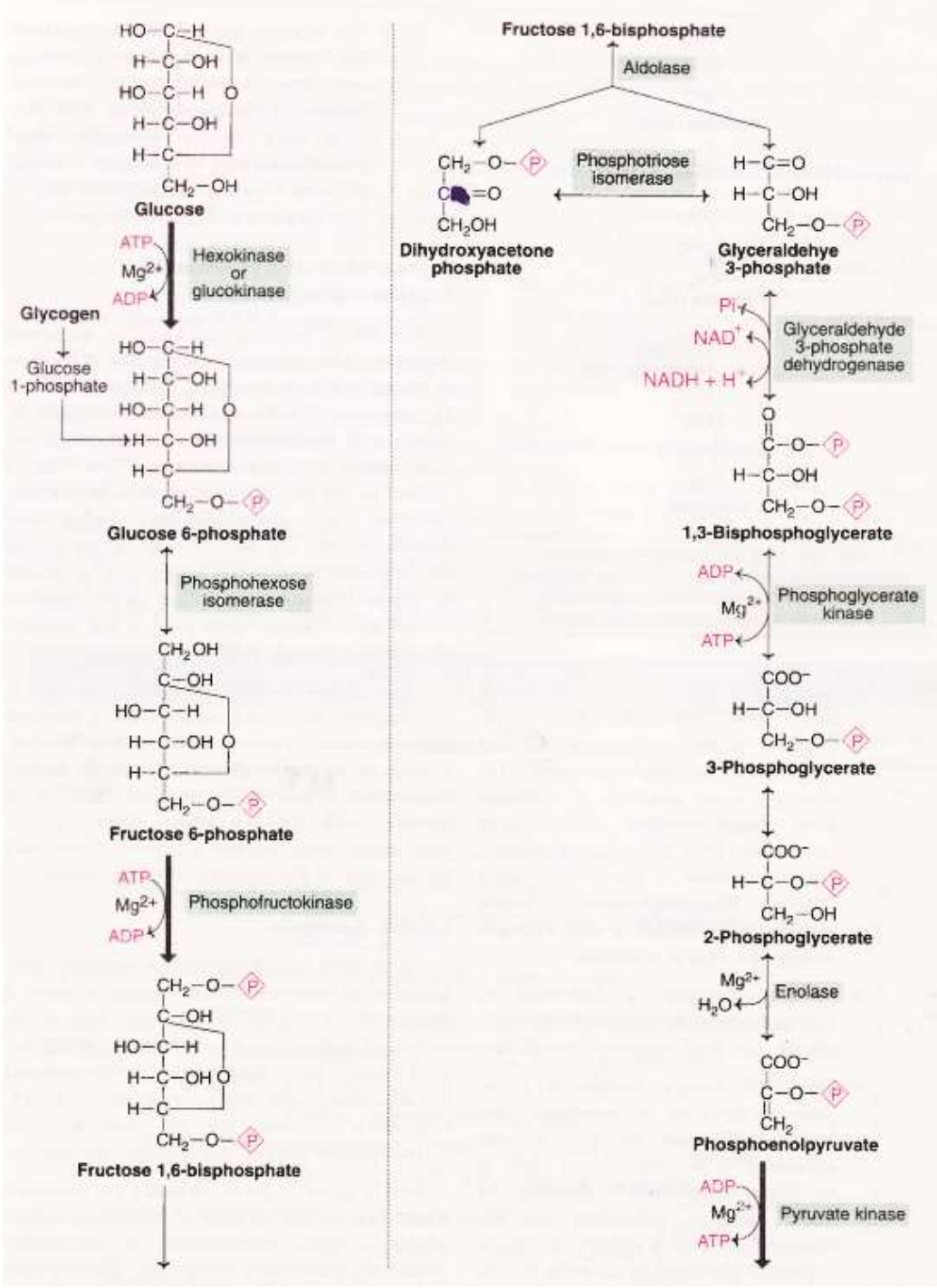
4. Glycolysis is very ***essential for brain*** which is dependent on glucose for energy. The glucose in brain has to undergo glycolysis before it is oxidized to CO_2 and H_2O.

5. Glycolysis (anaerobic) may be summarized by the net reaction

$$\text{Glucose} + 2\text{ADP} + 2\text{Pi} \longrightarrow 2\text{Lactate} + 2\text{ATP}$$

6. Glycolysis is a central metabolic pathway with many of its intermediates providing branch point to other pathways. Thus, the intermediates of glycolysis are useful for the synthesis of amino acids and fat.

7. Reversal of glycolysis along with the alternate arrangements at the irreversible steps, will result in the synthesis of glucose (gluconeogenesis).



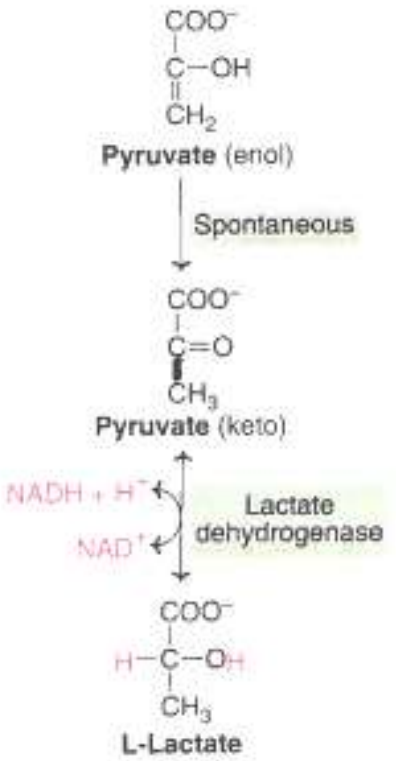


***Fig. 13.2** : The reactions in the pathway of glycolysis (The three steps catalysed by hexokinase, phosphofructokinase and pyruvate kinase, shown in thick lines are irreversible).*

Glycolysis and shuttle pathways

In the presence of mitochondria and oxygen, the NADH produced in glycolysis can participate in the shuttle pathways (***Refer Chapter 11***) for the synthesis of ATP. If the cytosolic NADH uses malate-aspartate shuttle, 3 ATP are generated from each molecule of NADH. This is in contrast to glycerolphosphate shuttle that produces only 2 ATP.

Reactions of glycolysis

The sequence of reactions of glycolysis is given in ***Fig.13.2***. The pathway can be divided into three distinct phases

A. Energy investment phase or priming stage

B. Splitting phase

C. Energy generation phase.

The sequence of reactions are discussed below.

A. **Energy investment phase**

1. Glucose is phosphorylated to glucose 6-phosphate by ***hexokinase*** or ***glucokinase*** (both are ***isoenzymes***). This is an irreversible reaction, dependent on ATP and Mg^{2+}. The enzyme hexokinase is present in almost all the tissues. It catalyses the phosphorylation of various hexoses (fructose, mannose etc.), has low K_m for substrates (about 0.1 mM) and is inhibited by glucose 6-phosphate.

   Glucokinase present in liver, catalyses the phosphorylation of only glucose, has high K_m for glucose (10 mM) and is not inhibited by glucose 6-phosphate.

   Due to high affinity (low K_m), glucose is utilized by hexokinase even at low concentration, whereas glucokinase acts only at higher levels of glucose i.e., after a meal when blood glucose concentration is above 100 mg/dl.

   Glucose 6-phosphate is impermeable to the cell membrane. It is a ***central molecule with a variety of metabolic fates***—glycolysis, glycogenesis, gluconeogenesis and pentose phosphate pathway.

2. Glucose 6-phosphate undergoes isomerization to give fructose 6-phosphate in the presence of the enzyme phosphohexose isomerase and Mg^{2+}.

3. Fructose 6-phosphate is phosphorylated to fructose 1,6-bisphosphate by phosphofructokinase (PFK). This is an irreversible and a regulatory step in glycolysis.

B. **Splitting phase**

4. The six carbon fructose 1,6-bisphosphate is split (hence the name glycolysis) to two three-carbon compounds, glyceraldehyde 3-phosphate and dihydroxyacetone phosphate by the enzyme aldolase (fructose 1,6-bisphosphate aldolase).

5. The enzyme phosphotriose isomerase catalyses the reversible interconversion of glyceraldehyde 3-phosphate and dihydroxyacetone phosphate. Thus, two molecules of glyceraldehyde 3-phosphate are obtained from one molecule of glucose.

C. **Energy generation phase**

6. Glyceraldehyde 3-phosphate dehydrogenase converts glyceraldehyde 3-phosphate to 1,3-bisphosphoglycerate. This step is important as it is involved in the formation of NADH + H^+ and a high energy compound 1,3-bisphosphoglycerate. ***Iodoacetate*** and ***arsenate inhibit*** the enzyme glyceraldehyde 3-phosphate dehydrogenase. In aerobic condition, NADH passes through the electron transport chain and 6 ATP (2 × 3 ATP) are synthesized by oxidative phosphorylation.

7. The enzyme phosphoglycerate kinase acts on 1,3-bisphosphoglycerate resulting in the synthesis of ATP and formation of 3-phosphoglycerate. This step is a good example of ***substrate level phosphorylation***, since ATP is synthesized from the substrate without the involvement of electron transport chain. ***Phosphoglycerate kinase reaction is reversible, a rare example among the kinase reactions.***

8. 3-Phosphoglycerate is converted to 2-phosphoglycerate by phosphoglycerate mutase. This is an isomerization reaction.

9. The high energy compound phosphoenol pyruvate is generated from 2-phosphoglycerate by the enzyme ***enolase***. This enzyme requires Mg^{2+} or Mn^{2+} and is ***inhibited*** by ***fluoride.*** For blood ***glucose estimation*** in the laboratory, fluoride is added to the blood to prevent glycolysis by the cells, so that blood glucose is correctly estimated.

Regulation of glycolysis

The three enzymes namely hexokinase (and glucokinase), phosphofructokinase and pyruvate kinase, catalysing the irreversible reactions regulate glycolysis.

Hexokinase is inhibited by glucose 6-phosphate. This enzyme prevents the accumulation of glucose 6-phosphate due to product inhibition. Glucokinase, which specifically phosphorylates glucose, is an inducible enzyme. The substrate glucose, probably through the involvement of insulin, induces glucokinase.

Phosphofructokinase (PFK) is the most important regulatory enzyme in glycolysis. This enzyme catalyses the ***rate limiting committed step.*** PFK is an allosteric enzyme regulated by allosteric effectors. ATP, citrate and H^+ ions (low pH) are the most important allosteric inhibitors, whereas, fructose 2,6-bisphosphate, ADP, AMP and Pi are the allosteric activators.

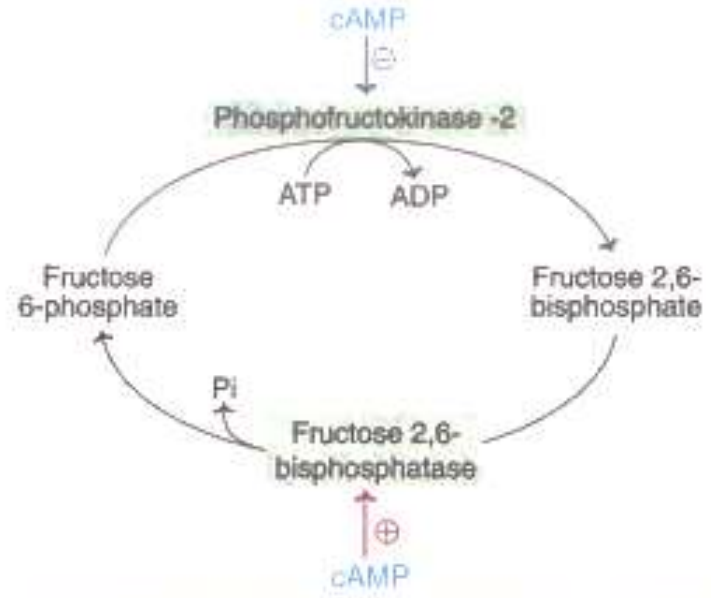


Fig. 13.3 : Regulation of fructose 2,6-bisphosphatase.

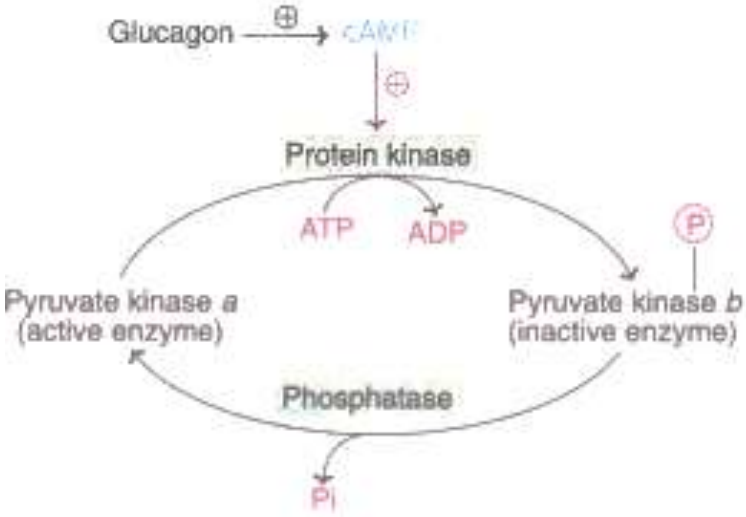


Fig. 13.4 : Regulation of pyruvate kinase.

Pasteur effect

The ***inhibition of glycolysis by oxygen*** (aerobic condition) is known as Pasteur effect. This effect was discovered by Louis Pasteur, more than a century ago, while studying fermentation by yeast. He observed that when anaerobic yeast cultures (metabolizing yeast) were exposed to air, the utiliziation of glucose decreased by nearly seven fold.

In the aerobic condition, the levels of glycolytic intermediates from fructose 1,6-bisphosphate onwards decrease while the earlier intermediates accumulate. This clearly indicates that Pasteur effect is due to the inhibition of the enzyme phosphofructokinase. The inhibitory effect of citrate and ATP (produced in the presence of oxygen) on phosphofructokinase explains the Pasteur effect.

Crabtree effect

The phenomenon of inhibition of oxygen consumption by the addition of glucose to tissues having high aerobic glycolysis is known as Crabtree effect. Basically, this is ***opposite*** to that of ***Pasteur effect.*** Crabtree effect is due to increased competition of glycolysis for inorganic phosphate (Pi) and NAD^+ which limits their availability for phosphorylation and oxidation.

Conversion of pyruvate to lactate—significance

The fate of pyruvate produced in glycolysis depends on the presence or absence of oxygen in the cells. Under anaerobic conditions (lack of O_2), pyruvate is reduced by NADH to lactate in presence of the enzyme lactate dehydrogenase (competitive inhibitor—oxamate). The NADH utilized in this step is obtained from the reaction catalysed by glyceraldehyde 3-phosphate dehydrogenase. The formation of lactate allows the regeneration of NAD^+ which can be reused by glyceraldehyde 3-phosphate dehydrogenase so that glycolysis proceeds even in the absence of oxygen to supply ATP.

The occurrence of uninterrupted glycolysis is very essential in skeletal muscle during strenous exercise where oxygen supply is very limited. ***Glycolysis in the erythrocytes leads to lactate production***, since mitochondria—the centres for aerobic oxidation—are absent. Brain, retina, skin, renal medulla and gastrointestinal tract derive most of their energy from glycolysis.

Lactic acidosis

Lactic acid is a three carbon hydroxy acid. Elevation of lactic acid in the circulation (normal plasma 4–15 mg/dl) may occur due to its increased production or decreased utilization. Mild forms of lactic acidosis (not life-threatening) are associated with strenuous exercise, shock, respiratory diseases, cancers, low pyruvate dehydrogenase activity, von Gierke's disease etc.

Cancer and glycolysis

Cancer cells display increased uptake of glucose, and glycolysis. As the tumors grow rapidly, the blood vessels are unable to supply adequate oxygen, and thus a condition of hypoxia exists. Due to this, anaerobic glycolysis predominantly occurs to supply energy. The

RAPAPORT-LEUBERING CYCLE

This is a supplementary pathway to glycolysis which is operative in the erythrocytes of man and other mammals. Rapaport-Leubering cycle is mainly concerned with the synthesis of ***2,3-bisphosphoglycerate (2,3-BPG)*** in the RBC. 1,3-Bisphosphoglycerate (1,3-BPG) produced in glycolysis is converted to 2,3-BPG by the enzyme 2,3-bisphosphoglycerate mutase (***Fig.13.5***). 2,3-BPG is hydrolysed to 3-phosphoglycerate by bisphosphoglycerate phosphatase. [**Note** : There is a difference between the usages—bisphosphate and diphosphate. A bisphosphate has two phosphates held separately (e.g. 2,3-BPG), in contrast to diphosphate (e.g. ADP) where the phosphates are linked together].

It is now believed that bisphosphoglycerate mutase is a bifunctional enzyme with mutase and phosphatase activities catalysed by two different sites present on the same enzyme.

About 15-25% of the glucose that gets converted to lactate in erythrocytes goes via 2,3-BPG synthesis.

Significance of 2,3-BPG

1. Production of 2,3-BPG allows the glycolysis to proceed without the synthesis of ATP. This is advantageous to erythrocytes since glycolysis occurs when the need for ATP is minimal. Rapaport-Leubering cycle is, therefore, regarded as ***a shunt pathway of glycolysis*** to dissipate or waste the energy not needed by erythrocytes.

2. 2,3-BPG, however, is not a waste molecule in RBC. It combines with hemoglobin (Hb) and reduces Hb affinity with oxygen. Therefore, in the presence of 2,3-BPG, ***oxyhemoglobin unloads more oxygen to the tissues.***

Increase in erythrocyte 2,3-BPG is observed in hypoxic condition, high altitude, fetal tissues, anemic conditions etc. In all these cases, 2,3-BPG will enhance the supply of oxygen to the tissues.

3. Glycolysis in the erythrocytes is linked with 2,3-BPG production and oxygen transport. In the deficiency of the enzyme hexokinase, glucose is not phosphorylated, hence the synthesis and concentration of 2,3-BPG are low in RBC. The hemoglobin exhibits high oxygen affinity in hexokinase-defective patients. On the other hand, in the patients with pyruvate kinase deficiency, the level of 2,3-BPG in erythrocytes is high, resulting in low oxygen affinity.

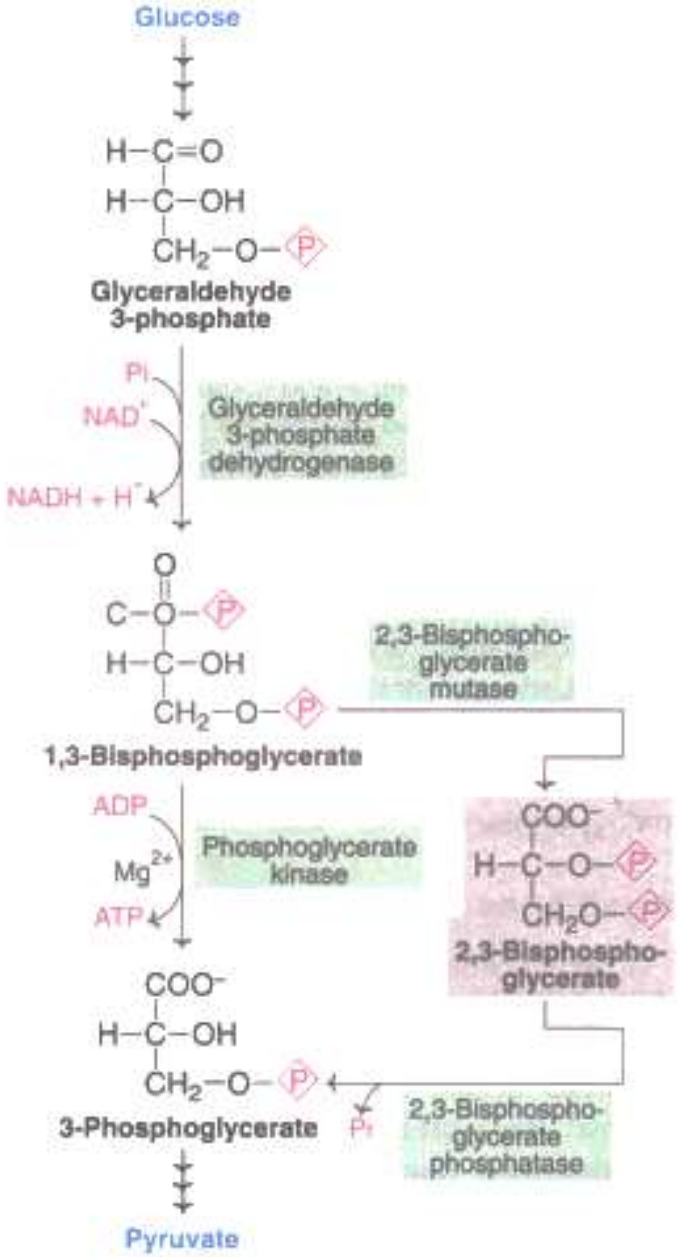


***Fig. 13.5** : Rapaport-Leubering cycle for the synthesis of 2,3-bisphosphoglycerate (2,3-BPG).*

CONVERSION OF PYRUVATE TO ACETYL CoA

Pyruvate is converted to acetyl CoA by ***oxidative decarboxylation***. This is an irreversible reaction, catalysed by a multienzyme complex, known as ***pyruvate dehydrogenase complex*** (PDH), which is found only in the mitochondria. High activities of PDH are found in cardiac muscle and kidney. The enzyme PDH requires five cofactors (coenzymes), namely—TPP, lipoamide, FAD, coenzyme A and NAD^+ (lipoamide contains lipoic acid linked to ε-amino group of lysine). The overall reaction of PDH is

$$\text{Pyruvate} + NAD^+ + CoA \xrightarrow{\text{PDH}} \text{Acetyl CoA} + CO_2 + NADH + H^+$$

Biochemical importance of PDH

1. Lack of TPP (due to deficiency of thiamine) inhibits PDH activity resulting in the accumulation of pyruvate.

2. In the thiamine deficient alcoholics, pyruvate is rapidly converted to lactate, resulting in lactic acidosis.

3. In patients with inherited deficiency of PDH, lactic acidosis (usually after glucose load) is observed.

4. PDH activity can be inhibited by arsenic and mercuric ions. This is brought about by binding of these ions with −SH groups of lipoic acid.

Regulation of PDH

Pyruvate dehydrogenase is a good example for ***end product*** (acetyl CoA, NADH) ***inhibition***. Besides this, PDH is also regulated by phosphorylation and dephosphorylation (***Fig.13.7***) PDH is active as a dephosphoenzyme while it is inactive as a phosphoenzyme. PDH phosphatase activity is promoted by Ca^{2+}, Mg^+ and insulin (in adipose tissue). It is of interest to note that calcium released during muscle contraction stimulates PDH (by increasing phosphatase activity) for energy production.

PDH kinase (responsible to form inactive PDH) is promoted by ATP, NADH and acetyl CoA, while it is inhibited by NAD^+, CoA and pyruvate. The net result is that in the presence of high energy signals (ATP, NADH), the PDH is turned off.

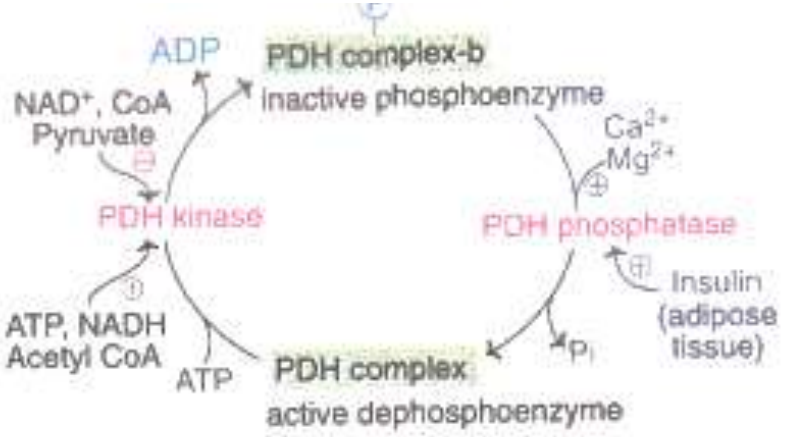


Fig. 13.7 : Regulation of pyruvate dihydrogenase (PDH) complex.

The intermediates of PDH catalysed reaction are not free but bound with enzyme complex. ***In mammals, the PDH complex has an approximate molecular weight of 9×10^6. It contains 60 molecules of dihydrolipoyltransacetylase and about 20–30 molecules each of the other two enzymes (pyruvate dehydrogenase and dihydrolipoyl dehydrogenase).***

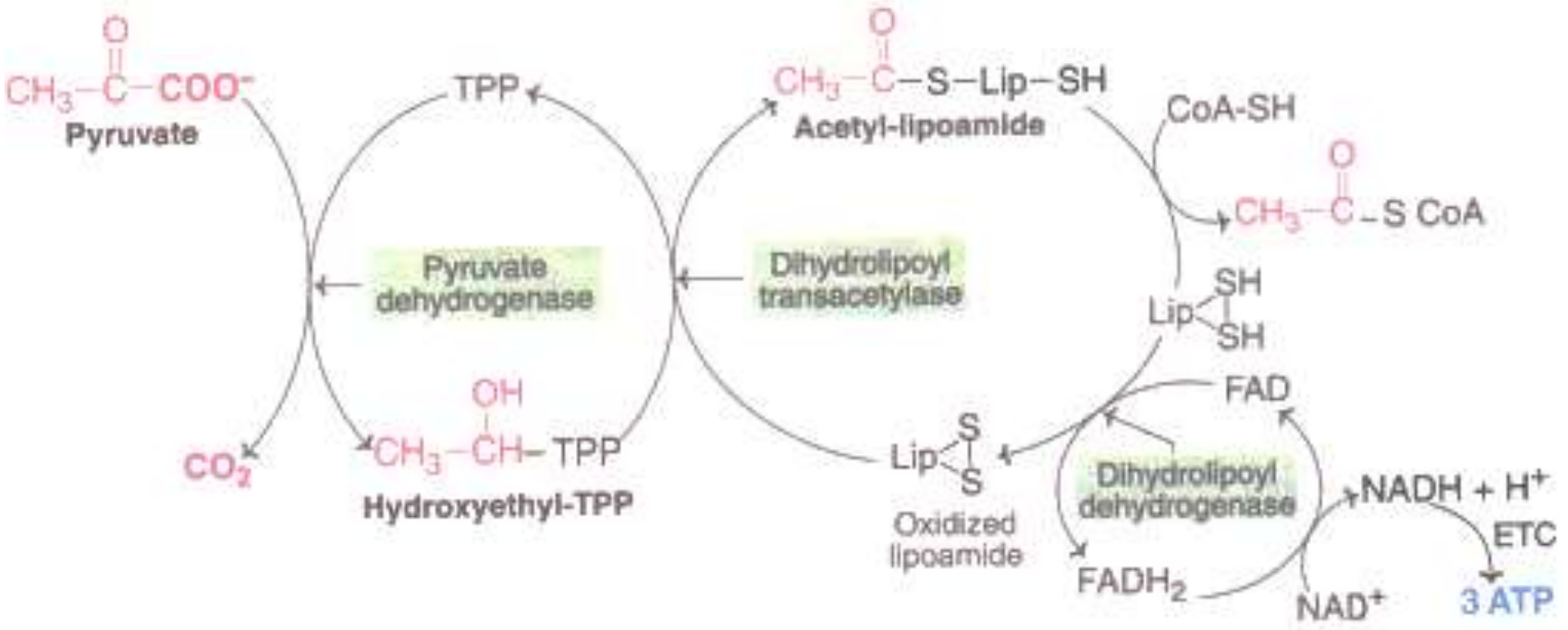


***Fig. 13.6** : The mechanism of action of pyruvate dehydrogenase complex. (**Note** : The reaction involving the conversion of pyruvate to acetyl CoA requires five coenzymes—TPP, lipoamide, CoASH, FAD and NAD⁺).*

TRANSPORT OF REDUCING EQUIVALENTS—SHUTTLE PATHWAYS

The inner mitochondrial membrane is impermeable to NADH. Therefore, the NADH produced in the cytosol cannot directly enter the mitochondria. Two pathways—namely ***glycerol-phosphate shuttle*** and ***malate-aspartate shuttle***—are operative to do this job. They transport the reducing equivalents from cytosol to mitochondria and not vice versa.

I. Glycerol-phosphate shuttle

Cytosolic glycerol 3-phosphate dehydrogenase oxidizes NADH to NAD^+. The reducing equivalents are transported through glycerol 3-phosphate into the mitochondria. Glycerol 3-phosphate dehydrogenase—present on outer surface of inner mitochondrial membrane—reduces FAD to $FADH_2$. Dihydroxyacetone phosphate escapes into the cytosol and the shuttling continues as depicted in ***Fig.11.12***. $FADH_2$ gets oxidized via ETC to generate ***2 ATP.***

II. Malate-aspartate shuttle

In the cytosol, oxaloacetate accepts the reducing equivalents (NADH) and becomes malate. Malate then enters mitochondria where it is oxidized by mitochondrial malate dehydrogenase. In this reaction, NADH and oxaloacetate are regenerated. NADH gets oxidized via electron transport chain and ***3 ATP*** are produced. This is in contrast to glycerol-phosphate shuttle where only 2 ATP are produced.

In the mitochondria, oxaloacetate participates in transamination reaction with glutamate to produce aspartate and α-ketoglutarate. The aspartate enters the cytosol and transaminates with α-ketoglutarate to give oxaloacetate and glutamate. The malate-aspartate shuttle is shown in ***Fig.11.13***.

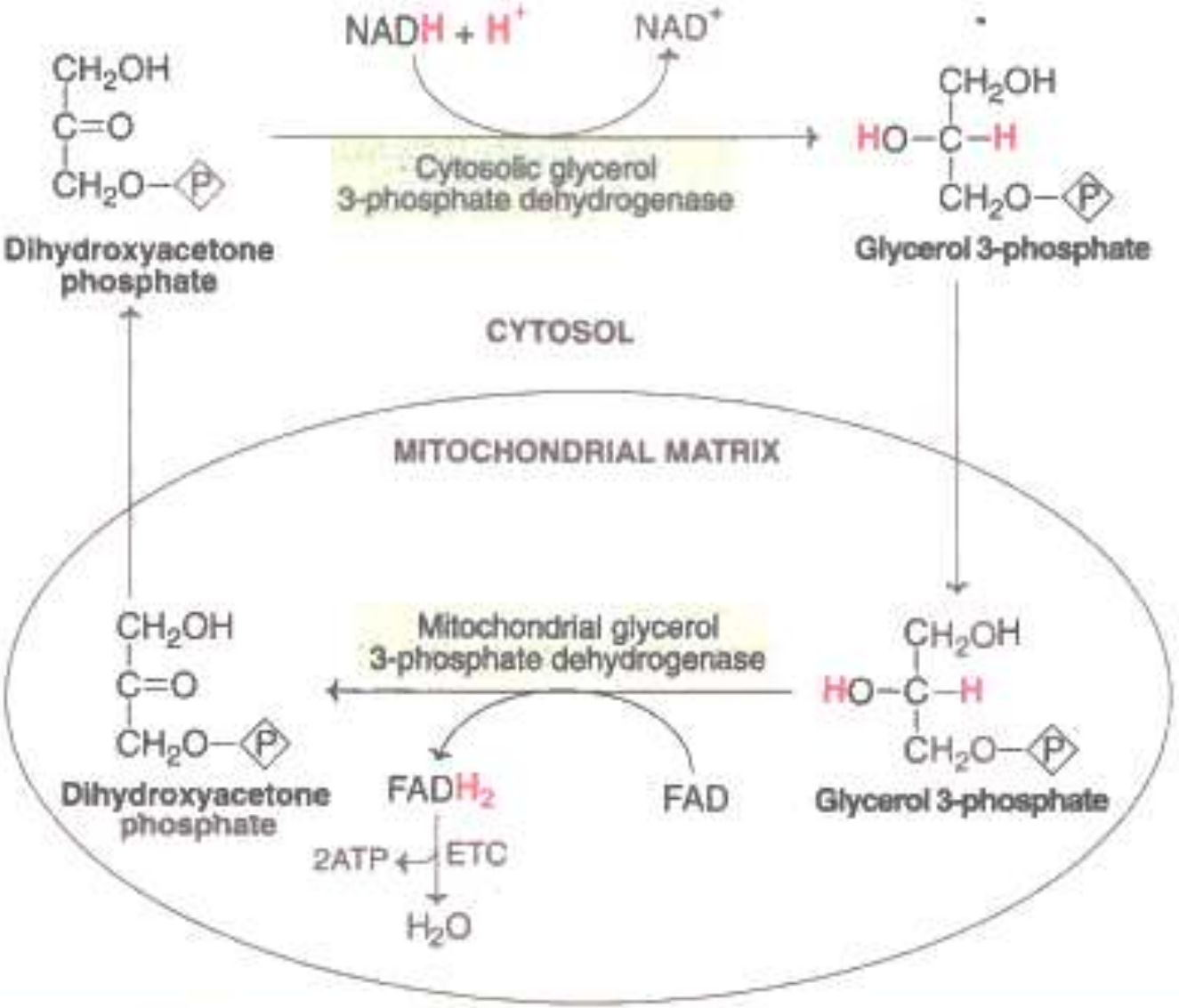


Fig. 11.12 : Glycerol-phosphate shuttle (reducing equivalents transported are shown in Blue).

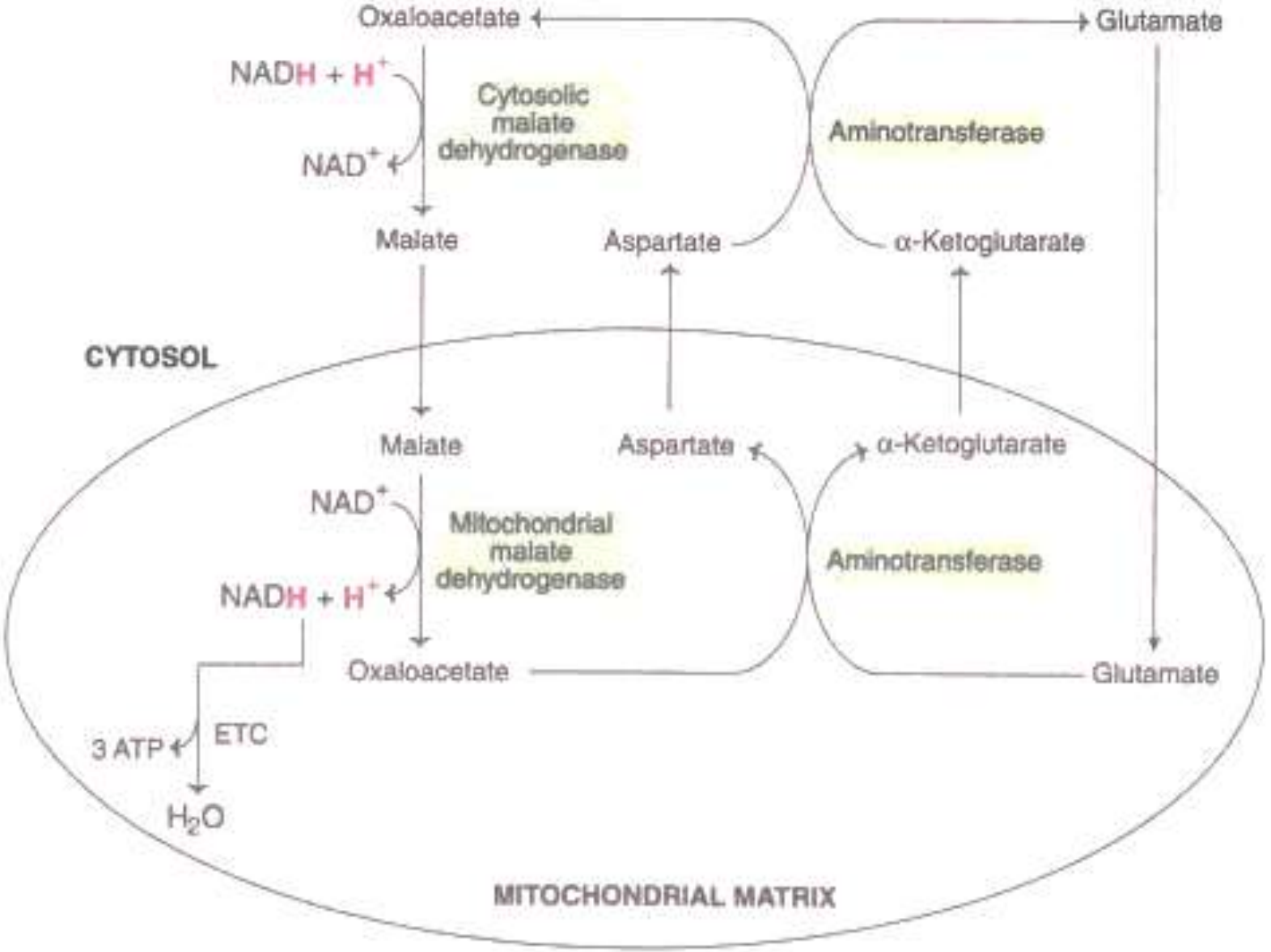


Fig. 11.13 : Malate-aspartate shuttle.

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METABOLISM OF CARBOHYDRATES -2

TEJASVI NAVADHITAMASTU

"Let our (the teacher and the taught) learning be radiant"

Let our efforts at learning be luminous and filled with joy, and endowed with the force of purpose

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E –content

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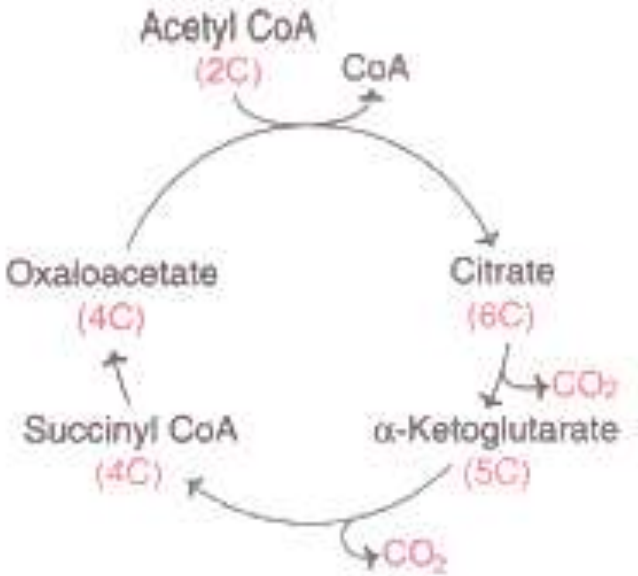
CITRIC ACID CYCLE

The citric acid cycle (Krebs cycle or tricarboxylic acid—TCA cycle) is the most important metabolic pathway for the energy supply to the body. About 65-70% of the ATP is synthesized in Krebs cycle. ***Citric acid cycle essentially involves the oxidation of acetyl CoA to CO_2 and H_2O.*** This cycle utilizes about two-thirds of total oxygen consumed by the body. The name TCA cycle is used, since, at the outset of the cycle, tricarboxylic acids (citrate, cis-aconitate and isocitrate) participate.



TCA cycle—an overview

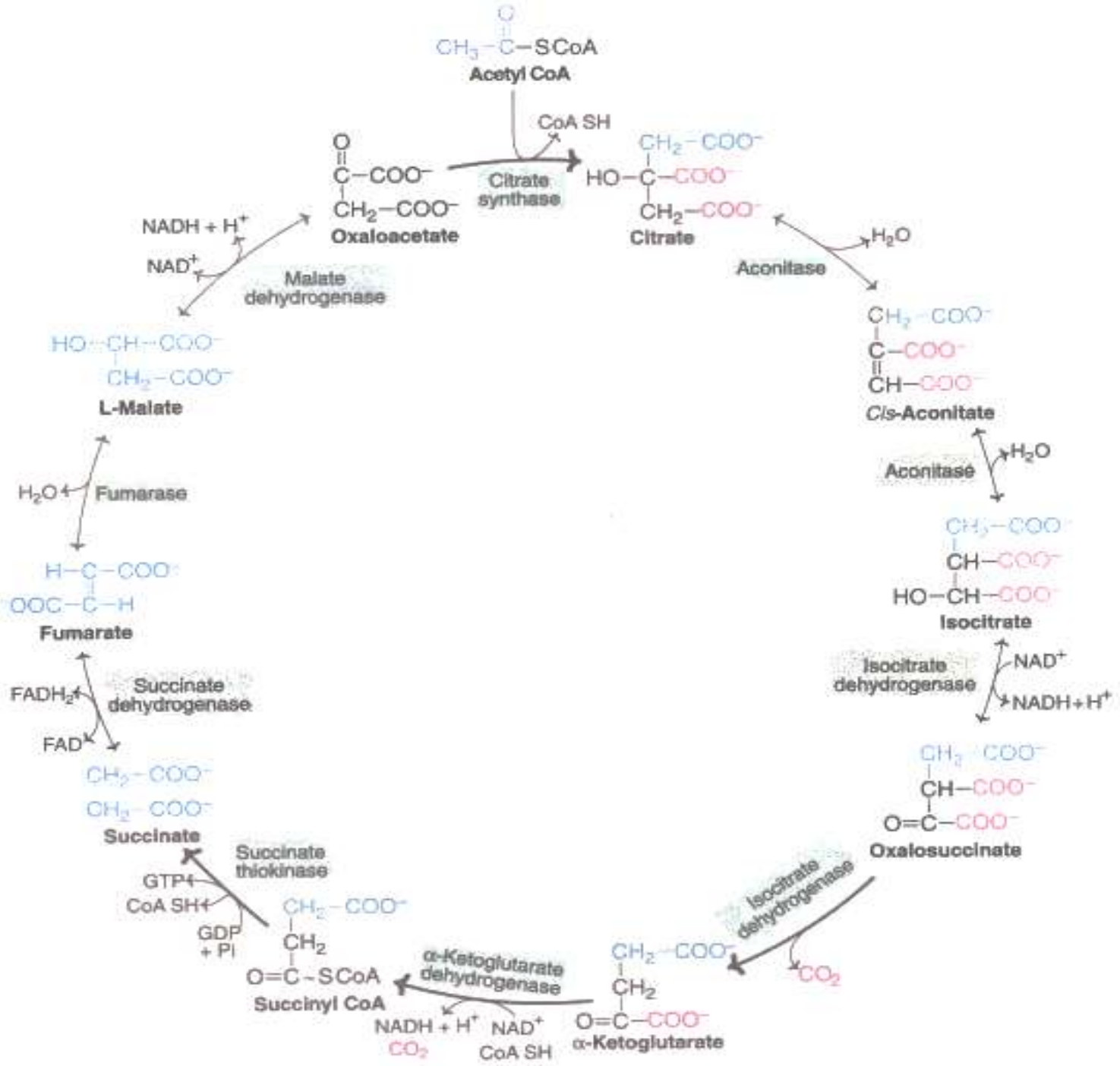
Krebs cycle basically involves the combination of a two carbon acetyl CoA with a four carbon oxaloacetate to produce a six carbon tricarboxylic acid, citrate. In the reactions that follow, the two carbons are oxidized to CO_2 and oxaloacetate is regenerated and recycled. ***Oxaloacetate is considered to play a catalytic role in citric acid cycle.*** An overview of Krebs cycle is depicted in ***Fig.13.8***.

TCA cycle—an open cycle

Krebs cycle is a cyclic process. However, it should not be viewed as a closed circle, since many compounds enter the cycle and leave. TCA cycle is comparable to a heavy traffic circle in a national highway with many connecting roads. Each intermediate of the cycle connecting another pathway is a road!

Amphibolic nature of the citric acid cycle

The citric acid cycle provides various intermediates for the synthesis of many compounds needed by the body. Krebs cycle is ***both catabolic and anabolic in nature***, hence regarded as ***amphibolic***.


Acetyl CoA
CoA SH
Citrate synthase
Oxaloacetate
Citrate
NADH + H+
NAD+
Malate dehydrogenase
H2O
Aconitase
L-Malate
Cis-Aconitate
H2O
Aconitase
Fumarase
Isocitrate
Fumarate
Isocitrate dehydrogenase
NAD+
NADH+H+
FADH2
Succinate dehydrogenase
FAD
Oxalosuccinate
Succinate
Succinate thiokinase
GTP
CoA SH
GDP + Pi
Isocitrate dehydrogenase
CO2
α-Ketoglutarate dehydrogenase
Succinyl CoA
NADH + H+
CO2
NAD+
CoA SH
α-Ketoglutarate

Reactions of citric acid cycle

Oxidative decarboxylation of pyruvate to acetyl CoA by pyruvate dehydrogenase complex is discussed above. This step is a connecting link between glycolysis and TCA cycle. A few authors, however, describe the conversion of pyruvate to acetyl CoA along with citric acid cycle. The events of TCA cycle are described hereunder (***Fig.13.9***).

1. **Formation of citrate :** Krebs cycle proper starts with the condensation of acetyl CoA and oxaloacetate, catalysed by the enzyme citrate synthase.

2. and 3. **Citrate is isomerized to isocitrate** by the enzyme aconitase. This is achieved in a two stage reaction of dehydration followed by hydration through the formation of an intermediate—*cis*-aconitate.

4. and 5. **Formation of α-ketoglutarate :** The enzyme isocitrate dehydrogenase (ICD) catalyses the conversion (oxidative decarboxylation) of isocitrate to oxalosuccinate and then to α-ketoglutarate. The formation of NADH and the liberation of CO_2 occur at this stage.

6. **Conversion of α-ketoglutarate to succinyl CoA** occurs through oxidative decarboxylation, catalysed by α-ketoglutarate dehydrogenase complex. This enzyme is dependent on five cofactors—TPP, lipoamide, NAD^+, FAD and CoA. The mechanism of the reaction is analogous to the conversion of pyruvate to acetyl CoA (***See Fig.13.6***). At this stage of the TCA cycle, second NADH is produced and the second CO_2 is liberated.

7. **Formation of succinate :** Succinyl CoA is converted to succinate by succinate thiokinase. This reaction is coupled with the phosphorylation of GDP to GTP. This is a ***substrate level phosphorylation***. GTP is converted to ATP by the enzyme nucleoside diphosphate kinase.

$$GTP + ADP \leftrightarrow ATP + GDP$$

8. **Conversion of succinate to fumarate :** Succinate is oxidized by succinate dehydrogenase to fumarate. This reaction results in the production of $FADH_2$ and not NADH.

9. **Formation of malate :** The enzyme fumarase catalyses the conversion of fumarate to malate with the addition of H_2O.

10. **Conversion of malate to oxaloacetate :** Malate is then oxidized to oxaloacetate by malate dehydrogenase. The third and final synthesis of NADH occurs at this stage. The oxaloacetate is regenerated which can combine with another molecule of acetyl CoA, and continue the cycle.

Summary of TCA cycle

The events of Krebs cycle may be summarized as given in the next column

$$\text{Acetyl CoA} + 3\ NAD^+ + FAD + GDP + Pi + 2H_2O \longrightarrow 2CO_2 + 3NADH + 3H^+ + FADH_2 + GTP + CoA$$

OXIDATIVE PHOSPHORYLATION ATP YIELD

	ELECTRON CARRIERS	I	II	III	IV	TOTAL H^+ PUMPED	ATP SYNTHASE $4H^+ \rightarrow 1ATP$
GLYCOLYSIS	$2NADH + H^+$	$8H^+$	-	$8H^+$	$4H^+$	$20H^+$	$20/4$: 5 ATP
PREPARATORY STEP	$2NADH + H^+$	$8H^+$	-	$8H^+$	$4H^+$	$20H^+$	$20/4$: 5 ATP
KREBS CYCLE	$6NADH + H^+$	$24H^+$	-	$24H^+$	$12H^+$	$60H^+$	$60/4$: 15 ATP
	$2FADH_2$	-	-	$8H^+$	$4H^+$	$12H^+$	$12/4$: 3 ATP

Requirement of O_2 by TCA cycle

There is no direct participation of oxygen in Krebs cycle. However, the cycle operates only under ***aerobic conditions.*** This is due to the fact that NAD^+ and FAD (from NADH and $FADH_2$, respectively) required for the operation of the cycle can be regenerated in the respiratory chain only in the presence of O_2. Therefore, citric acid cycle is strictly aerobic in contrast to glycolysis which operates in both aerobic and anaerobic conditions.

Inhibitors of Krebs cycle

The important enzymes of TCA cycle inhibited by the respective inhibitors are listed

Enzyme	*Inhibitor*
Aconitase	Fluoroacetate (non-competitive)
α-Ketoglutarate dehydrogenase	Arsenite (non-competitive)
Succinate dehydrogenase	Malonate (competitive)

Regulation of citric acid cycle

The cellular demands of ATP are crucial in controlling the rate of citric acid cycle. The regulation is brought about either by enzymes or the levels of ADP. Three enzymes—namely ***citrate synthase, isocitrate dehydrogenase*** and ***α-ketoglutarate dehydrogenase***—regulate citric acid cycle.

1. **Citrate synthase** is inhibited by ATP, NADH, acetyl CoA and succinyl CoA.

2. **Isocitrate dehydrogenase** is activated by ADP, and inhibited by ATP and NADH.

3. **α-Ketoglutarate dehydrogenase** is inhibited by succinyl CoA and NADH.

4. **Availability of ADP** is very important for the citric acid cycle to proceed. This is due to the fact that unless sufficient levels of ADP are available, oxidation (coupled with phosphorylation of ADP to ATP) of NADH and $FADH_2$ through electron transport chain stops. The accumulation of NADH and $FADH_2$ will lead to inhibition of the enzymes (as stated above) and also limits the supply of NAD^+ and FAD which are essential for TCA cycle to proceed.

Amphibolic nature of the citric acid cycle

The citric acid cycle provides various intermediates for the synthesis of many compounds needed by the body. Krebs cycle is ***both catabolic and anabolic in nature***, hence regarded as ***amphibolic***.

TCA cycle is actively involved in gluconeogenesis, transamination and deamination.

The most important synthetic (anabolic) reactions connected with TCA cycle are given (***Fig.13.10***)

1. Oxaloacetate and α-ketoglutarate, respectively, serve as precursors for the synthesis of aspartate and glutamate which, in turn, are required for the synthesis of other non-essential amino acids, purines and pyrimidines.

2. Succinyl CoA is used for the synthesis of porphyrins and heme.

3. Mitochondrial citrate is transported to the cytosol, where it is cleaved to provide acetyl CoA for the biosynthesis of fatty acids, sterols etc.

Anaplerosis or anaplerotic reactions

The synthetic reactions described above deplete the intermediates of citric acid cycle. The cycle will cease to operate unless the intermediates drawn out are replenished. ***The reactions concerned to replenish or to fill up the intermediates of citric acid cycle are called anaplerotic reactions or anaplerosis*** (*Greek* : fill up). In ***Fig.13.10***, the important synthetic pathways that draw the intermediates of TCA cycle and the anaplerotic reactions to fill them up are given.

The salient features of important anaplerotic reactions are described

1. Pyruvate carboxylase catalyses the conversion of pyruvate to oxaloacetate. This is an ATP dependent carboxylation reaction.

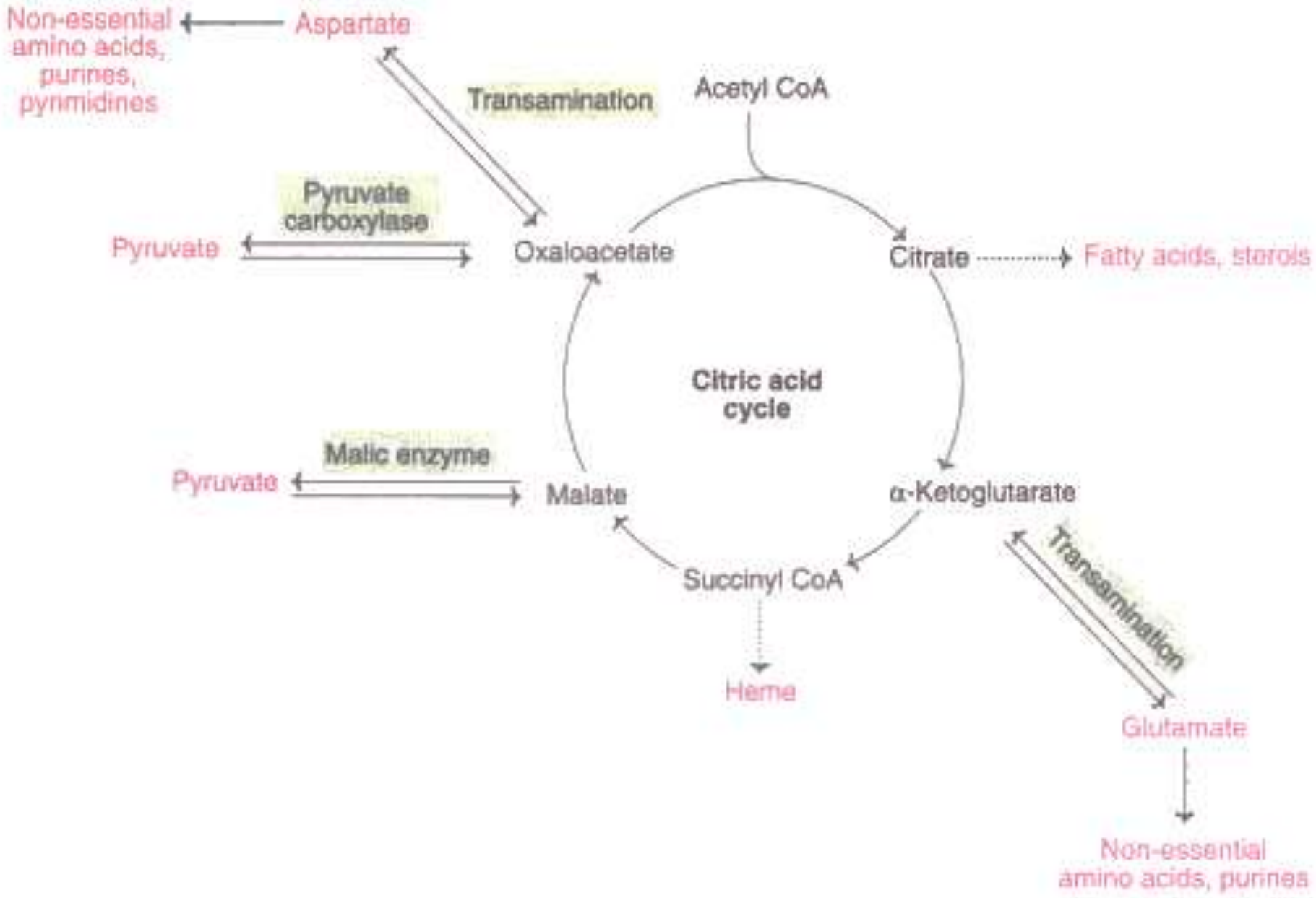
$$\text{Pyruvate} + CO_2 + \text{ATP} \longrightarrow \text{Oxaloacetate} + \text{ADP} + \text{Pi}$$

The details of the above reaction are described under gluconeogenesis.

2. Pyruvate is converted to malate by $NADP^+$ dependent malate dehydrogenase (malic enzyme).

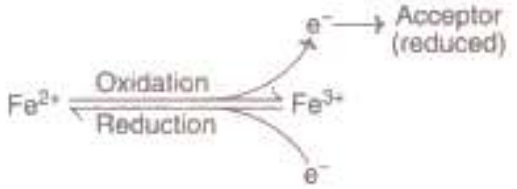
$$\text{Pyruvate} + CO_2 + \text{NADPH} + H^+ \rightleftharpoons \text{Malate} + NADP^+ + H_2O$$

3. Transamination is a process wherein an amino acid transfers its amino group to a keto acid and itself gets converted to a keto acid. The formation of α-ketoglutarate and oxaloacetate occurs by this mechanism.


Non-essential amino acids, purines, pyrimidines
Aspartate
Transamination
Acetyl CoA
Pyruvate carboxylase
Pyruvate
Oxaloacetate
Citrate
Fatty acids, sterols
Citric acid cycle
Malic enzyme
Pyruvate
Malate
α-Ketoglutarate
Transamination
Succinyl CoA
Heme
Glutamate
Non-essential amino acids, purines

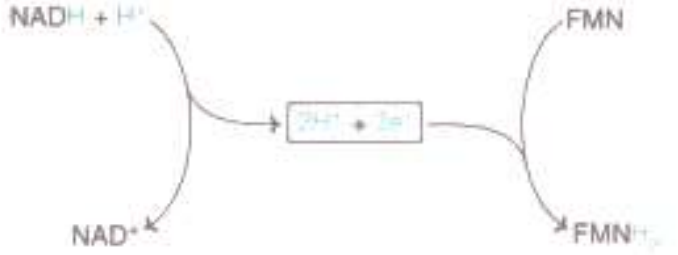
BIOLOGICAL OXIDATION

Oxidation is defined as the loss of electrons and reduction as the gain of electrons. This may be illustrated by the interconversion of ferrous ion (Fe^{2+}) to ferric ion (Fe^{3+}).



The electron lost in the oxidation is accepted by an acceptor which is said to be reduced. Thus the oxidation-reduction is a tightly coupled process.

The general principle of oxidation-reduction is applicable to biological systems also. The oxidation of NADH to NAD^+ coupled with the reduction of FMN to $FMNH_2$ is illustrated



In the above illustration, there are two redox pairs NADH/NAD^+ and FMN/$FMNH_2$. The redox pairs differ in their tendency to lose or gain electrons.

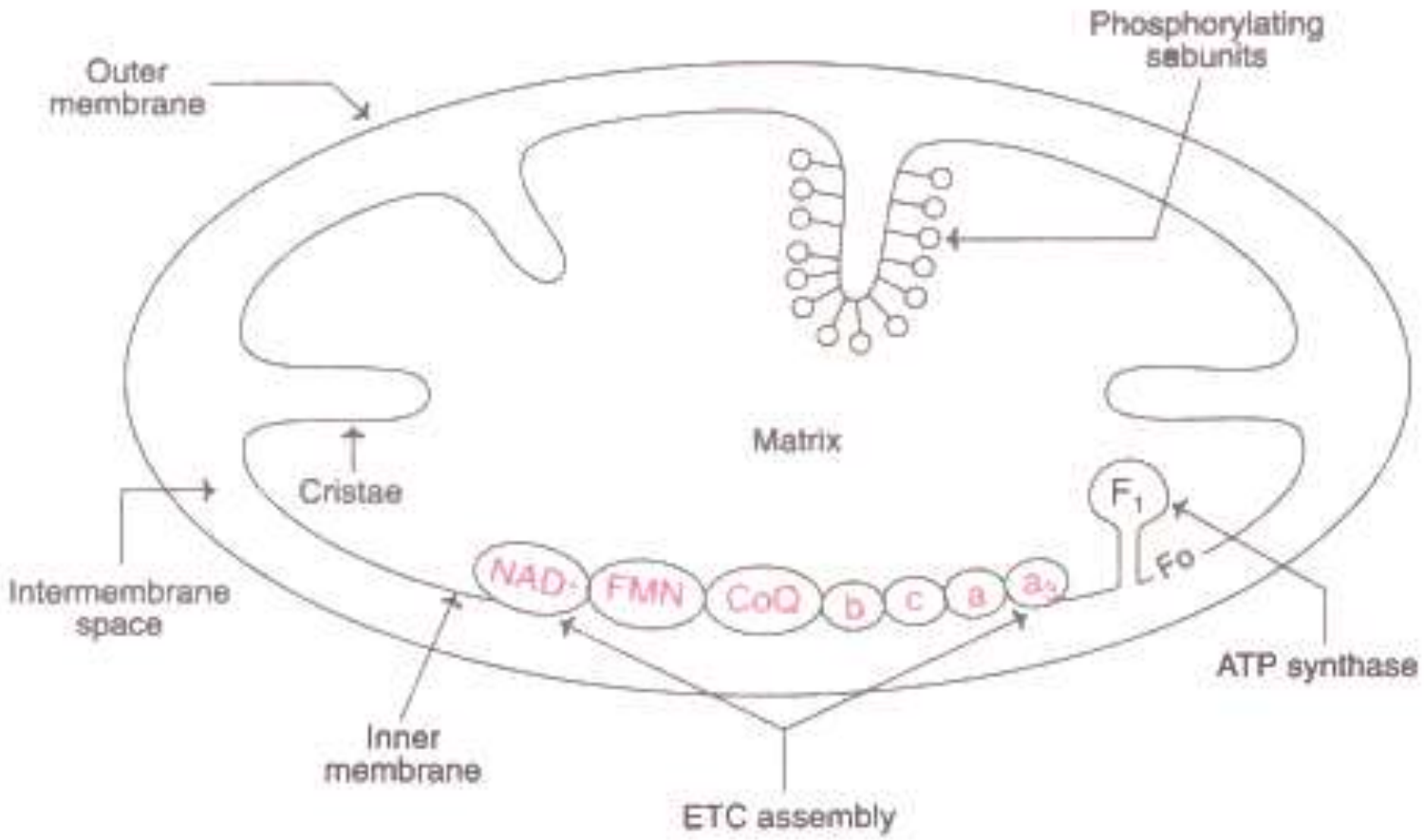
Redox potential (E_0)

The ***oxidation-reduction*** potential or, simply, redox potential, is a quantitative measure of the tendency of a redox pair to lose or gain electrons. The redox pairs are assigned specific ***standard redox potential*** (E_0 volts) at pH 7.0 and 25°C.

Table 11.3 Standard redox potential (E_0) of some oxidation-reduction systems

Redox pair	*E_0 Volts*
Succinate/α-ketoglutarate	– 0.67
$2H^+/H_2$	– 0.42
NAD^+/NADH	– 0.32
$NADP^+$/NADPH	– 0.32
FMN/$FMNH_2$ (enzyme bound)	– 0.30
Lipoate (ox/red)	– 0.29
FAD/$FADH_2$	– 0.22
Pyruvate/lactate	– 0.19
Fumarate/succinate	+ 0.03
Cytochrome b (Fe^{3+}/Fe^{2+})	+ 0.07
Coenzyme Q (ox/red)	+ 0.10
Cytochrome c_1 (Fe^{3+}/Fe^{2+})	+ 0.23
Cytochrome c (Fe^{3+}/Fe^{2+})	+ 0.25
Cytochrome a (Fe^{3+}/Fe^{2+})	+ 0.29
$\frac{1}{2}O_2/H_2O$	+ 0.82

ELECTRON TRANSPORT CHAIN



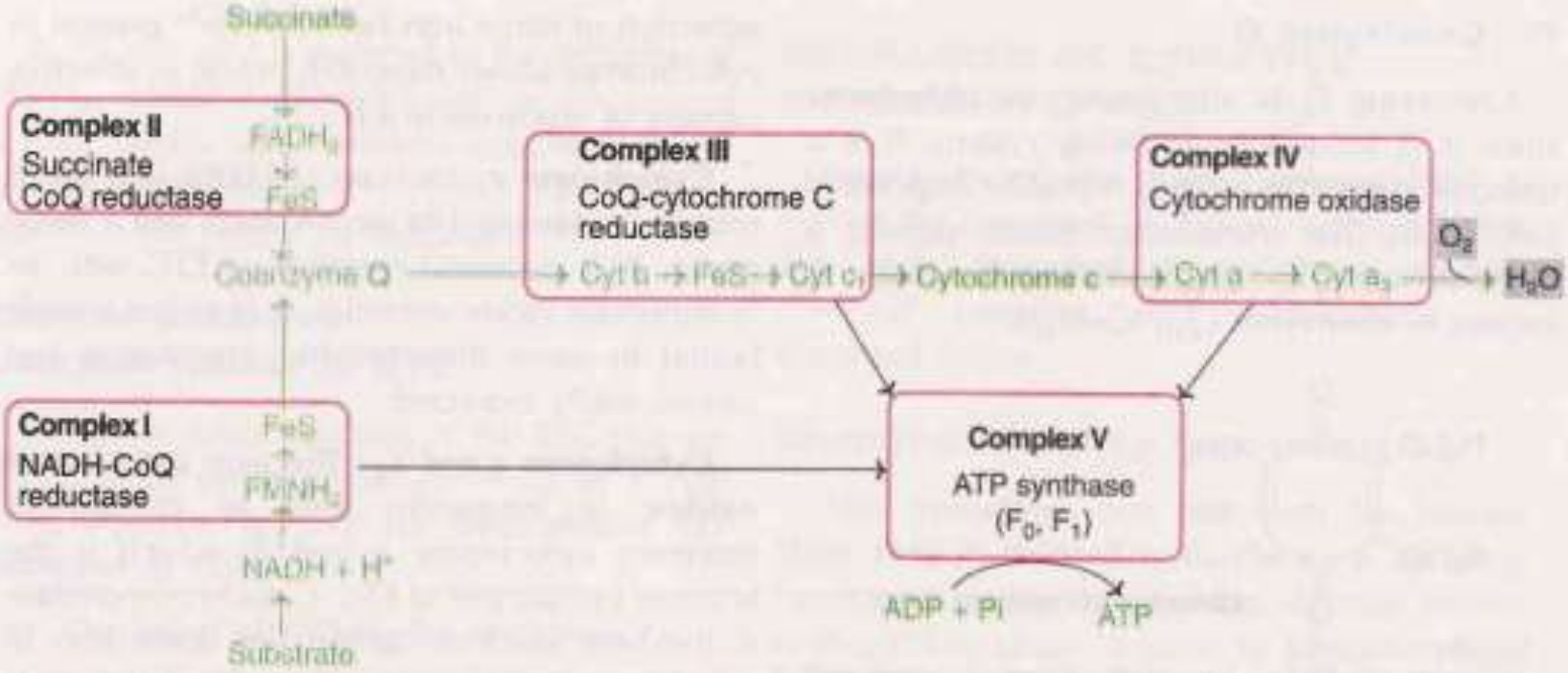


Fig. 11.6 : Multiprotein complexes in electron transport chain.

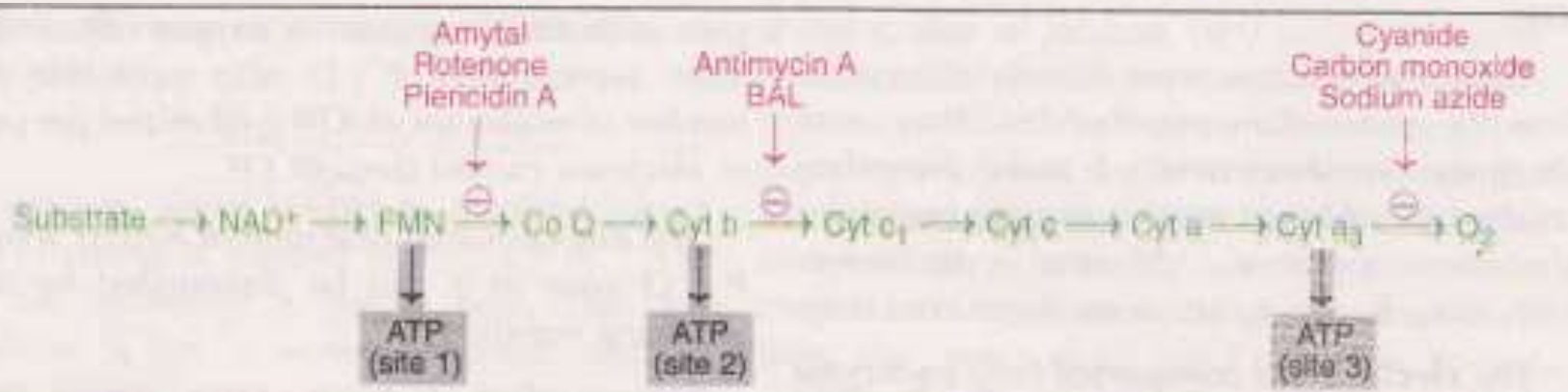


Fig. 11.7 : Electron transport chain with sites of ATP synthesis and inhibitors (BAL–British antilewisite).

The Electron-Transport Chain Can Be Isolated in Four Complexes

The electron-transport chain involves several different molecular species, including:

1. **Flavoproteins,** which contain tightly bound FMN or FAD as prosthetic groups and which may participate in one- or two-electron transfer events.
2. **Coenzyme Q,** also called **ubiquinone** (and abbreviated **CoQ** or **UQ**) (see Figure 20.5), which can function in either one- or two-electron transfer reactions.
3. Several **cytochromes** (proteins containing heme prosthetic groups [see Chapter 5], which function by carrying or transferring electrons), including cytochromes *b*, *c*, c_1, *a*, and a_3. Cytochromes are one-electron transfer agents in which the heme iron is converted from Fe^{2+} to Fe^{3+} and back.
4. A number of **iron–sulfur proteins,** which participate in one-electron transfers involving the Fe^{2+} and Fe^{3+} states.
5. Protein-bound **copper,** a one-electron transfer site that converts between Cu^{+} and Cu^{2+}.

MECHANISM OF OXIDATIVE PHOSPHORYLATION

Several hypotheses have been put forth to explain the process of oxidative phosphorylation. The most important among them—namely, chemical coupling, and chemiosmotic—are discussed below.

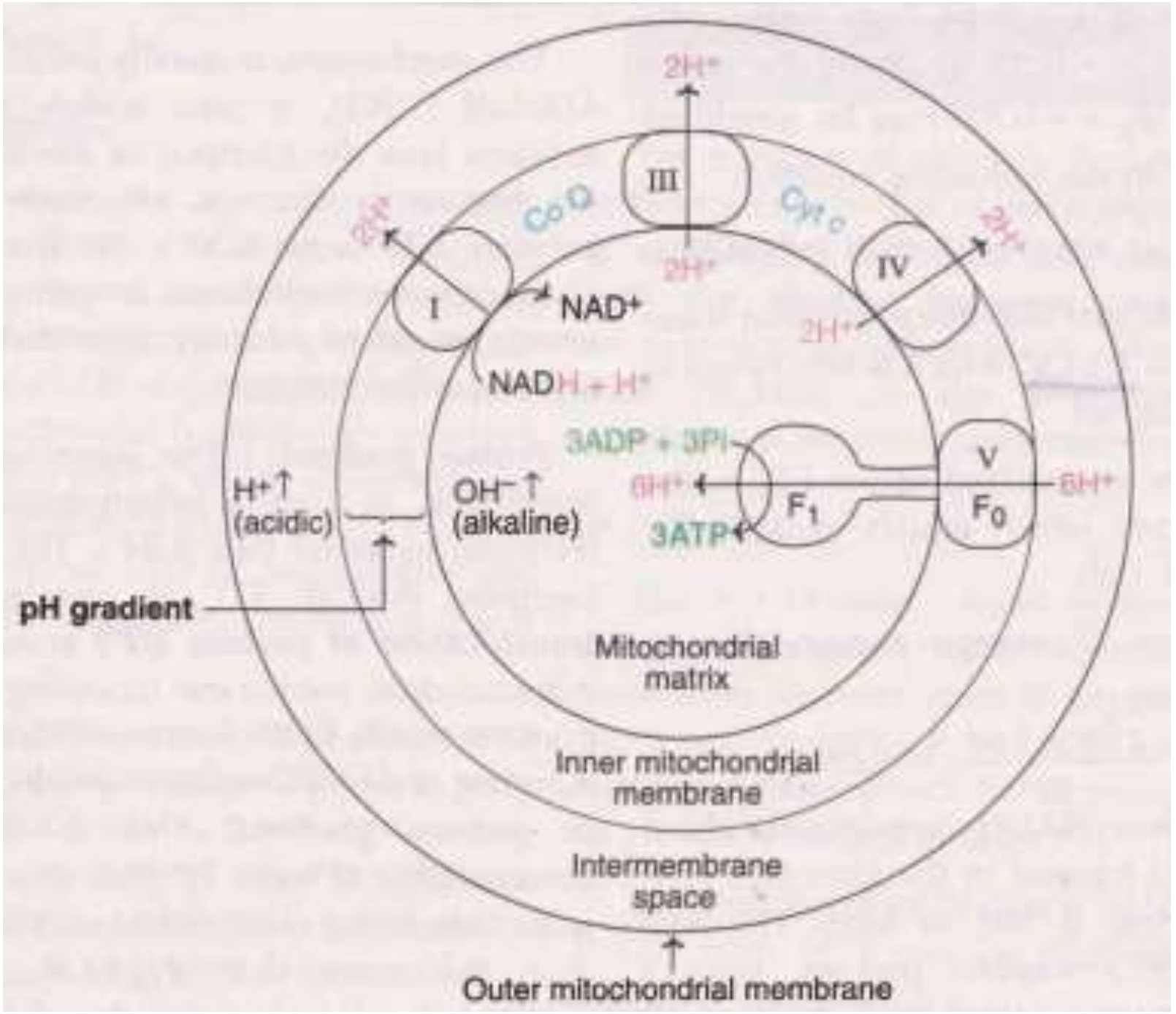
Chemical coupling hypothesis

This hypothesis was put forth by Edward Slater (1953). According to chemical coupling hypothesis, during the course of electron transfer in respiratory chain, a series of ***phosphorylated high-energy intermediates*** are first produced which are utilized for the synthesis of ATP. These reactions are believed to be analogous to the substrate level phosphorylation that occurs in glycolysis or citric acid cycle. However, this hypothesis lacks experimental evidence, since all attempts, so far, to isolate any one of the high-energy intermediates have not been successful.

Chemiosmotic hypothesis

This mechanism, originally proposed by Peter Mitchell (1961), is now widely accepted. It explains how the transport of electrons through the respiratory chain is effectively utilized to produce ATP from ADP + Pi. The concept of chemiosmotic hypothesis is comparable with energy stored in a battery separated by positive and negative charges.

Proton gradient : The inner mitochondrial membrane, as such, is impermeable to protons (H^+) and hydroxyl ions (OH^-). The transport of electrons through ETC is coupled with the ***translocation of protons (H^+)*** across the inner mitochondrial membrane (coupling membrane) from the matrix to the intermembrane space. The pumping of protons results in an ***electrochemical or proton gradient.*** This is due to the accumulation of more H^+ ions (low pH) on the outer side of the inner mitochondrial membrane than the inner side (***Fig.11.8***). The proton gradient developed due to the electron flow in the respiratory chain is sufficient to result in the synthesis of ATP from ADP and Pi.


2H+
III
CoQ
Cyt c
2H+
2H+
IV
2H+
I
NAD+
NADH + H+
3ADP + 3Pi
V
4H+
4H+
F1
F0
3ATP
H+↑
(acidic)
OH⁻↑
(alkaline)
pH gradient
Mitochondrial
matrix
Inner mitochondrial
membrane
Intermembrane
space
Outer mitochondrial membrane

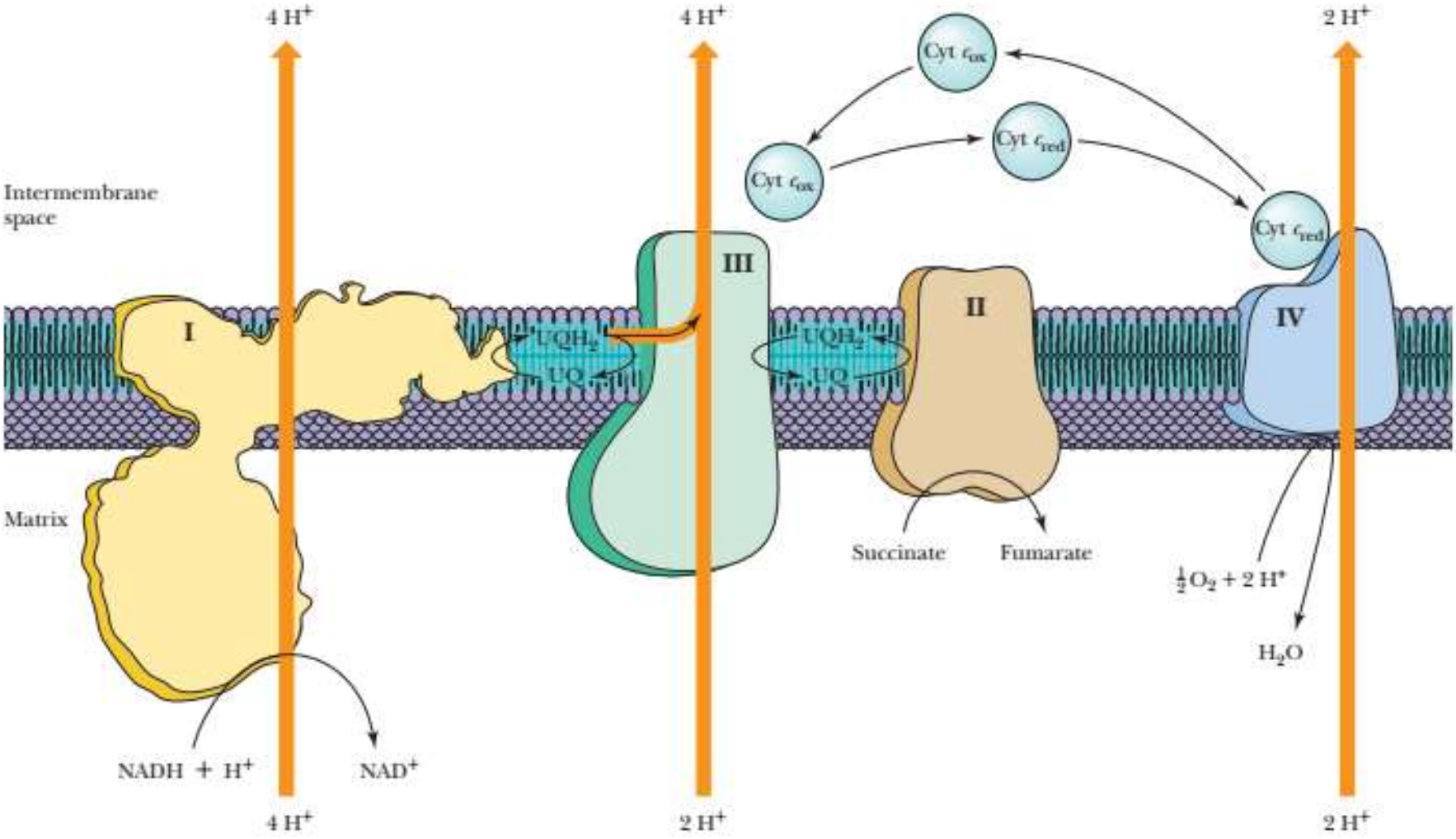


FIGURE 20.19 A model for the electron-transport pathway in the mitochondrial inner membrane. UQ/UQH_2 and cytochrome *c* are mobile electron carriers and function by transferring electrons between the complexes. The proton transport driven by Complexes I, III, and IV is indicated.

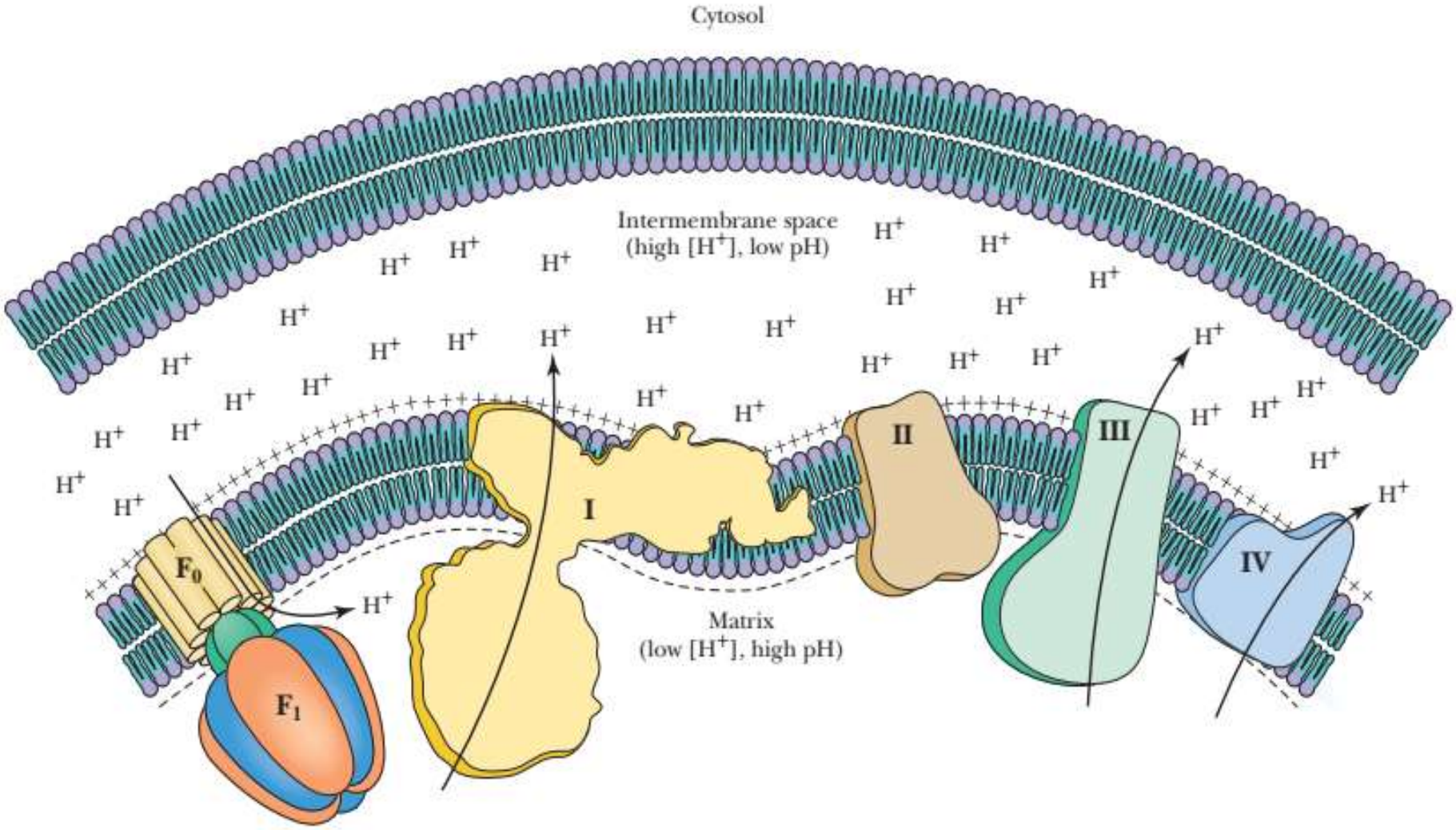


FIGURE 20.20 The proton and electrochemical gradients existing across the inner mitochondrial membrane. The electrochemical gradient is generated by the transport of protons across the membrane by Complexes I, III, and IV in the inner mitochondrial membrane.

Rotary motor model for ATP generation

Paul Boyer in 1964 proposed (Nobel Prize, 1997) that a conformational change in the mitochondrial membrane proteins leads to the synthesis of ATP. The original Boyer hypothesis, now considered as ***rotary motor/engine driving model*** or ***binding change model***, is widely accepted for the generation ATP.

The enzyme ATP synthase is F_0F_1 complex (of complex V). The F_0 subcomplex is composed of channel protein 'C' subunits to which F_1-ATP synthase is attached (***Fig.11.10***). F_1-ATP synthase consists of a central γ subunit surrounded by alternating α and β subunits (α_3 and β_3).

In response to the proton flux, the γ subunit physically rotates. This induces conformational changes in the β_3 subunits that finally lead to the release of ATP.

According to the binding change mechanism, the three β subunits of F_1-ATP synthase adopt different conformations. One subunit has ***open*** (***O***) conformation, the second has ***loose*** (***L***) conformation while the third one has ***tight*** (***T***) ***conformation*** (***Fig.11.11***).

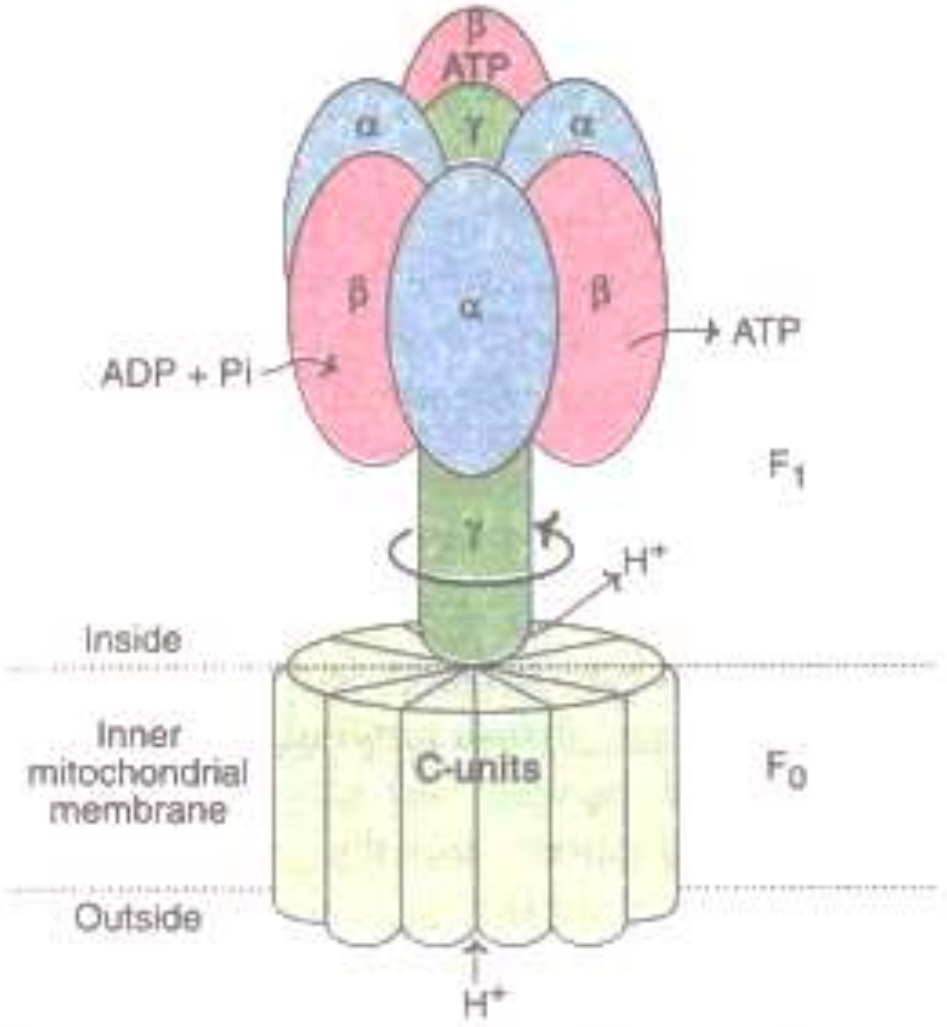


Fig. 11.10 : Structure of mitochondrial ATP synthase (F_0F_1) complex (C units-channel protein subunits; α, β, and γ are the subunits of F_1-ATP synthase).

By an unknown mechanism, protons induce the rotation of γ subunit, which in turn induces conformation changes in β subunits. The substrates ADP and Pi bind to β subunit in L-conformation. The L site changes to T conformation, and this leads to the synthesis of ATP. The O site changes to L conformation which binds to ADP and Pi. The T site changes to O conformation, and releases ATP. This cycle of conformation changes of β subunits is repeated. And three ATP are generated for each revolution (***Fig.11.11***).

It may be noted that the ATP release in O conformation is energy dependent (and not ATP synthesis) and very crucial in rotary motor model for ATP generation.

The enzyme ATP synthase acts as a proton-driving motor, and is an example of rotary catalysis. Thus, ***ATP synthase*** is the world's smallest ***molecular motor***.

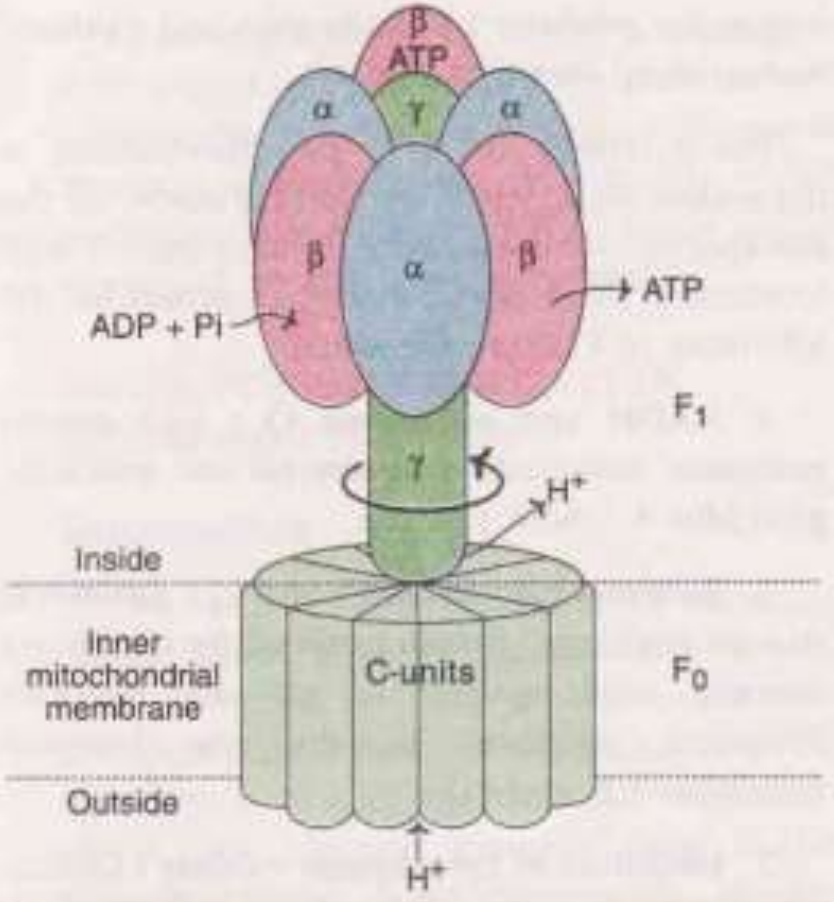


Fig. 11.10 : *Structure of mitochondrial ATP synthase (F_0F_1) complex (C units-channel protein subunits; α, β, and γ are the subunits of F_1-ATP synthase).*

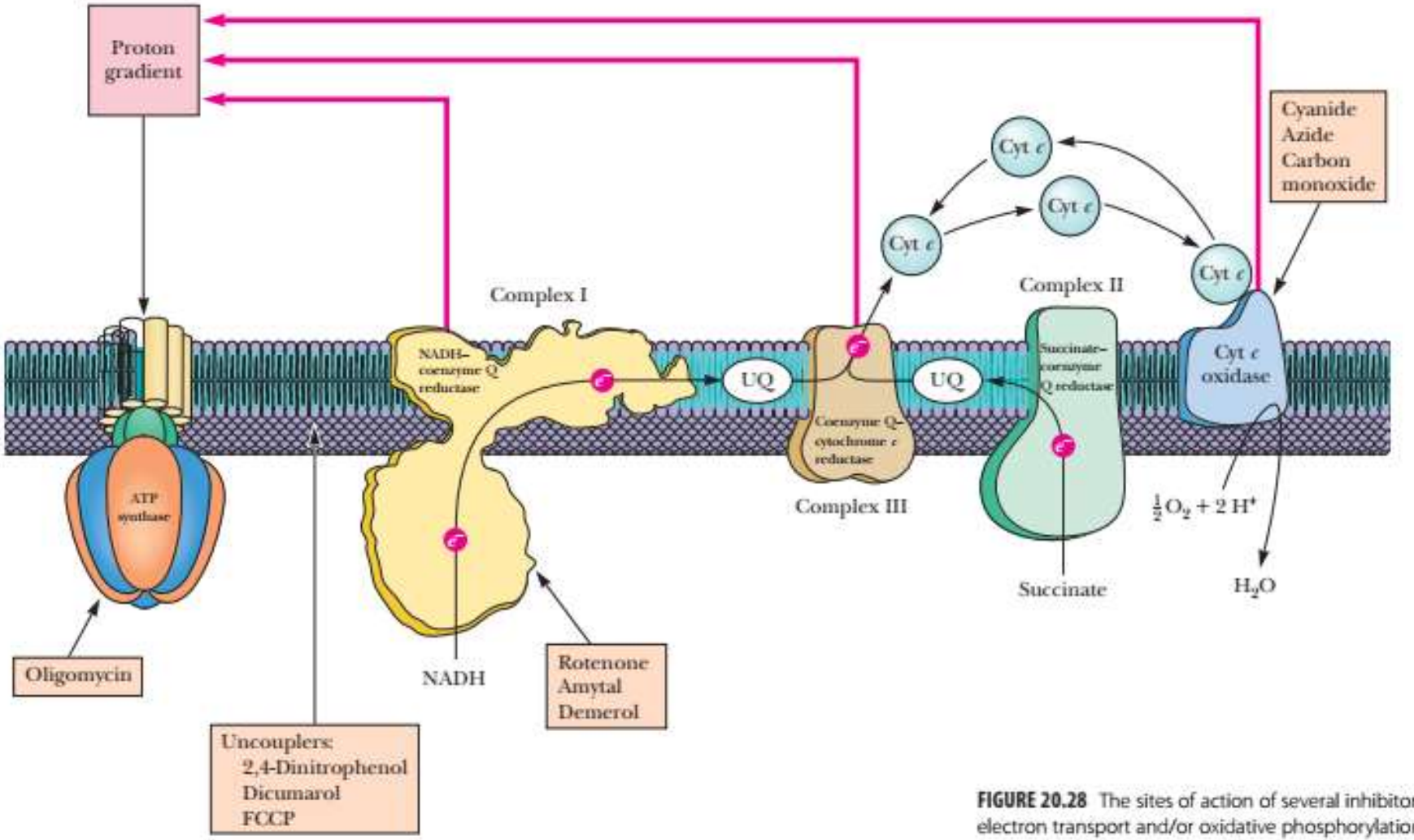


FIGURE 20.28 The sites of action of several inhibitors of electron transport and/or oxidative phosphorylation.

INHIBITORS OF OXIDATIVE PHOSPHORYLATION

Uncouplers

The mitochondiral transport of electrons is tightly coupled with oxidative phosporylation (ATP synthesis). In other words, oxidation and phosphorylation proceed simultaneously. There are certain compounds that can uncouple (or delink) the electron transport from oxidative phosphorylation. Such compounds, known as uncouplers, increase the permeability of inner mitochondrial membrane to protons (H^+). The result is that ATP synthesis does not occur. The energy linked with the transport of electrons is dissipated as heat. ***The uncouplers allow*** (often at accelerated rate) ***oxidation of substrates*** (via NADH or $FADH_2$) ***without ATP formation.***

The uncoupler, ***2,4-dinitrophenol (DNP)***, has been extensively studied. It is a small lipophilic molecule. DNP is a proton-carrier and can easily diffuse through the inner mitochondrial membrane. In the people seeking to lose weight, DNP was used as a drug. However, this is now discontinued, as it produces hyperthermia and other side effects. In fact, Food and Drug Administration (USA) has banned the use of DNP.

The other uncouplers include dinitrocresol, pentachlorophenol, trifluorocarbonylcyanide phenylhydrazone (FCCP). The last compound (FCCP) is said to be 100 times more effective as an uncoupler than dinitrophenol. When administered ***in high doses***, the drug ***aspirin*** acts as an uncoupler.

Physiological uncouplers : Certain physiological substances which act as uncouplers at higher concentration have been identified. These include ***thermogenin***, ***thyroxine*** and ***long chain free fatty acids***. The unconjugated bilirubin is also believed to act as an uncoupler. This is, however, yet to be proved beyond doubt.

Significance of uncoupling

Uncoupling of respiration from oxidative phosphorylation under natural conditions assumes biological significance. The maintenance of body temperature is particularly important in hairless animals, hibernating animals and the animals adapted to cold. These animals possess a specialized tissue called ***brown adipose tissue*** in the upper back and neck portions. The mitochondria of brown adipose tissue are rich in electron carriers and are specialized to carry out an ***oxidation uncoupled from phosphorylation***. This causes liberation of heat when fat is oxidized in the brown adipose tissue. Brown adipose tissue may be considered as a site of ***non-shivering thermogenesis***. The presence of active brown adipose tissue in certain individuals is believed to protect them from becoming obese. The excess calories consumed by these people are burnt and ***liberated as heat***, instead of being stored as fat.

Thermogenin (or uncoupling protein, UCP) is a natural uncoupler located in the inner mitochondrial membrane of brown adipose tissue. It acts like an uncoupler, blocks the formation of ATP, and liberates heat.

Ionophores : The term 'ionophores' is used to collectively represent the lipophilic substances that promote the transport of ions across biological membranes.

All the uncouplers (described above) are, in fact, proton ionophores.

The antibiotics ***valinomycin*** and ***nigercin*** act as ionophores for K^+ ions. Both these compounds are also capable of dissipating proton gradient across the inner mitochondrial membrane and inhibit oxidative phosphorylation.

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3. David L. Nelson; Michael M. Cox “Lehninger Principles of Biochemistry” Seventh Edition, Macmillan 2017, ISBN:9781464187957

All the original contributors of the concept and findings published are gratefully acknowledged while preparing the e-content for the students of Biotechnology and allied sciences

METABOLISM OF CARBOHYDRATES -3

TEJASVI NAVADHITAMASTU

"Let our (the teacher and the taught) learning be radiant"

Let our efforts at learning be luminous and filled with joy, and endowed with the force of purpose

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E –content

Course: M.Sc.
Subject: Biochemistry; Biotechnology
Topic: Metabolism of Carbohydrates
Subtopic: Glycogen Metabolism
Prepared by: Prof. Rajesh Sharma
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Faculty : Science
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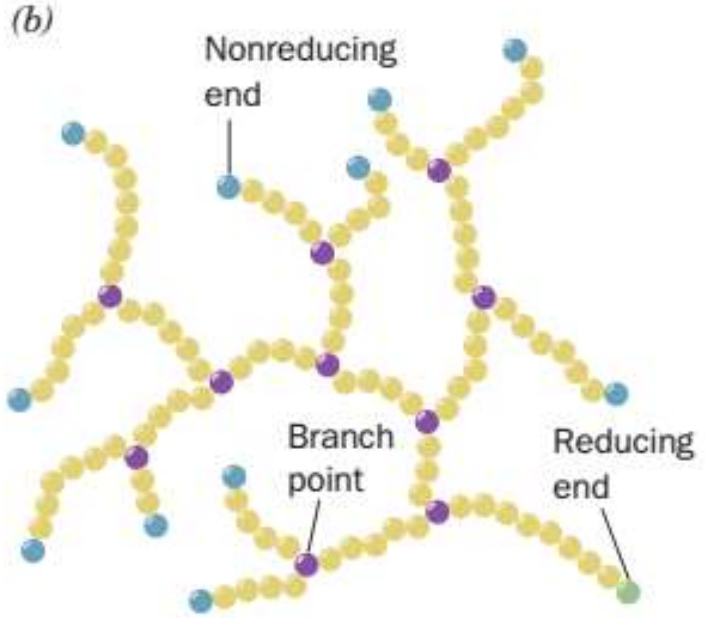
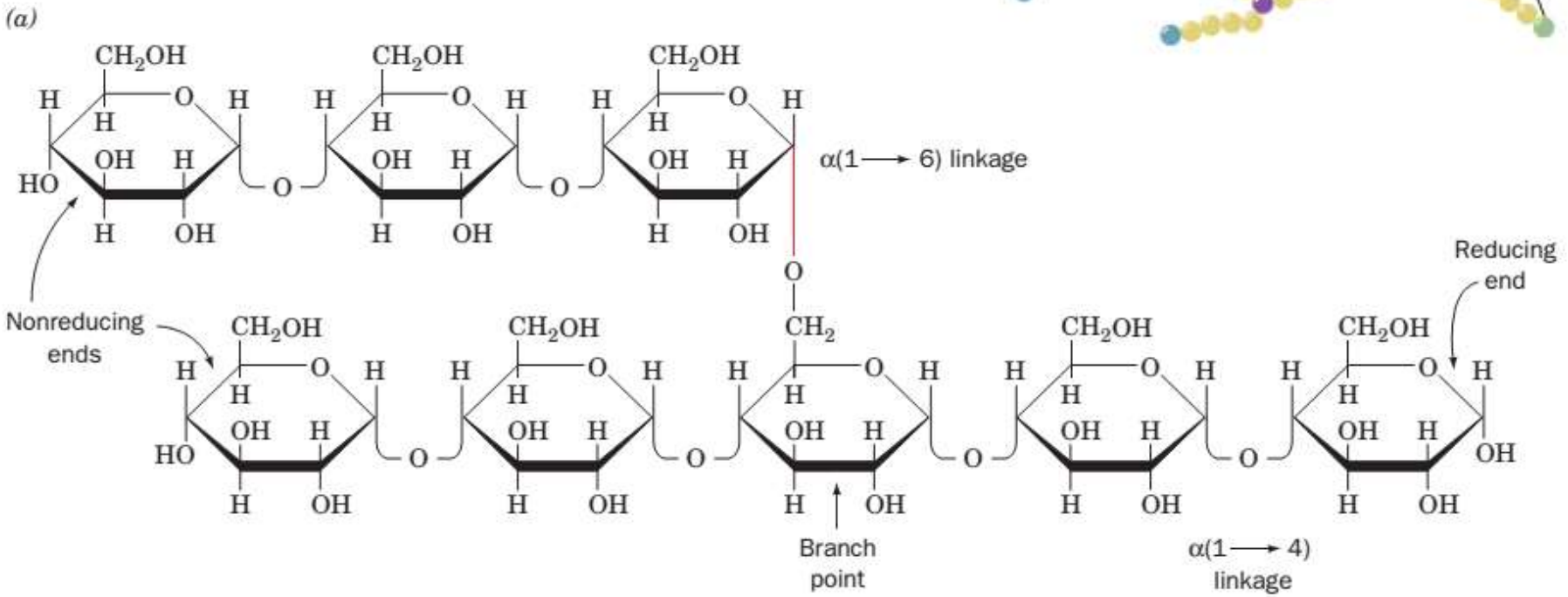
Note-This E-content has been prepared as a reading material for students without any commercial interest. Original contributors are acknowledged.

Glycogen Metabolism

1. Glycogenesis
2. Glycogenolysis
3. Regulation Of Glycogen Metabolism
4. Glycogen Storage Disease





Glycogen forms a left-handed helix with 6.5 glucose residues per turn, similar to α-amylose (Fig. 11-18*b*). An

GLYCOGENESIS

The ***synthesis of glycogen*** from glucose is glycogenesis (***Fig.13.14***). Glycogenesis takes place in the cytosol and requires ATP and UTP, besides glucose.

1. **Synthesis of UDP-glucose :** The enzymes hexokinase (in muscle) and glucokinase (in liver) convert glucose to glucose 6-phosphate. Phosphoglucomutase catalyses the conversion of glucose 6-phosphate to glucose 1-phosphate. Uridine diphosphate glucose (UDPG) is synthesized from glucose 1-phosphate and UTP by UDP-glucose pyrophosphorylase.

2. **Requirement of primer to initiate glycogenesis :** A small fragment of pre-existing glycogen must act as a '***primer***' to initiate glycogen synthesis. It is recently found that in the absence of glycogen primer, a specific protein—namely '***glycogenin***'—can accept glucose from UDPG. The hydroxyl group of the amino acid tyrosine of glycogenin is the site at which the initial glucose unit is attached. The enzyme glycogen initiator synthase transfers the first molecule of glucose to glycogenin. Then glycogenin itself takes up a few glucose residues to form a fragment of primer which serves as an acceptor for the rest of the glucose molecules.

3. **Glycogen synthesis by glycogen synthase :** Glycogen synthase is responsible for the formation of 1,4-glycosidic linkages. This enzyme transfers the glucose from UDP-glucose to the non-reducing end of glycogen to form α-1,4 linkages.

4. **Formation of branches in glycogen :** Glycogen synthase can catalyse the synthesis of a linear unbranched molecule with 1,4 α-glycosidic linkages. Glycogen, however, is a branched tree-like structure. The formation of branches is brought about by the action of a branching enzyme, namely ***glucosyl α-4-6 transferase***. (amylo α 1,4 → 1,6 transglucosidase). This enzyme transfers a small fragment of five to eight glucose residues from the non-reducing end of glycogen chain (by breaking α-1,4 linkages) to another glucose residue where it is linked by α-1,6 bond. This leads to the formation of a new non-reducing end, besides the existing one. Glycogen is further elongated and branched, respectively, by the enzymes glycogen synthase and glucosyl 4-6 transferase.

The overall reaction of the glycogen synthesis for the addition of each glucose residue is

$$(\text{Glucose})_n + \text{Glucose} + 2\text{ATP} \longrightarrow (\text{Glucose})_{n+1} + 2\text{ ADP} + \text{Pi}$$

Why store glycogen as a fuel reserve?

As such, fat is the fuel reserve of the body. However, fat is not preferred, instead glycogen is chosen for a routine, and day to day use of energy for the following reasons

- Glycogen can be rapidly mobilized
- Glycogen can generate energy in the absence of oxygen
- Brain depends on continuous glucose supply (which mostly comes from glycogen.)

On the other hand, fat mobilization is slow, needs O_2 for energy production and cannot produce glucose (to a significant extent). Thus, fat may be considered as a fixed deposit while glycogen is in the current/saving account in a bank!

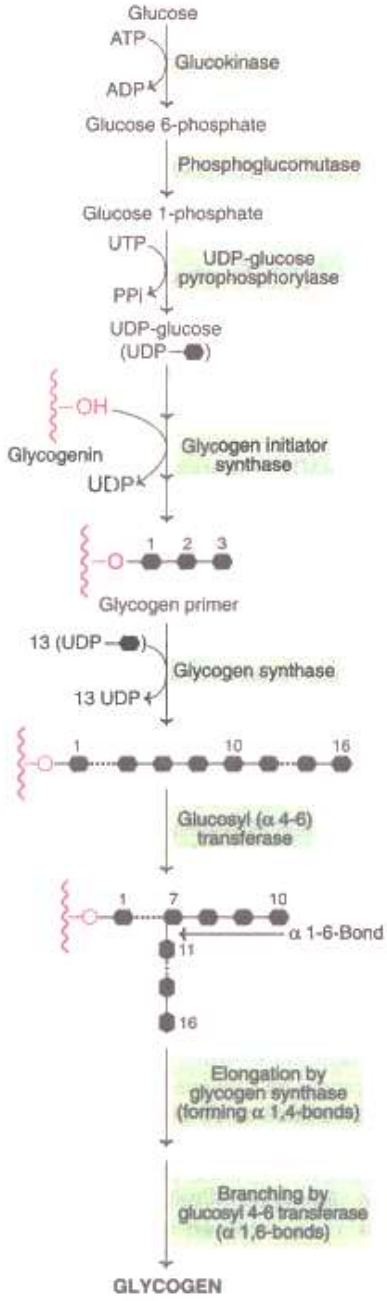
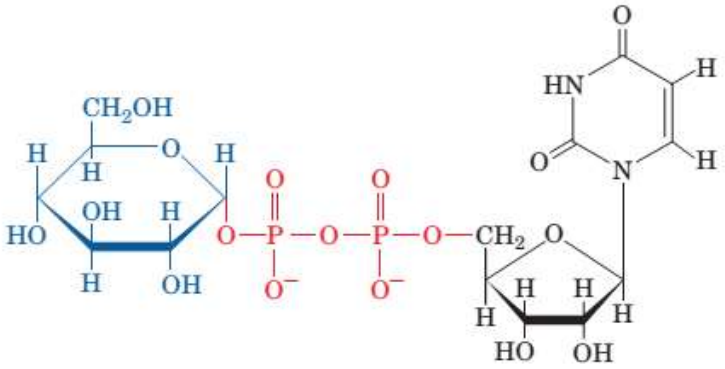
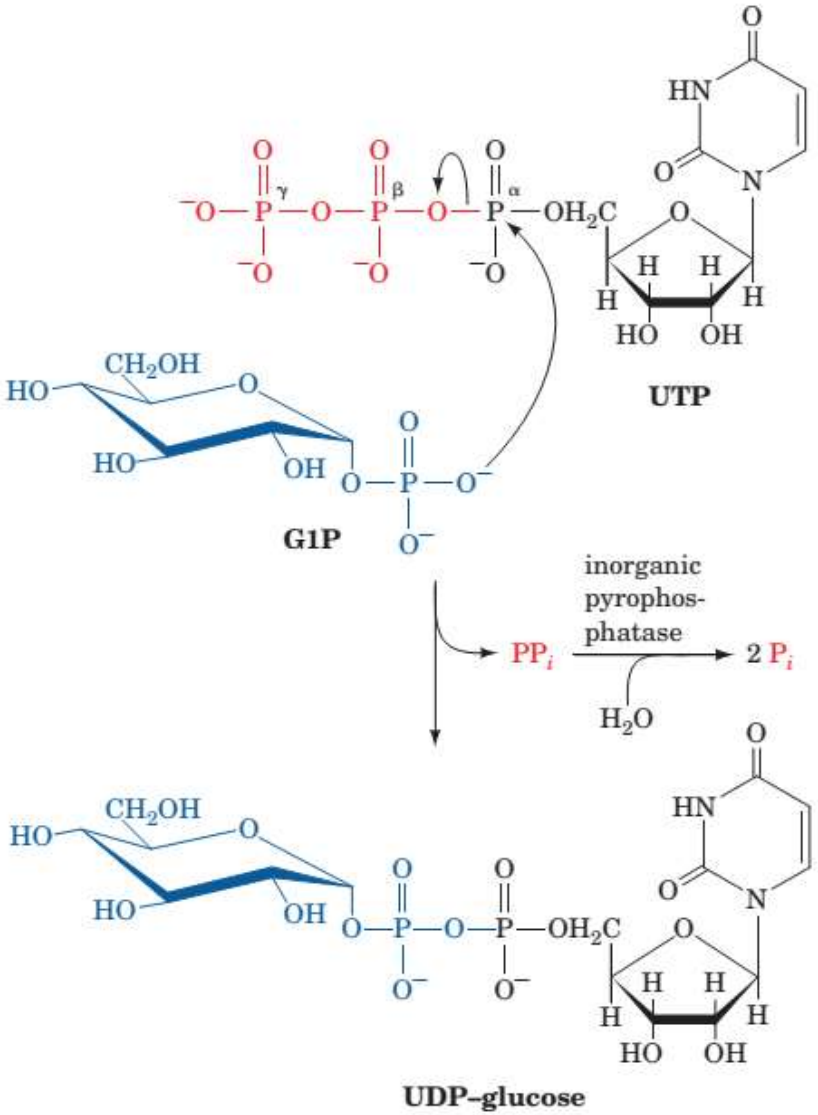


Fig. 13.14 : Glycogen synthesis from glucose (glycogenesis).

1- Synthesis of UDP-glucose

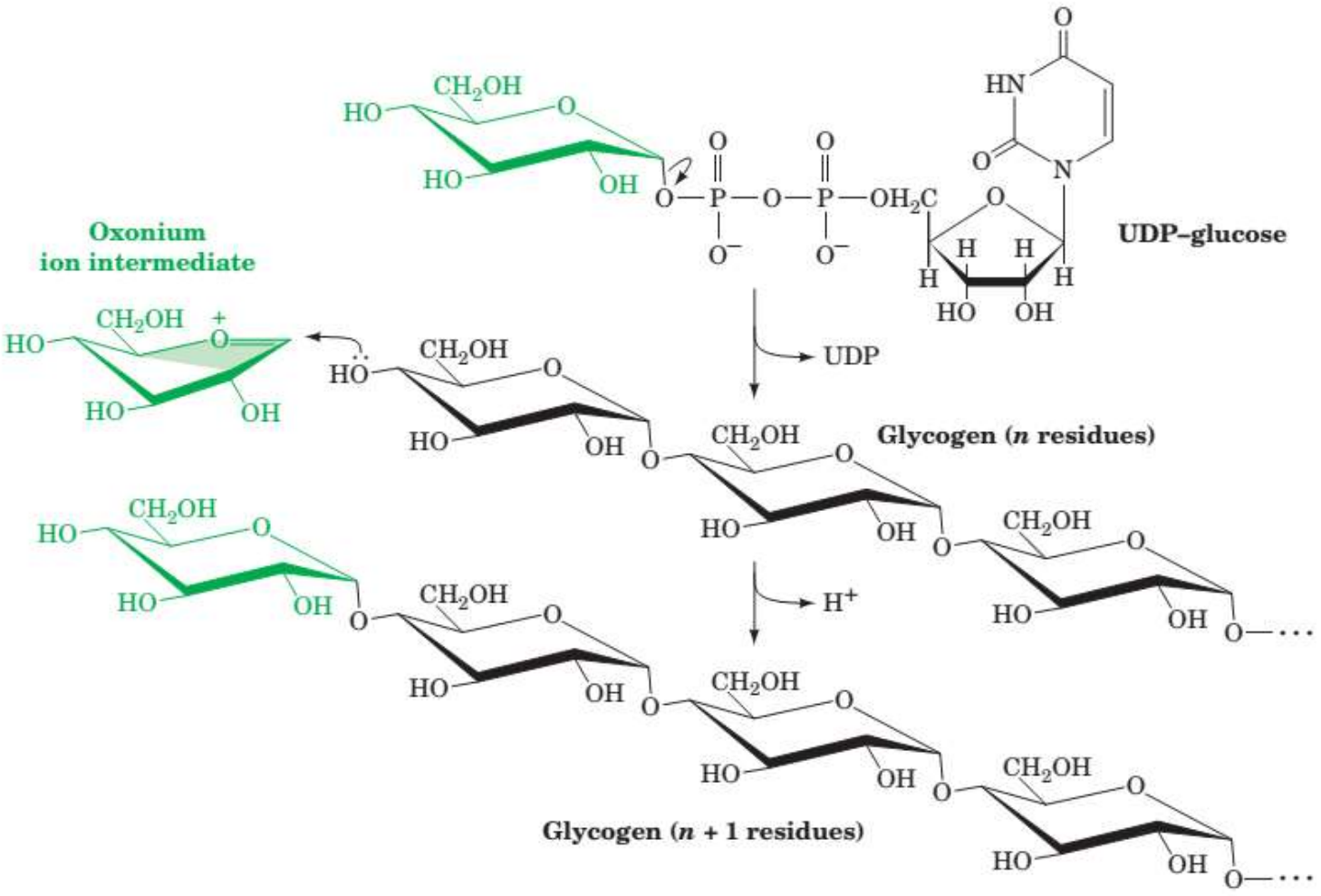


Uridine diphosphate glucose (UDPG)



	$\Delta G^{\circ\prime}$ (kJ · mol^{-1})
G1P + UTP $\rightleftharpoons$ UDPG + PP_i	~0
H_2O + PP_i $\rightleftharpoons$ $2P_i$	−19.2
Overall: G1P + UTP $\rightleftharpoons$ UDPG + $2P_i$	−19.2

2-Glycogen synthesis by Glycogensynthase



3- Formation of branches in glycogen

Why store glycogen as a fuel reserve?

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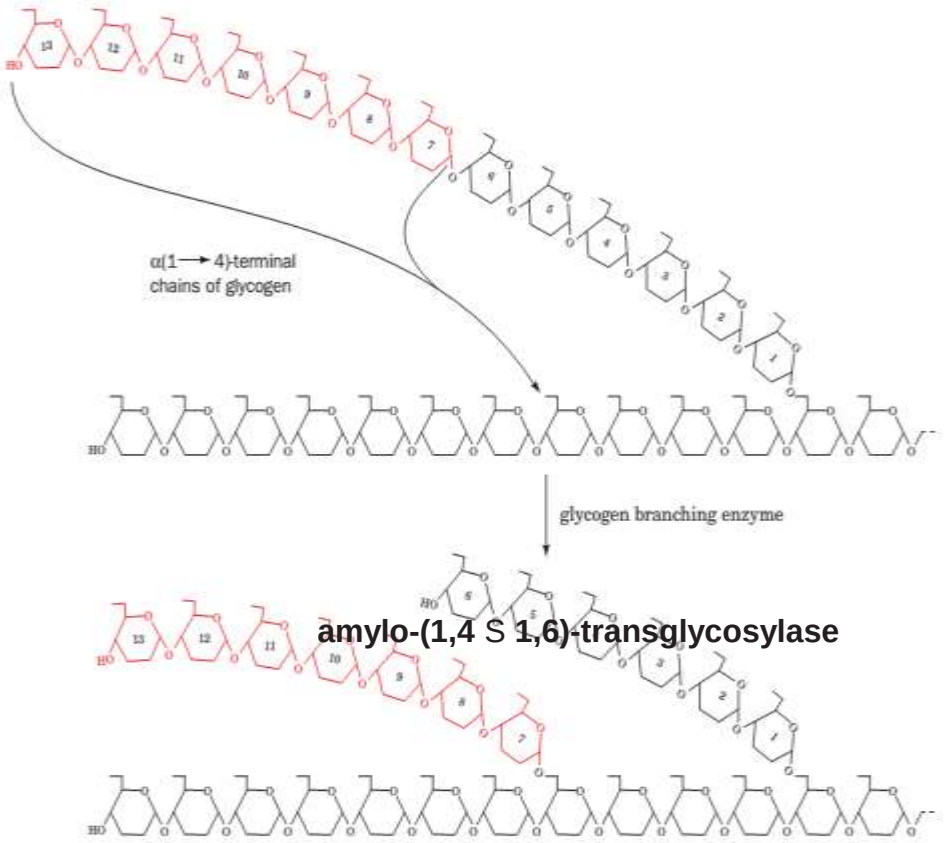
- Glycogen can be rapidly mobilized
- Glycogen can generate energy in the absence of oxygen
- Brain depends on continuous glucose supply (which mostly comes from glycogen.)

On the other hand, fat mobilization is slow, needs O_2 for energy production and cannot produce glucose (to a significant extent). Thus, fat may be considered as a fixed deposit while glycogen is in the current/saving account in a bank!

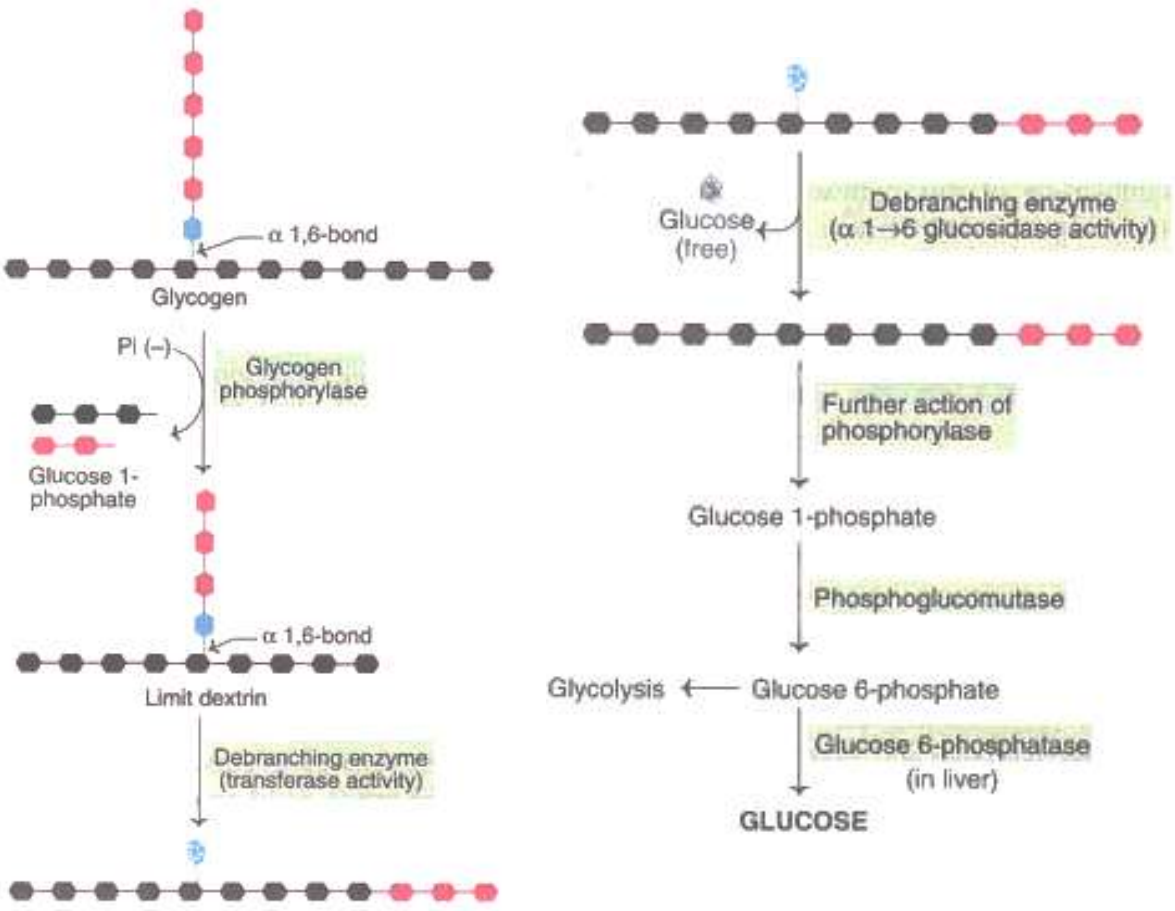
$$(Glucose)_n + Glucose + 2ATP \longrightarrow (Glucose)_{n+1} + 2\ ADP + Pi$$



Gycogenolysis

Glycogen Breakdown

A. Glycogen Phosphorylase
B. Phosphoglucomutase
C. Glycogen Debranching Enzyme



The ***degradation of stored glycogen*** in liver and muscle constitutes glycogenolysis. The pathways for the synthesis and degradation of glycogen are not reversible. An independent set of enzymes present in the cytosol carry out glycogenolysis. Glycogen is degraded by breaking α-1,4- and α-1,6-glycosidic bonds (***Fig.13.15***).

1. **Action of glycogen phosphorylase :** The α-1,4-glycosidic bonds (from the non-reducing ends) are cleaved sequentially by the enzyme glycogen phosphorylase to yield glucose 1-phosphate. This process—called ***phosphorolysis***—continues until four glucose residues remain on either side of branching point (α-1,6-glycosidic link). The glycogen so formed is known as ***limit dextrin*** which cannot be further degraded by phosphorylase. Glycogen phosphorylase possesses a molecule of pyridoxal phosphate, covalently bound to the enzyme.

2. **Action of debranching enzyme :** The branches of glycogen are cleaved by two enzyme activities present on a single polypeptide called ***debranching enzyme,*** hence it is a ***bifunctional enzyme.***

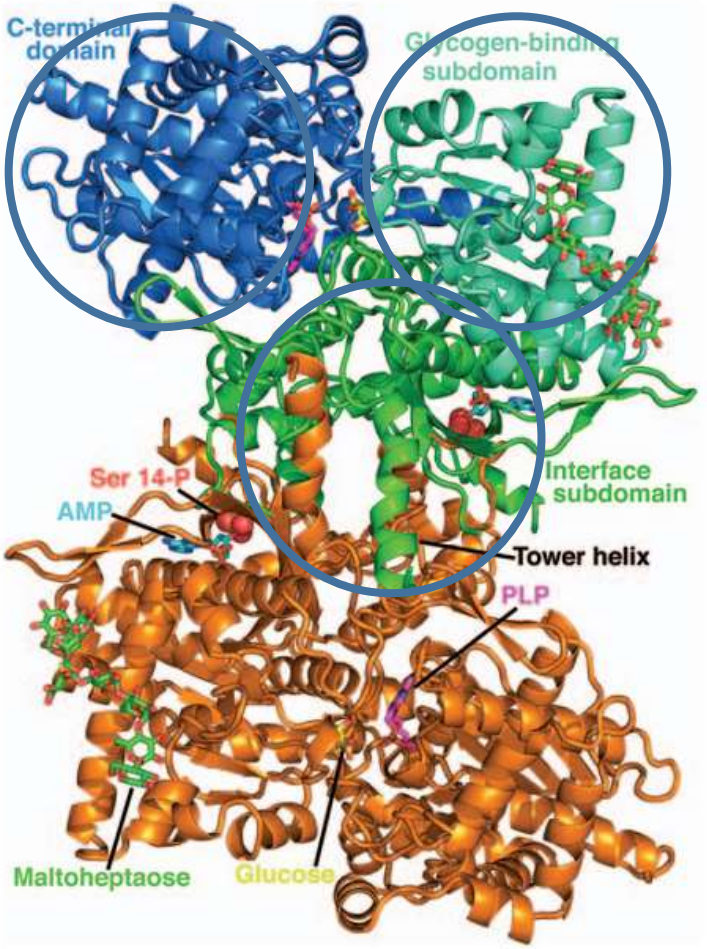
Glycosyl 4 : 4 transferase (oligo α-1,4 → 1,4 glucan transferase) activity removes a fragment of three or four glucose residues attached at a branch and transfers them to another chain. Here, one α-1,4-bond is cleaved and the same α-1,4 bond is made, but the places are different.

Amylo α-1,6-glucosidase breaks the α-1,6 bond at the branch with a single glucose residue and ***releases a free glucose.***

The remaining molecule of glycogen is again available for the action of phosphorylase and debranching enzyme to repeat the reactions stated in 1 and 2.

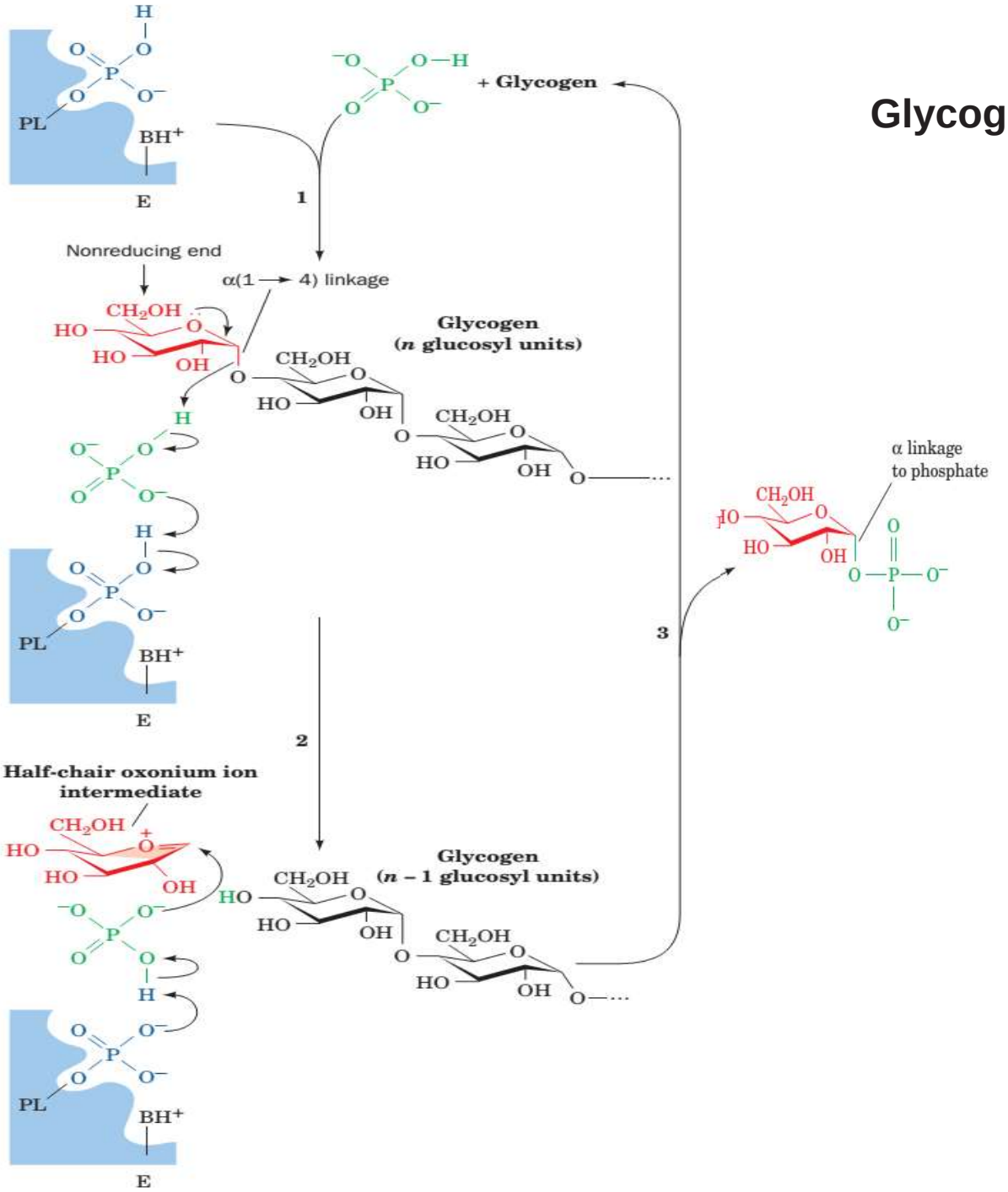
Glycogen Phosphorylase

Figure 18-2 **X-ray structure of rabbit muscle glycogen phosphorylase *a*.** The homodimeric enzyme is drawn in ribbon form and viewed along its 2-fold axis. Each subunit consists of an N-terminal domain, which is subdivided into an interface subdomain (residues 1–315) and a glycogen-binding subdomain (residues 316–484), and a C-terminal domain (residues 485–842). The enzyme's several ligands are drawn in stick form colored according to type with N blue, O red, P orange, and C atoms as indicated. The active site is marked by a bound glucose molecule (C yellow). Pyridoxal phosphate (PLP) is covalently linked to the side chain of Lys 678 in the C-terminal domain (C magenta). In addition, the enzyme binds its allosteric effector AMP (C cyan) and **maltoheptaose** (C green), an $\alpha(1 \rightarrow 4)$-linked glucose heptamer, which is bound in the enzyme's glycogen storage site. Ser 14-P, the phosphoryl group on Ser 14, is drawn in space-filling form. [X-ray structure coordinates courtesy of Stephen Sprang, University of Texas Southwest Medical Center.] **See Kinemage Exercise 14-1**

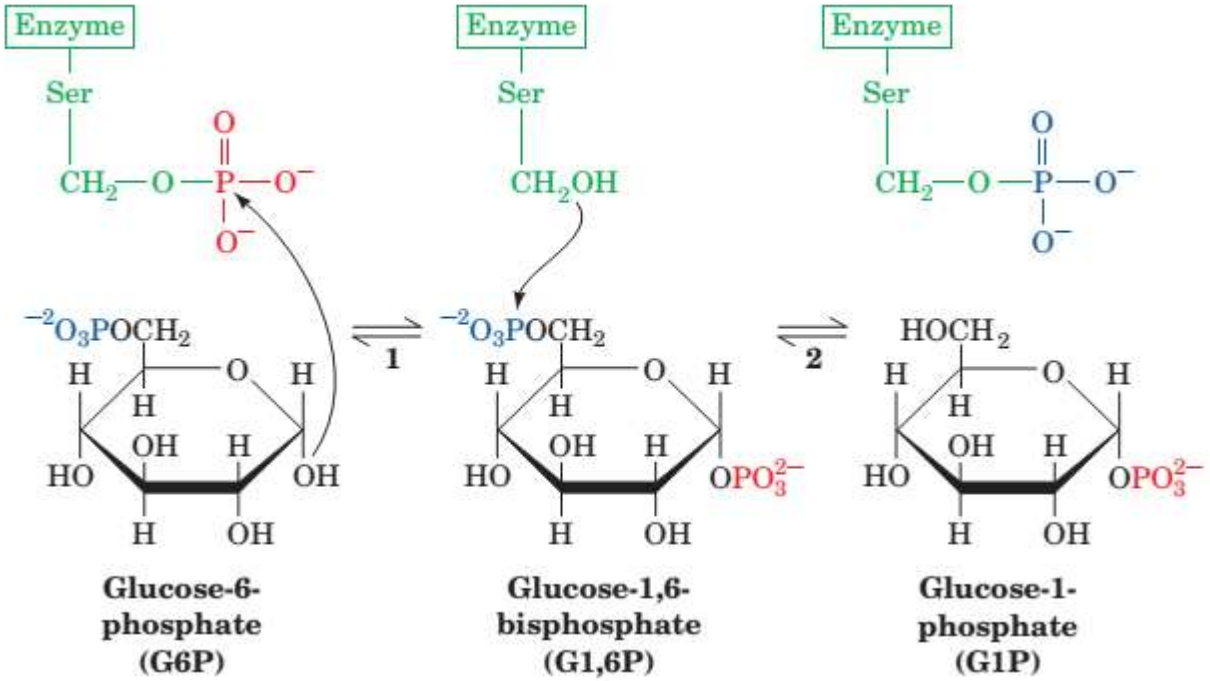


- Glycogen phosphorylase is a dimer of identical 842-residue (97-kD) subunits that catalyzes the controlling step in glycogen breakdown.
- It is regulated both by allosteric interactions and by covalent modification.
- *The enzyme-catalyzed modification/demodification process yields two forms of phosphorylase:* ***phosphorylase a, which has a phosphoryl group esterified to Ser 14 in each of its subunits, and phosphorylase b, which lacks these phosphoryl groups.***

Glycogen Phosphorylase



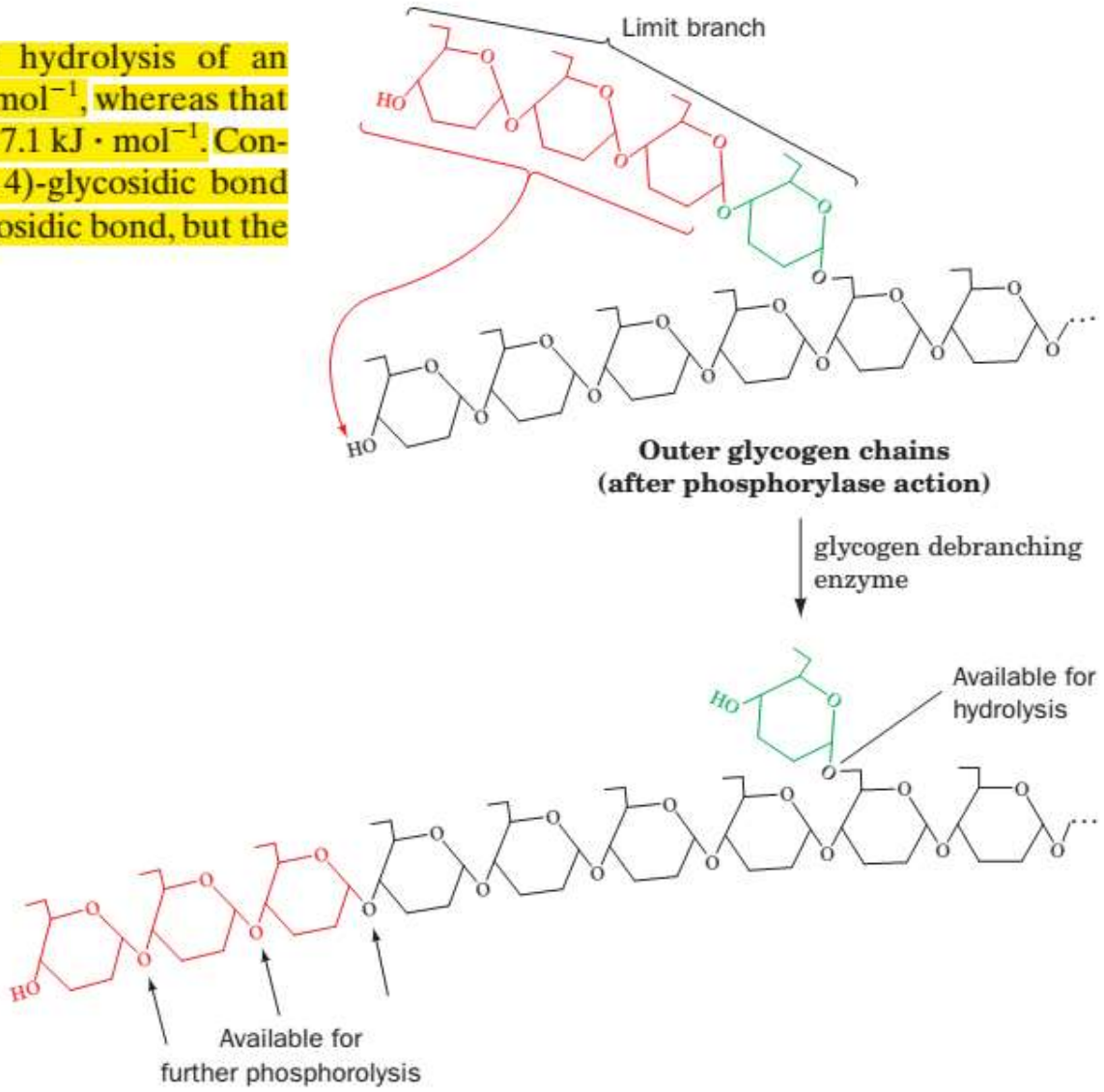
2. *Phosphoglucomutase*



G1,6P occasionally dissociates from phosphoglucomutase, resulting in the inactivation of this enzyme. The presence of small amounts of G1,6P is therefore necessary to keep phosphoglucomutase fully active. This intermediate is provided by **phosphoglucokinase,** which catalyzes the phosphorylation of the C6—OH group of G1P by ATP.

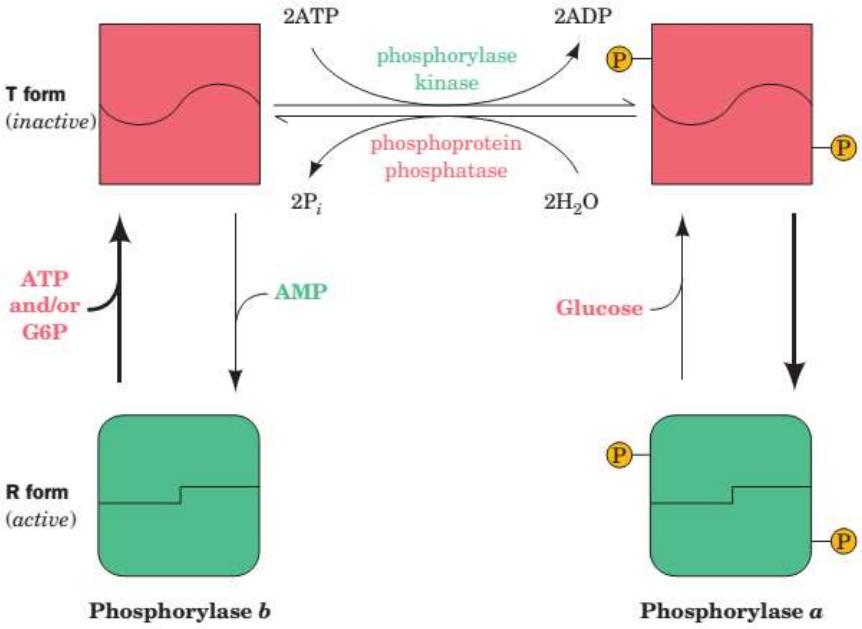
Glycogen Debranching Enzyme

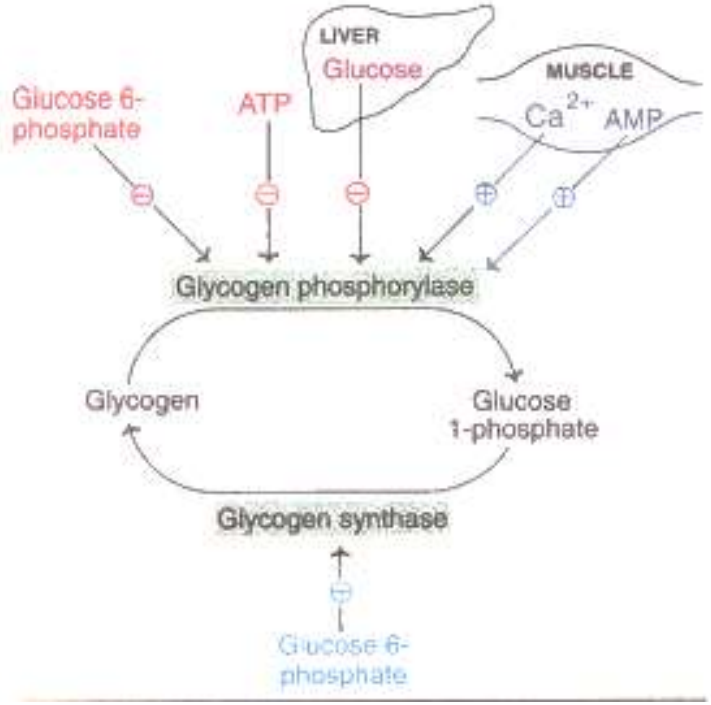
these reactions. The free energy of hydrolysis of an $\alpha(1 \rightarrow 4)$-glycosidic bond is $-15.5\ kJ \cdot mol^{-1}$, whereas that of an $\alpha(1 \rightarrow 6)$-glycosidic bond is only $-7.1\ kJ \cdot mol^{-1}$. Consequently, the hydrolysis of an $\alpha(1 \rightarrow 4)$-glycosidic bond drives the synthesis of an $\alpha(1 \rightarrow 6)$-glycosidic bond, but the reverse reaction is endergonic.



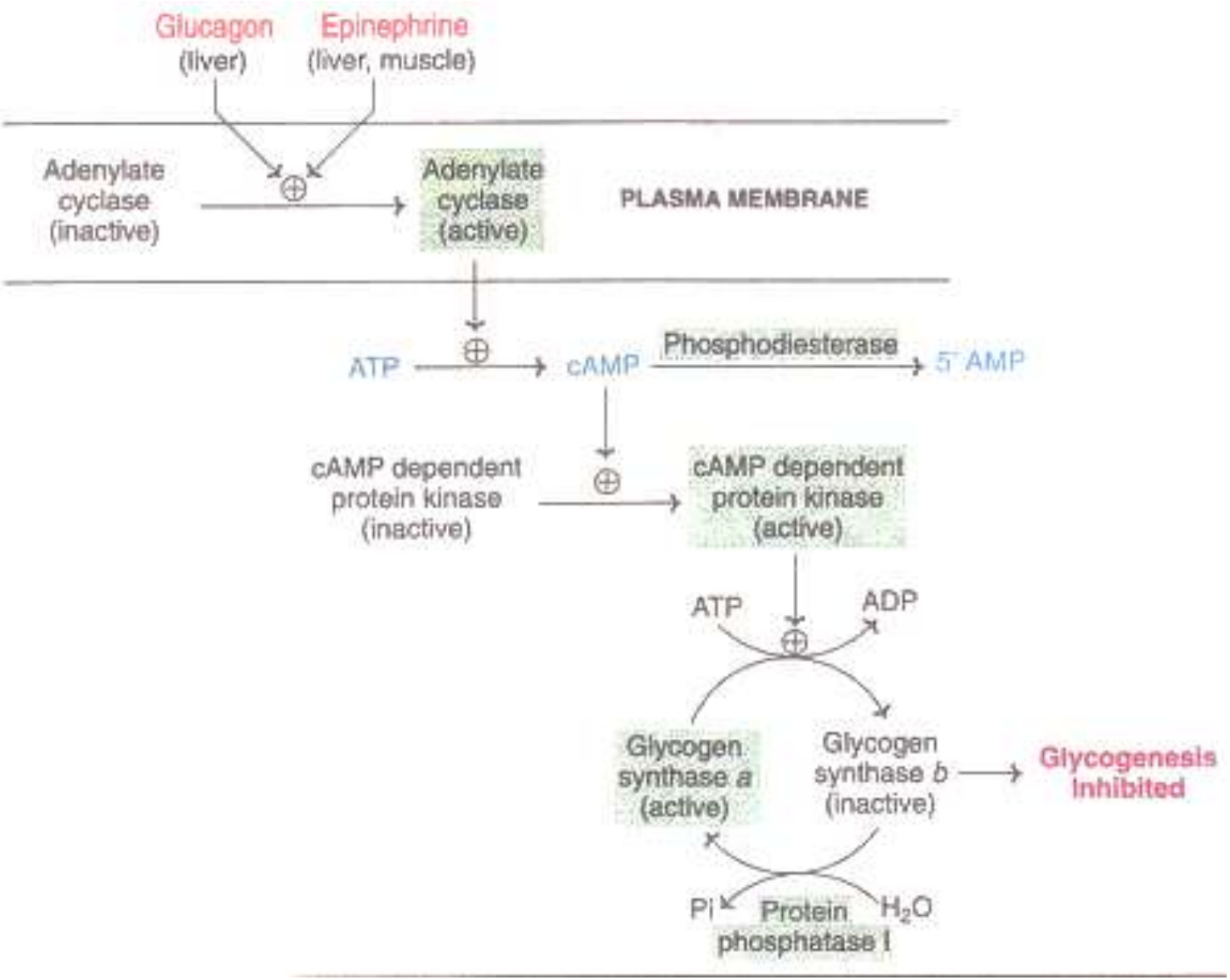
REGULATION OF GLYCOGEN METABOLISM

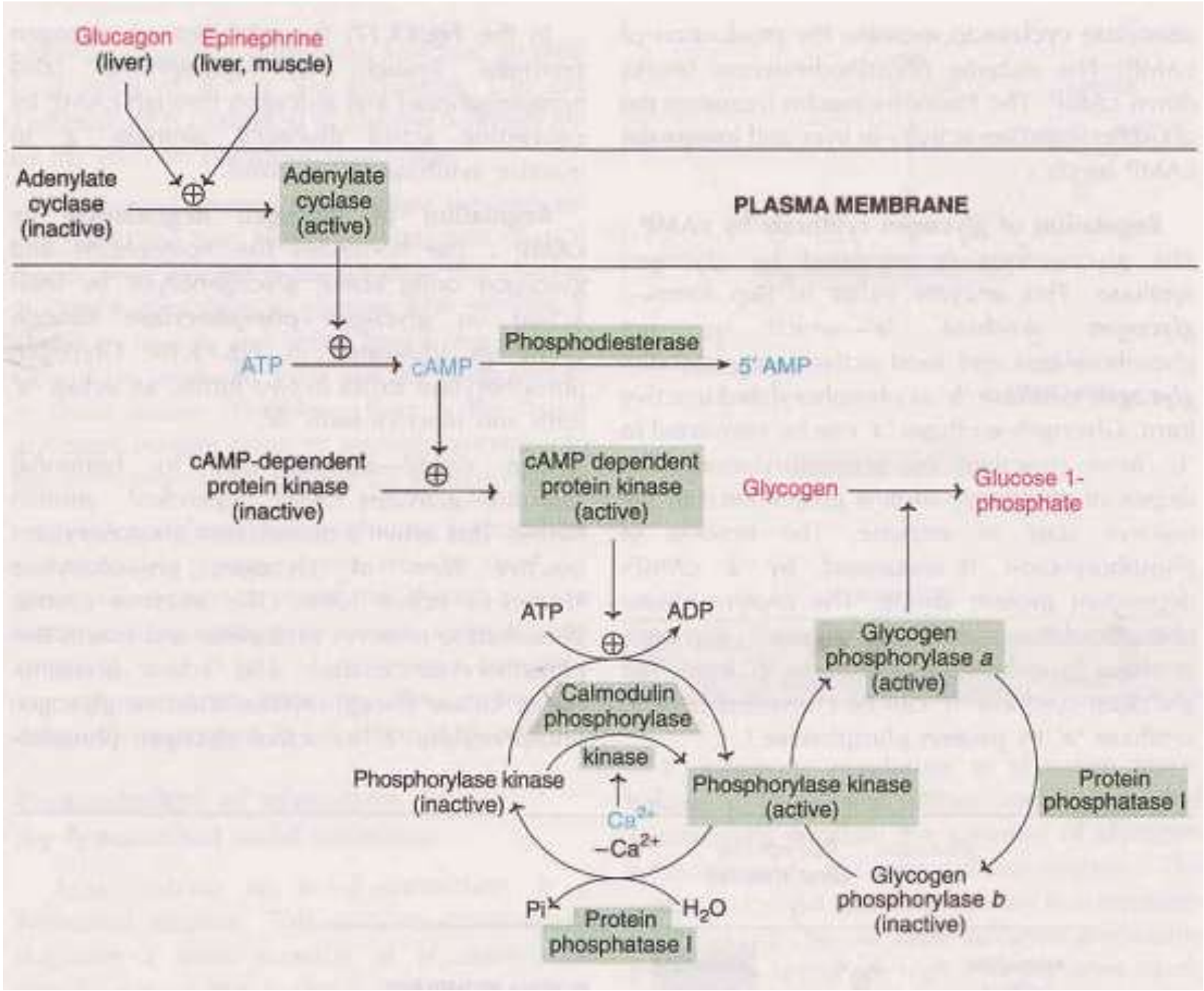
1. Allosteric Regulation





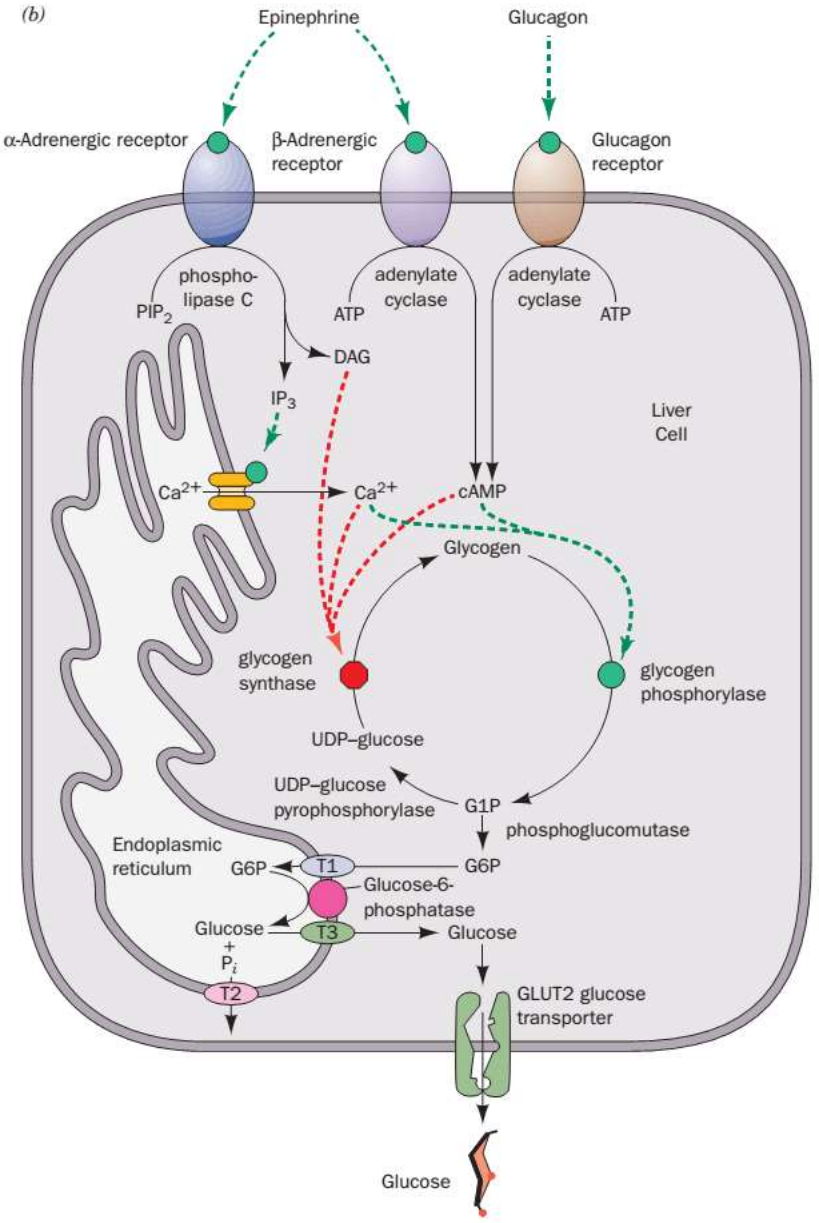
2- Hormonal Regulation




Glucagon (liver)
Epinephrine (liver, muscle)
Adenylate cyclase (inactive)
Adenylate cyclase (active)
PLASMA MEMBRANE
ATP
cAMP
Phosphodiesterase
5' AMP
cAMP-dependent protein kinase (inactive)
cAMP dependent protein kinase (active)
Glycogen
Glucose 1-phosphate
ATP
ADP
Calmodulin
Glycogen phosphorylase a (active)
phosphorylase kinase
Phosphorylase kinase (inactive)
Phosphorylase kinase (active)
Protein phosphatase I
Ca^{2+}
$-Ca^{2+}$
Glycogen phosphorylase b (inactive)
Pi
H_2O
Protein phosphatase I

3- Regulation by Ca^{++}

Figure 18-27 **The liver's response to stress.** (*a*) Stimulation of α-adrenergic receptors by epinephrine activates phospholipase C to hydrolyze phosphatidylinositol-4,5-bisphosphate (PIP_2) to inositol-1,4,5-trisphosphate (IP_3) and diacylglycerol (DAG). (*b*) The participation of two second messenger systems: the cAMP-mediated stimulation of glycogenolysis and inhibition of glycogen synthesis triggered by glucagon and β-adrenergic receptor activation; and the IP_3, DAG, and Ca^{2+}-mediated stimulation of glycogenolysis and inhibition of glycogen synthesis triggered by α-adrenergic receptor activation. IP_3 stimulates the release of Ca^{2+} from the endoplasmic reticulum, whereas DAG, together with Ca^{2+}, activates protein kinase C to phosphorylate and thereby inactivate glycogen synthase. G6Pase occupies the endoplasmic reticulum. Consequently, the cytosolically produced G6P is transported into the endoplasmic reticulum via the **T1 G6P translocase,** where it is hydrolyzed to glucose and P_i. The P_i and glucose are then returned to the cytosol by the **T2** and **T3 transporters,** respectively, and the glucose is exported from the cell via the GLUT2 glucose transporter.



GLYCOGEN STORAGE DISEASES

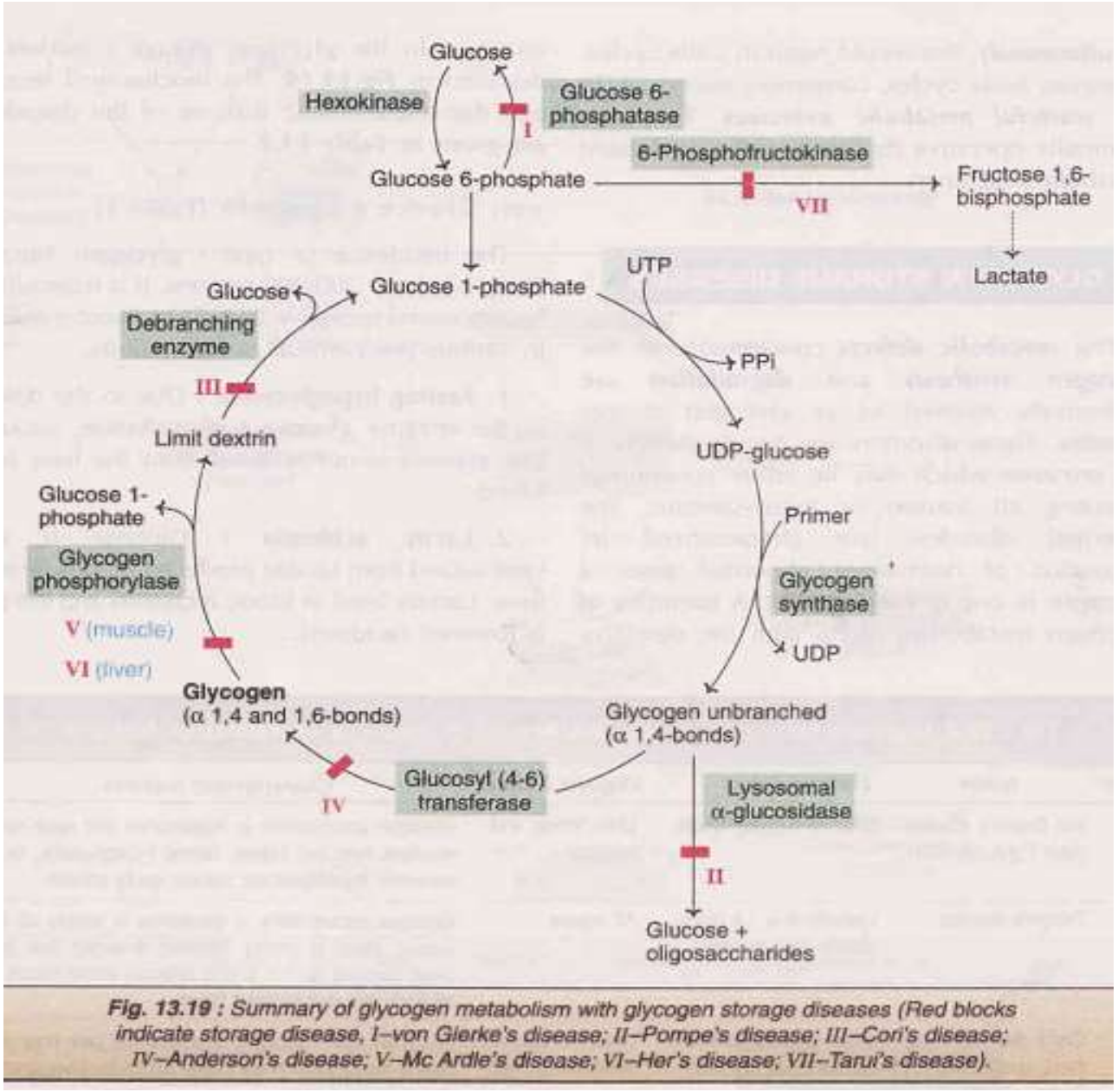


Fig. 13.19 : *Summary of glycogen metabolism with glycogen storage diseases (Red blocks indicate storage disease, I–von Gierke's disease; II–Pompe's disease; III–Cori's disease; IV–Anderson's disease; V–Mc Ardle's disease; VI–Her's disease; VII–Tarui's disease).*

TABLE 13.2 Glycogen storage diseases – biochemical lesions and characteristic features

Type	*Name*	*Enzyme defect*	*Organ(s) involved*	*Characteristic features*
I	von Gierke's disease (type I glycogenosis)	Glucose 6-phosphatase	Liver, kidney and intestine	Glycogen accumulates in hepatocytes and renal cells, enlarged liver and kidney, fasting hypoglycemia, lactic acidemia; hyperlipidemia; ketosis; gouty arthritis.
II	Pompe's disease	Lysosomal α-1,4 glucosidase (acid maltase)	All organs	Glycogen accumulates in lysosomes in almost all the tissues; heart is mostly involved; enlarged liver and heart, nervous system is also affected; death occurs at an early age due to heart failure.
III	Cori's disease (limit dextrinosis, Forbe's disease)	Amylo α-1,6-glucosidase (debranching enzyme)	Liver, muscle, heart, leucocytes	Branched chain glycogen accumulates; liver enlarged; clinical manifestations are similar but milder compared to von Gierke's disease.
IV	Anderson's disease (amylopectinosis)	Glucosyl 4-6 transferase (branching enzyme)	Most tissues	A rare disease, glycogen with only few branches accumulate; cirrhosis of liver, impairment in liver function.
V	McArdle's disease (type V glycogenosis)	Muscle glycogen phosphorylase	Skeletal muscle	Muscle glycogen stores very high, not available during exercise; subjects cannot perform strenous exercise; suffer from muscle cramps; blood lactate and pyruvate do not increase after exercise; muscles may get damaged due to inadequate energy supply.
VI	Her's disease	Liver glycogen phosphorylase	Liver	Liver enlarged; liver glycogen cannot form glucose (pyruvate and lactate can be precursors for glucose); mild hypoglycemia and ketosis seen, not a very serious disease.
VII	Tarui's disease	Phosphofructokinase	Skeletal muscle, erythrocytes	Muscle cramps due to exercise; blood lactate not elevated; hemolysis occurs.

Rare glycogen disorders VIII, IX, X and XI have been identified. They are due to defects in the enzymes concerned with activating and deactivating liver phosphorylase.

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2. Donald Voet, Judith G. Voet “Biochemistry”, 4th Edition, John Wiley & Sons, Inc.,2010. ISBN: 978-0-470-57095-1
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METABOLISM OF CARBOHYDRATES - 4

TEJASVI NAVADHITAMASTU

"Let our (the teacher and the taught) learning be radiant"

Let our efforts at learning be luminous and filled with joy, and endowed with the force of purpose

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E – Content

Course: M.Sc.
Subject: Biochemistry; Biotechnology
Topic: Metabolism of Carbohydrates
Subtopic: Gluconeogenesis, HMP-Shunt, Lactose Metabolism
Prepared by: Prof. Rajesh Sharma
Department : Biotechnology
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GLUCONEOGENESIS

The synthesis of glucose from non-carbohydrate compounds is known as gluconeogenesis. The major ***substrates***/precursors for gluconeogenesis are ***lactate***, ***pyruvate***, ***glucogenic amino acids***, ***propionate*** and ***glycerol.***

Importance of gluconeogenesis

Glucose occupies a key position in the metabolism and its continuous supply is absolutely essential to the body for a variety of functions

1. Brain and central nervous system, erythrocytes, testes and kidney medulla are dependent on glucose for continuous supply of energy. Human brain alone requires about 120 g of glucose per day, out of about 160 g needed by the entire body.

2. Glucose is the only source that supplies energy to the skeletal muscle, under anaerobic conditions.

3. In fasting even more than a day, gluconeogenesis must occur to meet the basal requirements of the body for glucose and to maintain the intermediates of citric acid cycle. This is essential for the survival of humans and other animals.

4. Certain metabolites produced in the tissues accumulate in the blood, e.g. lactate, glycerol, propionate etc. Gluconeogenesis effectively clears them from the blood.

Gluconeogenesis from amino acids

The carbon skeleton of glucogenic amino acids (all except leucine and lysine) results in the formation of pyruvate or the intermediates of citric acid cycle (***Fig.13.11***) which, ultimately, result in the synthesis of glucose.

Gluconeogenesis from glycerol

Glycerol is liberated mostly in the adipose tissue by the hydrolysis of fats (triacylglycerols). The enzyme glycerokinase (found in liver and kidney, absent in adipose tissue) activates glycerol to glycerol 3-phosphate. The latter is converted to dihydroxyacetone phosphate by glycerol 3-phosphate dehydrogenase. Dihydroxyacetone phosphate is an intermediate in glycolysis which can be conveniently used for glucose production.

Gluconeogenesis from propionate

Oxidation of odd chain fatty acids and the breakdown of some amino acids (methionine, isoleucine) yields a three carbon propionyl CoA. Propionyl CoA carboxylase acts on this in presence of ATP and biotin and converts to methyl malonyl CoA which is then converted to succinyl CoA in presence of B_{12} coenzyme (***Refer Fig.7.38***). Succinyl CoA formed from propionyl CoA enters gluconeogenesis via citric acid cycle.

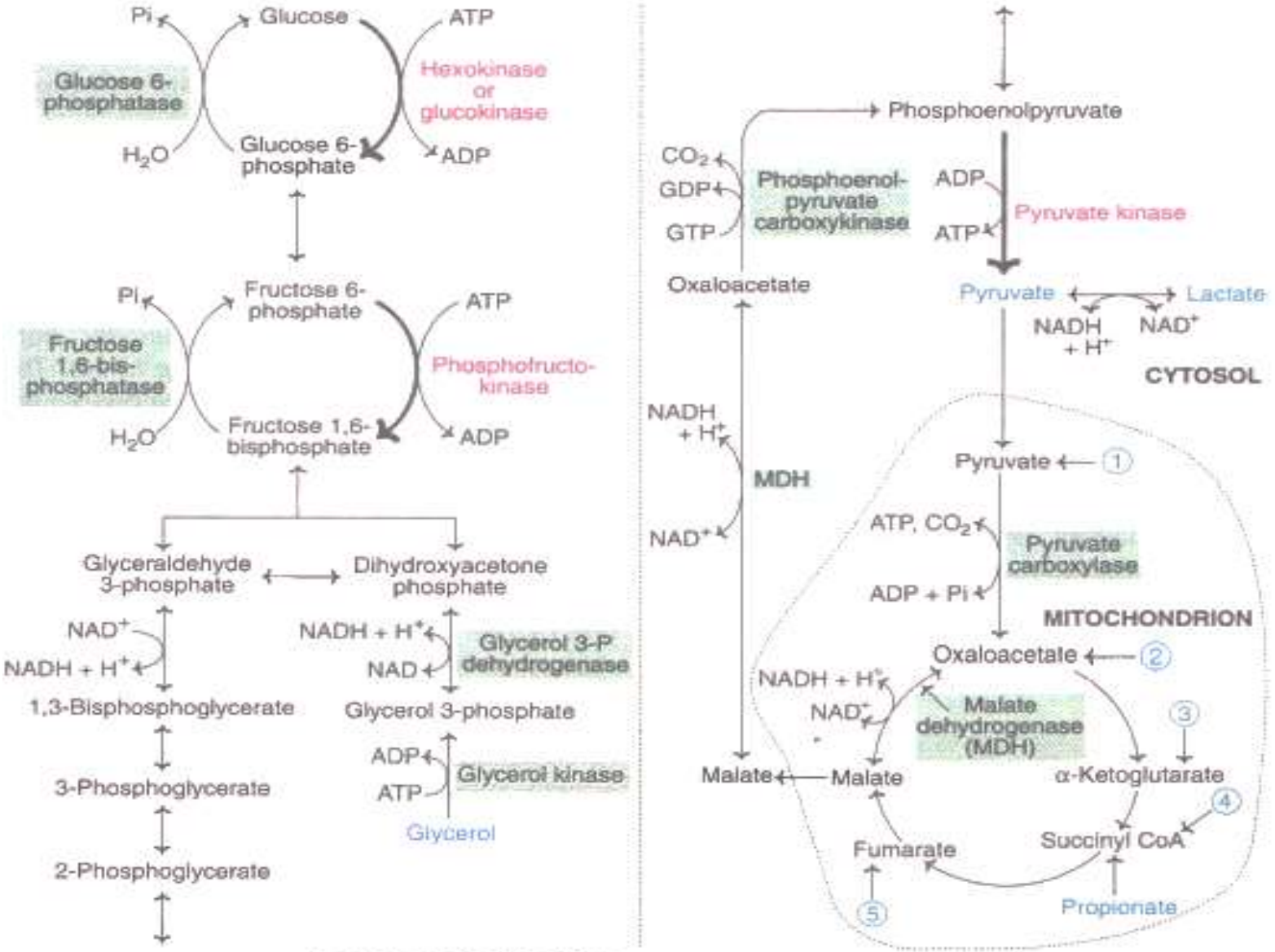


Fig. 13.11 contd. next column

Fig. 13.11 : *The pathway of gluconeogenesis. [The enzymes catalysing irreversible steps in glycolysis are shown in red. The important enzymes participating in gluconeogenesis are shown in shaded green. The substrates for gluconeogenesis are in blue. The numbers represent the entry of glucogenic amino acids : (1) Alanine, glycine, serine, cysteine, threonine and tryptophan; (2) Aspartate and asparagine; (3) Arginine, glutamate, glutamine, histidine, proline; (4) Isoleucine, methionine, valine; (5) Phenylalanine, tyrosine].*

Gluconeogenesis from lactate (Cori cycle)

Lactate produced by active skeletal muscle is a major precursor for gluconeogenesis. Under anaerobic conditions, pyruvate is reduced to lactate by lactate dehydrogenase (LDH)

$$\text{Pyruvate} + \text{NADH} + \text{H}^+ \overset{\text{LDH}}{\rightleftharpoons} \text{Lactate} + \text{NAD}^+$$

Lactate is a dead end in glycolysis, since it must be reconverted to pyruvate for its further metabolism. The very purpose of lactate production is to regenerate NADH so that glycolysis proceeds uninterrupted in skeletal muscle. Lactate or pyruvate produced in the muscle cannot be utilized for the synthesis of glucose due to the absence of the key enzymes of gluconeogenesis (glucose 6-phosphatase and fructose 1,6-bisphosphatase).

The plasma membrane is freely permeable to lactate. Lactate is carried from the skeletal muscle through blood and handed over to liver, where it is oxidized to pyruvate. Pyruvate, so produced, is converted to glucose by gluconeogenesis, which is then transported to the skeletal muscle.

The cycle involving the ***synthesis of glucose in liver from the skeletal muscle lactate*** and the reuse of glucose thus synthesized by the muscle for energy purpose is known as Cori cycle (***Fig.13.13***).

Glucose-alanine cycle

There is a continuous transport of amino acids from muscle to liver, which predominantly occurs during starvation. Alanine dominates among the transported amino acids. It is postulated that pyruvate in skeletal muscle undergoes transamination to produce alanine. Alanine is transported to liver and used for gluconeogenesis. This cycle is referred to as glucose-alanine cycle (***Fig.13.13***).

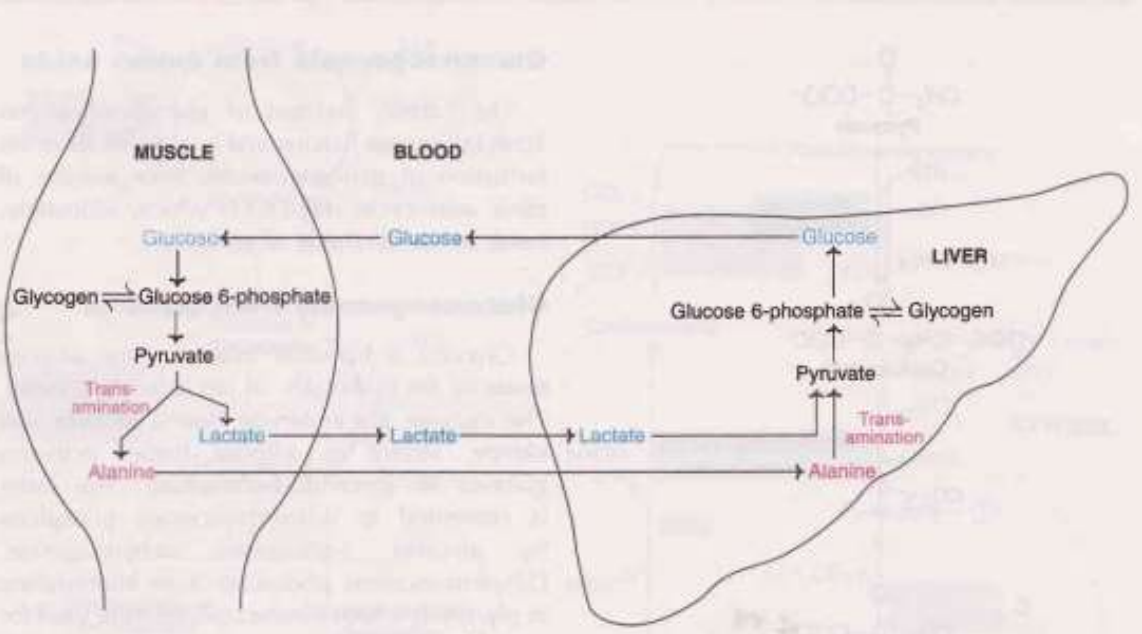


Fig. 13.13 : *The Cori cycle (blue) and glucose-alanine (red) cycle (other reactions common for both cycles).*

Why store glycogen as a fuel reserve?

As such, fat is the fuel reserve of the body. However, fat is not preferred, instead glycogen is chosen for a routine, and day to day use of energy for the following reasons

- Glycogen can be rapidly mobilized
- Glycogen can generate energy in the absence of oxygen
- Brain depends on continuous glucose supply (which mostly comes from glycogen.)

On the other hand, fat mobilization is slow, needs O_2 for energy production and cannot produce glucose (to a significant extent). Thus, fat may be considered as a fixed deposit while glycogen is in the current/saving account in a bank!

Alcohol inhibits gluconeogenesis

Ethanol oxidation in the liver to acetaldehyde by the enzyme alcohol dehydrogenase utilizes NAD^+. The excess NADH produced in the liver interferes with gluconeogenesis as illustrated below.

$$\text{Ethanol} + NAD^+ \longrightarrow \text{Acetaldehyde} + NADH + H^+$$

$$\text{Pyruvate} + NADH + H^+ \leftrightarrow \text{Lactate} + NAD^+$$

$$\text{Oxaloacetate} + NADH + H^+ \leftrightarrow \text{Malate} + NAD^+$$

Regulation of gluconeogenesis

The hormone ***glucagon*** and the availability of substrates mainly regulate gluconeogenesis, as discussed hereunder.

Influence of glucagon : This is a hormone, secreted by α-cells of the pancreatic islets. Glucagon stimulates gluconeogenesis by two mechanisms

1. Active form of pyruvate kinase is converted to inactive form through the mediation of cyclic AMP, brought about by glucagon. ***Decreased pyruvate kinase*** results in the reduced conversion of phosphoenol pyruvate to pyruvate and the former is diverted for the synthesis of glucose.

2. Glucagon reduces the concentration of fructose 2,6-bisphosphate. This compound allosterically inhibits phosphofructokinase and activates fructose 1,6-bisphosphatase, both favour increased gluconeogenesis.

Availability of substrates : Among the various substrates, glucogenic amino acids have stimulating influence on gluconeogenesis. This is particularly important in a condition like diabetes mellitus (decreased insulin level) where amino acids are mobilized from muscle protein for the purpose of gluconeogenesis.

Acetyl CoA promotes gluconeogenesis : During starvation—due to excessive lipolysis in adipose tissue—acetyl CoA accumulates in the liver. Acetyl CoA allosterically activates pyruvate carboxylase resulting in enhanced glucose production.

THE PENTOSE PHOSPHATE PATHWAY

Hexose monophosphate pathway or ***HMP shunt*** is also called ***pentose phosphate pathway*** or phosphogluconate pathway. ***This is an alternative pathway to glycolysis and TCA cycle for the oxidation of glucose.*** However, HMP shunt is more anabolic in nature, since it is concerned with the biosynthesis of NADPH and pentoses.

The enzymes of HMP shunt are located in the ***cytosol***. The tissues such as ***liver, adipose tissue, adrenal gland, erythrocytes, testes*** and ***lactating mammary gland***, are highly active in HMP shunt. Most of these tissues are involved in the biosynthesis of fatty acids and steroids which are dependent on the supply of NADPH.

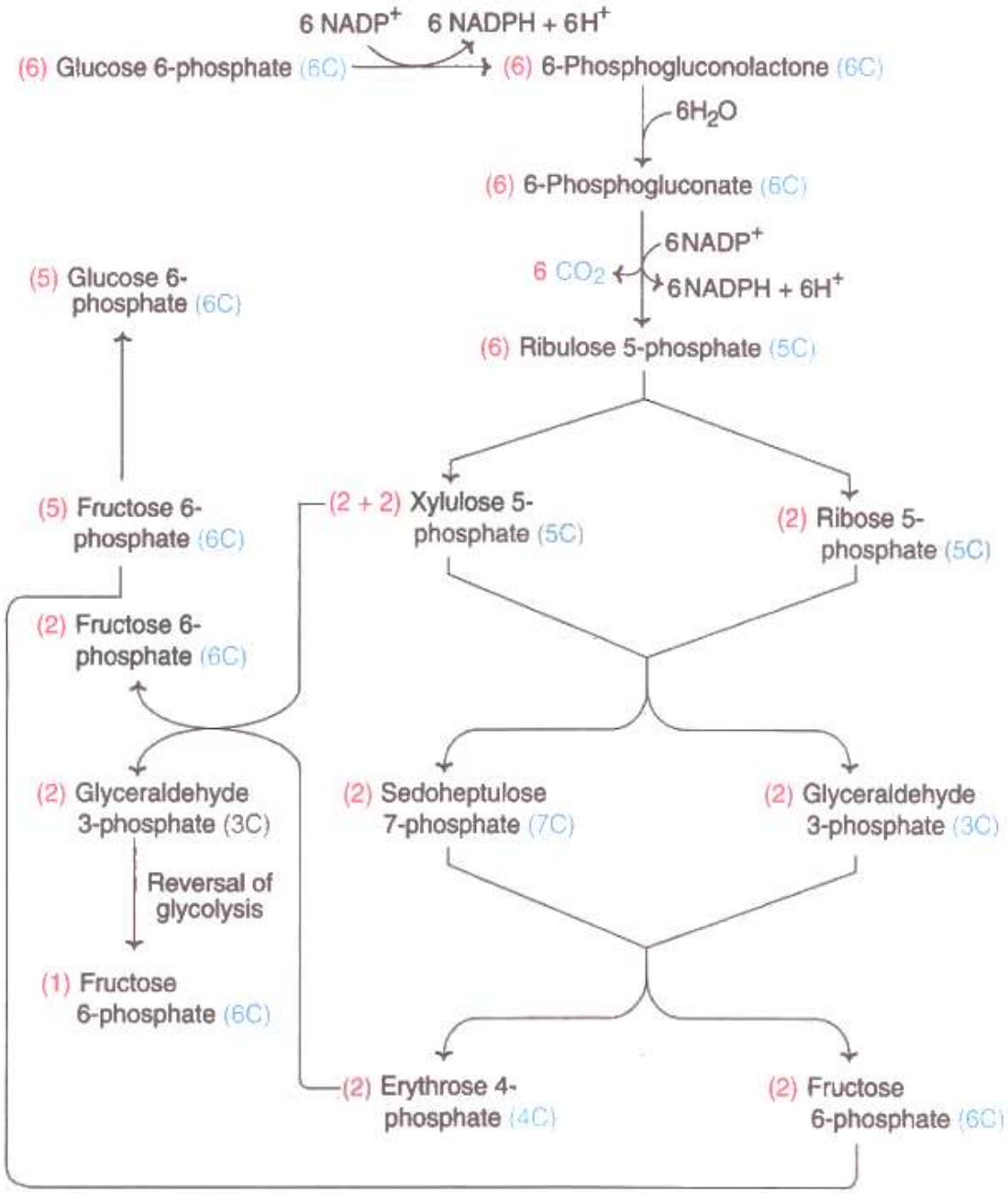
The overall reaction may be represented as

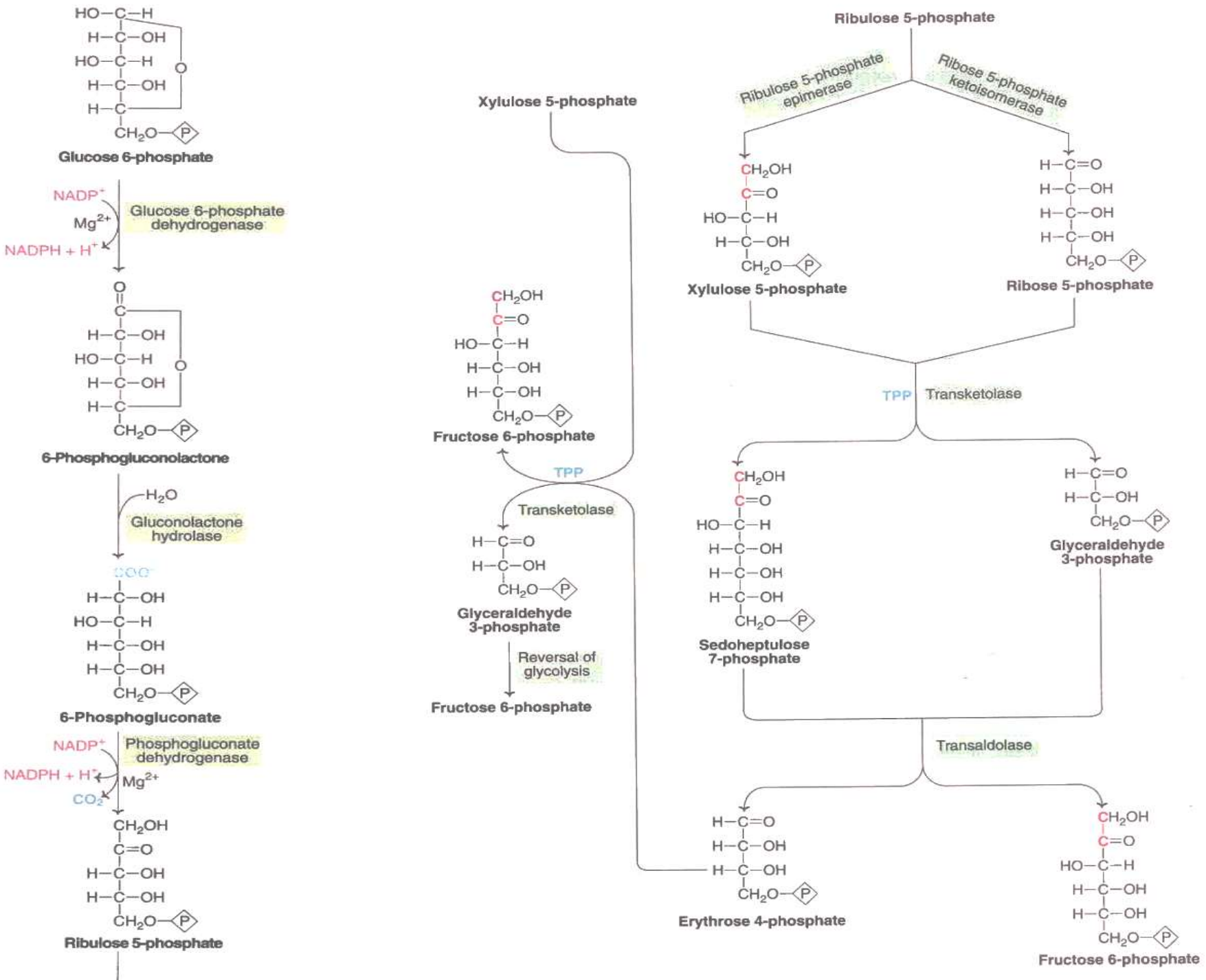
6 Glucose 6-phosphate + 12 $NADP^+$ + $6H_2O$ $\longrightarrow$ $6CO_2$ + 12 NADPH + $12H^+$ + 5 Glucose 6-phosphate.

1. **Oxidative phase :** Glucose 6-phosphate dehydrogenase (G6PD) is an NADP-dependent enzyme that converts glucose 6-phosphate to 6-phosphogluconolactone. The latter is then hydrolysed by the gluconolactone hydrolase to 6-phosphogluconate. The next reaction involving the synthesis of NADPH is catalysed by 6-phosphogluconate dehydrogenase to produce 3 keto 6-phosphogluconate which then undergoes decarboxylation to give ribulose 5-phosphate.

2. **Non-oxidative phase :** The non-oxidative reactions are concerned with the interconversion of three, four, five and seven carbon monosaccharides. Ribulose 5-phosphate is acted upon by an epimerase to produce xylulose 5-phosphate while ribose 5-phosphate ketoisomerase converts ribulose 5-phosphate to ribose 5-phosphate.

G6PD regulates HMP shunt : The first reaction catalysed by G6PD is most regulatory in HMP shunt. This enzyme catalyses an irreversible reaction. NADPH competitively inhibits G6PD. It is the ratio of NADPH/NAD^+ that ultimately determines the flux of this cycle.


6 NADP+
6 NADPH + 6H+
(6) Glucose 6-phosphate (6C)
(6) 6-Phosphogluconolactone (6C)
6H2O
(6) 6-Phosphogluconate (6C)
6NADP+
6 CO2
6NADPH + 6H+
(6) Ribulose 5-phosphate (5C)
(5) Glucose 6-phosphate (6C)
(5) Fructose 6-phosphate (6C)
(2 + 2) Xylulose 5-phosphate (5C)
(2) Ribose 5-phosphate (5C)
(2) Fructose 6-phosphate (6C)
(2) Glyceraldehyde 3-phosphate (3C)
(2) Sedoheptulose 7-phosphate (7C)
(2) Glyceraldehyde 3-phosphate (3C)
Reversal of glycolysis
(1) Fructose 6-phosphate (6C)
(2) Erythrose 4-phosphate (4C)
(2) Fructose 6-phosphate (6C)

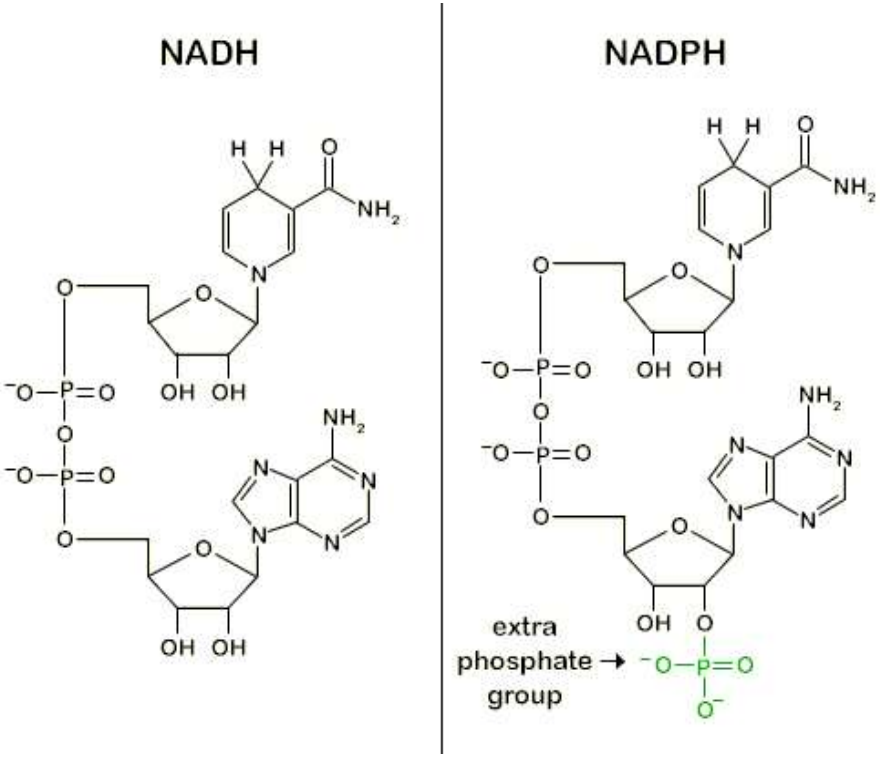

Glucose 6-phosphate
NADP+
Mg2+
NADPH + H+
Glucose 6-phosphate dehydrogenase
6-Phosphogluconolactone
H2O
Gluconolactone hydrolase
6-Phosphogluconate
NADP+
NADPH + H+
Mg2+
CO2
Phosphogluconate dehydrogenase
Ribulose 5-phosphate
Xylulose 5-phosphate
Fructose 6-phosphate
TPP
Transketolase
Glyceraldehyde 3-phosphate
Reversal of glycolysis
Fructose 6-phosphate
Ribulose 5-phosphate
Ribulose 5-phosphate epimerase
Ribose 5-phosphate ketoisomerase
Xylulose 5-phosphate
Ribose 5-phosphate
TPP
Transketolase
Sedoheptulose 7-phosphate
Glyceraldehyde 3-phosphate
Transaldolase
Erythrose 4-phosphate
Fructose 6-phosphate

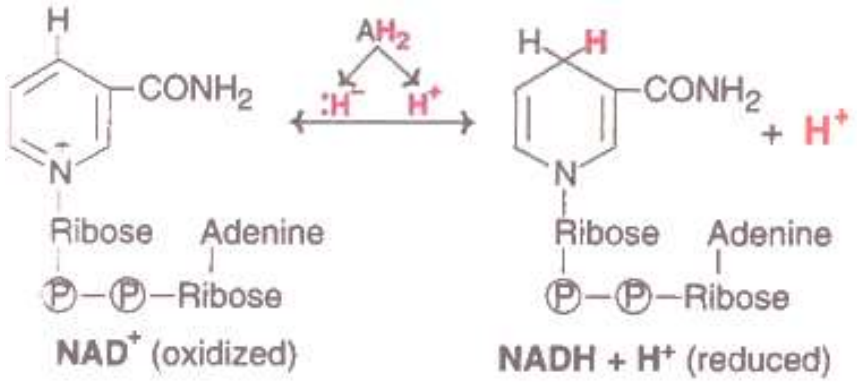
NADH vs. NADPH

NADH provides energy for Catabolic reactions and as for NADPH it provides energy for anabolic reactions

The phosphate group in NADPH doesn't affect the redox abilities of the molecule, it is too far away from the part of the molecule involved in the electron transfer. What the phosphate group does is to allow enzymes to discriminate between NADH and NADPH, which allows the cell to regulate both independently.

The ratio of NAD^+ to NADH inside the cell is high, while the ratio of $NADP^+$ to NADPH is kept low. The role of NADPH is mostly anabolic reactions, where NADPH is needed as a reducing agent, the role of NADH is mostly in catabolic reactions, where NAD^+ is needed as a oxidizing agent.





Significance of HMP shunt

Importance of pentoses

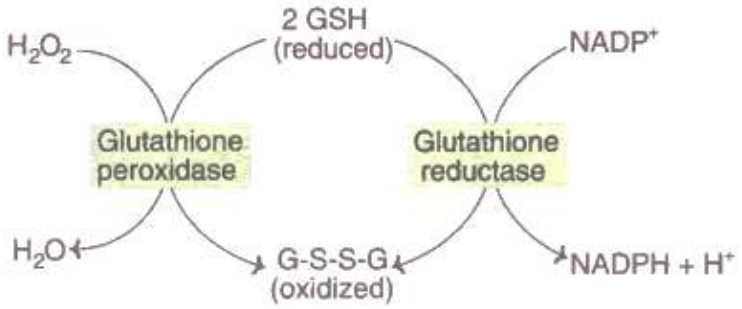
In the HMP shunt, hexoses are converted into pentoses, the most important being ribose 5-phosphate. This pentose or its derivatives are useful for the ***synthesis of nucleic acids*** (RNA and DNA) and many ***nucleotides*** such as ATP, NAD^+, FAD and CoA.

Importance of NADPH

1. NADPH is required for the reductive ***biosynthesis of fatty acids and steroids***, hence HMP shunt is more active in the tissues concerned with lipogenesis, e.g. adipose tissue, liver etc.

2. NADPH is used in the synthesis of certain amino acids involving the enzyme ***glutamate dehydrogenase.***

3. There is a continuous production of H_2O_2 in the living cells which can chemically damage unsaturated lipids, proteins and DNA. This is, however, prevented to a large extent through ***antioxidant reactions*** involving NADPH. Glutathione mediated reduction of H_2O_2 is given in the next column.



Glutathione (reduced, GSH) detoxifies H_2O_2, peroxidase catalyses this reaction. NADPH is responsible for the regeneration of reduced glutathione from the oxidized one.

4. Microsomal cytochrome P_{450} system (in liver) brings about the ***detoxification of drugs*** and foreign compounds by hydroxylation reactions involving NADPH.

5. **Phagocytosis** is the engulfment of foreign particles, including microorganisms, carried out by white blood cells. The process requires the supply of NADPH.

6. **Special functions of NADPH in RBC :** NADPH produced in erythrocytes has special functions to perform. It maintains the concentration of reduced glutathione (reaction explained in 3) which is essentially required to preserve the ***integrity of RBC membrane.*** NADPH is also necessary to keep the ferrous iron (Fe^{2+}) of hemoglobin in the reduced state so that accumulation of methemoglobin (Fe^{3+}) is prevented.

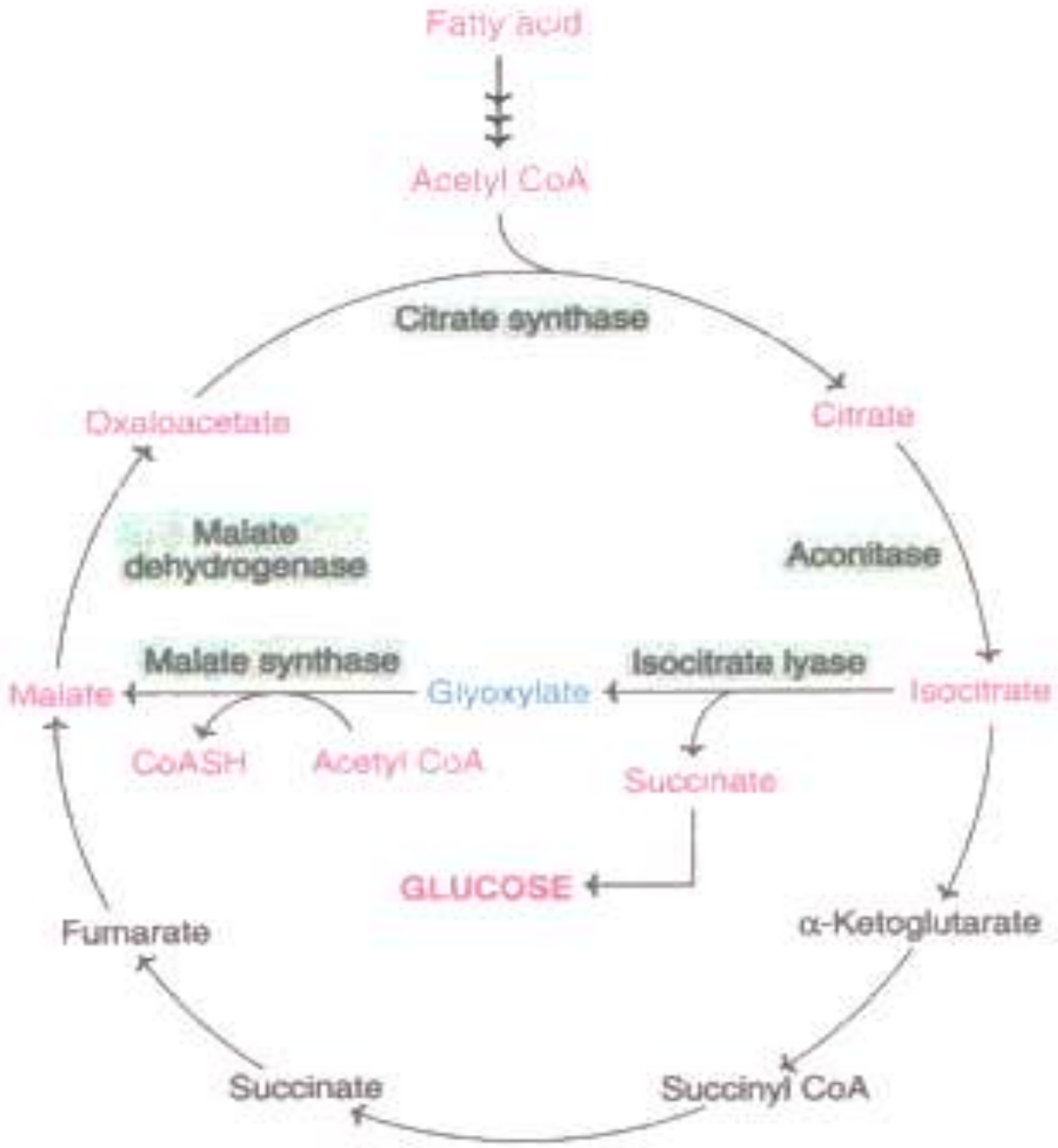
GLYOXYLATE CYCLE

The animals, including man, cannot carry out the net synthesis of carbohydrate from fat. However, the ***plants*** and many ***microorganisms*** are equipped with the metabolic machinery—namely the glyoxylate cycle—***to convert fat into carbohydrates***. This pathway is very significant in the germinating seeds where the stored triacylglycerol (fat) is converted to sugars to meet the energy needs.

Location of the cycle : The glyoxylate cycle occurs in ***glyoxysomes***, specialized cellular organelles, where fatty acid oxidation is also operative.

Reactions of the cycle : The glyoxylate cycle (***Fig.13.26***) is regarded as an anabolic variant of citric acid cycle. Acetyl CoA produced from fatty acid oxidation condenses with oxaloacetate to give citrate which is then converted to isocitrate. At this stage, isocitrate bypasses the citric acid cycle and is cleaved by isocitrate lyase to succinate and glyoxylate. Another molecule of acetyl CoA is now utilized to combine with glyoxylate to form malate. This reaction is catalysed by malate synthase and the malate so formed enters citric acid cycle.

The glyoxylate cycle is a cyclic pathway that results in the conversion of two 2-carbon fragments of acetyl CoA to 4-carbon compound, succinate. The succinate is converted to oxaloacetate and then to glucose involving the reactions of gluconeogenesis.


Fatty acid
Acetyl CoA
Citrate synthase
Oxaloacetate
Citrate
Malate dehydrogenase
Aconitase
Malate synthase
Isocitrate lyase
Malate
Glyoxylate
Isocitrate
CoASH
Acetyl CoA
Succinate
GLUCOSE
α-Ketoglutarate
Fumarate
Succinate
Succinyl CoA

URONIC ACID PATHWAY

This is an alternative oxidative pathway for glucose and is also known as ***glucuronic acid pathway*** (***Fig.13.22***). It is concerned with the synthesis of glucuronic acid, pentoses and vitamin, ascorbic acid (except in primates and guinea pigs). Dietary xylulose enters uronic acid pathway through which it can participate in other metabolisms. In most of the pathways of carbohydrate metabolism, phosphate esters participate, whereas, in uronic acid pathway, the free sugars or sugar acids are involved.

1. **Formation and importance of UDP-glucuronate :** Glucose 6-phosphate is first converted to glucose 1-phosphate. UDP-glucose is then synthesized by the enzyme UDP-glucose pyrophosphorylase. Till this step, the reactions are the same as described in glycogenesis (***Fig.13.14***). UDP-glucose dehydrogenase oxidizes UDP-glucose to UDP-glucuronate.

UDP-glucuronate is the metabolically active form of glucuronate which is utilized for conjugation with many substances like bilirubin, steroid hormones and certain drugs. Several insoluble compounds are converted to soluble ones through conjugation and, further, the drugs are detoxified. UDP-glucuronate is also required for the synthesis of glycosaminoglycans and proteoglycans.

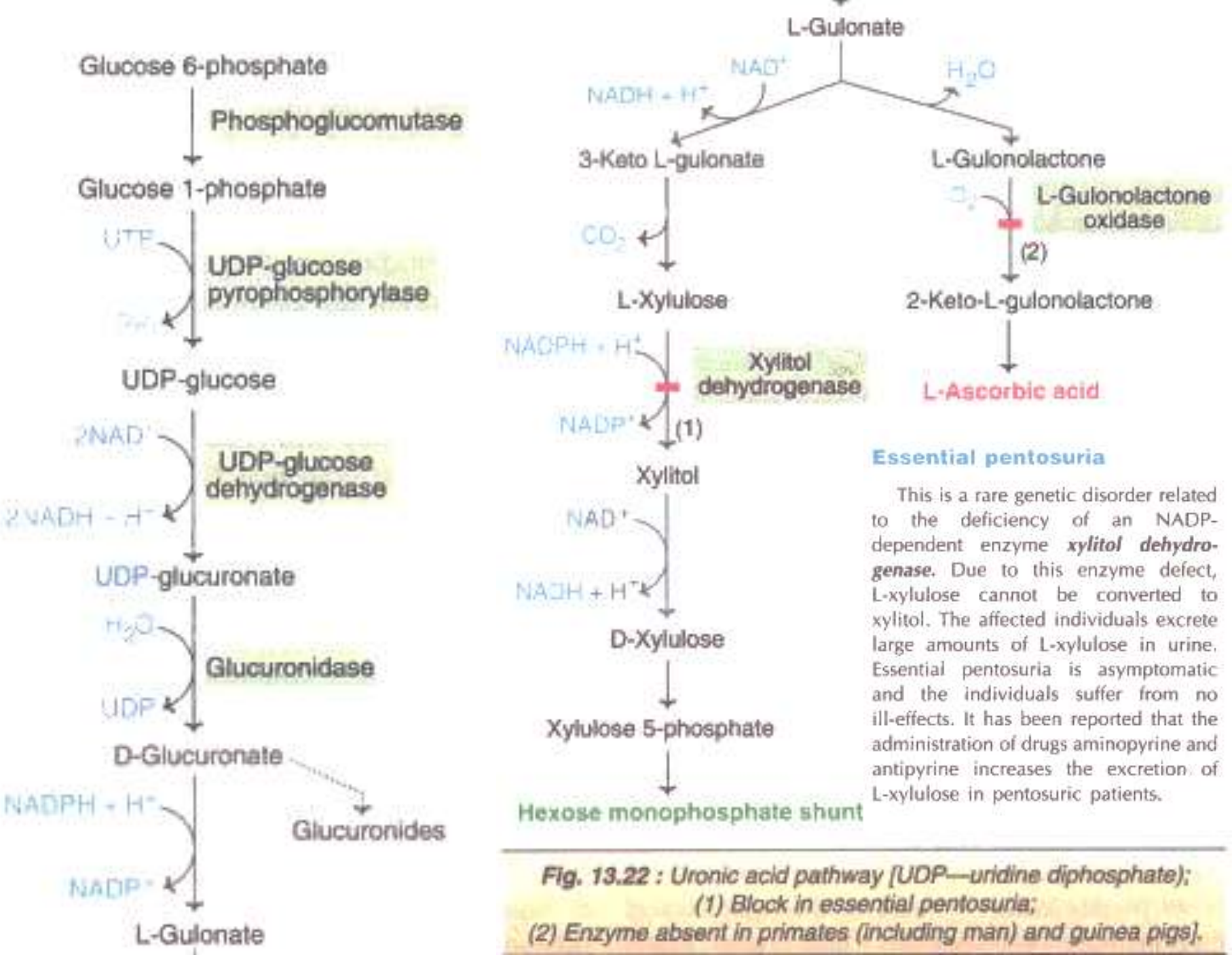
2. **Conversion of UDP-glucuronate to L-gulonate :** UDP-glucuronate loses its UDP moiety in a hydrolytic reaction and releases D-glucuronate which is reduced to L-gulonate by an NADPH-dependent reaction.

3. **Synthesis of ascorbic acid in some animals :** L-Gulonate is the precursor for the synthesis of ascorbic acid (vitamin C) in many animals. The enzyme ***L-gulonolactone oxidase***—which converts gulonate to ascorbic acid—is absent in man, other primates and guinea pigs. Therefore, vitamin C has to be supplemented in the diet for these animals.

4. **Oxidation of L-gulonate :** L-Gulonate is oxidized to 3-ketogulonate and then decarboxylated to a pentose, L-xylulose. L-Xylulose is converted to D-xylulose via xylitol by a reduction (NADPH-dependent) followed by an oxidation (NAD^+-dependent) reaction. This is necessary since the D-xylulose (and not L-form)—after getting phosphorylated—can enter the hexose monophosphate shunt, for further metabolism.

Effect of drugs on uronic acid pathway

Administration of drugs (barbital, chlorobutanol etc.) significantly increases the uronic acid pathway to achieve more synthesis of glucuronate from glucose. Certain drugs (aminopyrine, antipyrine) were found to enhance the synthesis of ascorbic acid in rats.



Essential pentosuria

This is a rare genetic disorder related to the deficiency of an NADP-dependent enzyme ***xylitol dehydrogenase.*** Due to this enzyme defect, L-xylulose cannot be converted to xylitol. The affected individuals excrete large amounts of L-xylulose in urine. Essential pentosuria is asymptomatic and the individuals suffer from no ill-effects. It has been reported that the administration of drugs aminopyrine and antipyrine increases the excretion of L-xylulose in pentosuric patients.

Fig. 13.22 : Uronic acid pathway [UDP—uridine diphosphate); (1) Block in essential pentosuria; (2) Enzyme absent in primates (including man) and guinea pigs].

Metabolism of Disaccharides: Lactose Synthesis

The lactose synthesis pathway is shown in the figure below. The following points are relevant to this figure (as indicated by the numbers on the figure; see below the figure for the legend of abbreviations):

1.One glucose is converted to UDP-glucose, which in turn is converted to one UDP-galactose. Another glucose is used for lactose synthesis without modification. Therefore, 2 glucoses are required for each lactose molecule synthesized.

2.Glucose passes across the Golgi membrane into the Golgi lumen by a glucose transporter (GLUT 1). **The presence of GLUT 1 on the Golgi membrane apparently is specific to the mammary epithelial cell, as most cells do not have this glucose transporter on the Golgi membrane.** The transport of glucose is not active (not requiring energy), and is apparently not rate limiting. But it is affected by glucose levels in the cytoplasm. The Golgi is shown in the image below, along with a secretory vesicle (SV) that contains a casein micelle (arrow).

3.UDP-galactose is actively transported into the Golgi lumen, and transport of UDP-galactose into the Golgi lumen may be rate limiting to lactose synthesis. UDP-glucose is not transported into the Golgi.

4.Lactose is a nonpermeable disaccharide which can not diffuse out of the Golgi membrane or out of secretory vesicles' membrane. This characteristic is important for milk synthesis because it is the synthesis of the nondiffusible lactose which results in water being drawn into the Golgi.

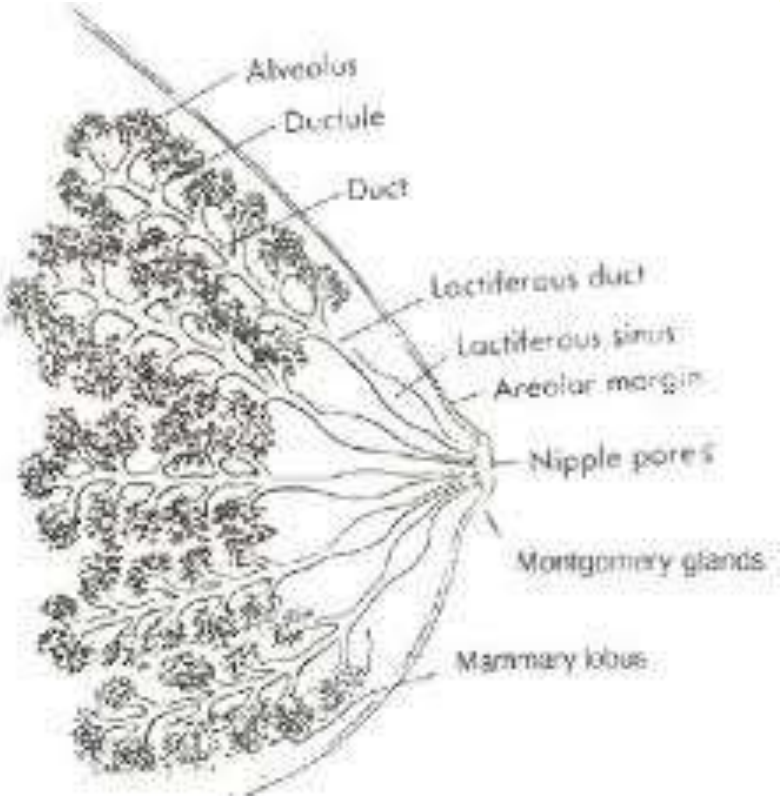
5.The UDP generated from lactose synthesis could be inhibitory to lactose synthesis if it accumulated in the Golgi lumen. However, UDP is rapidly hydrolyzed into UMP and inorganic P by nucleoside diphosphatase (NDPase). UMP is actively removed from the Golgi, while the inorganic P diffuses out of the Golgi.

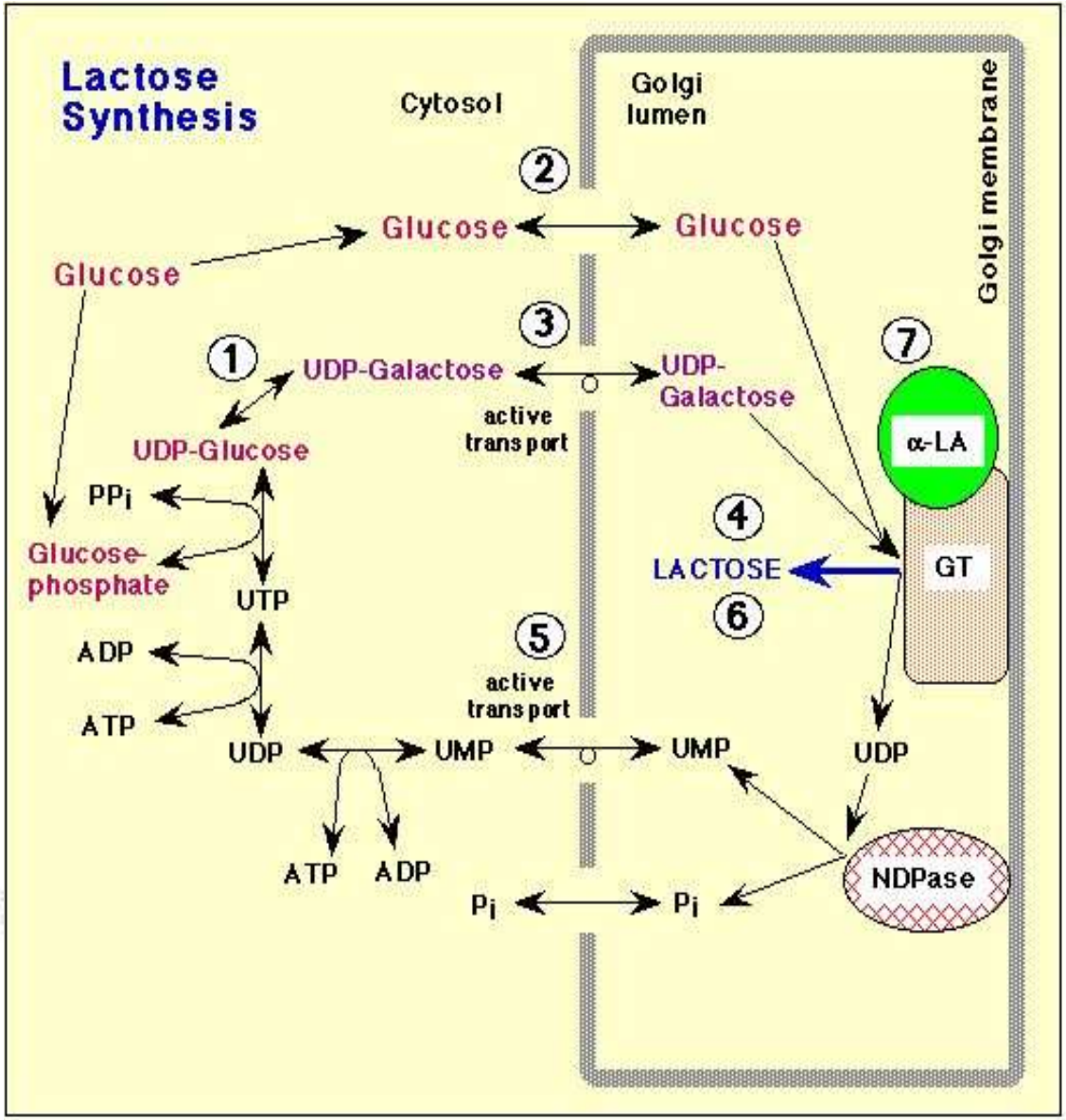
6.The lactose synthesis reaction is essentially one-way, that is, lactose is not hydrolyzed to form glucose and galactose. The very high levels of lactose do not inhibit its own synthesis.
7.The lactose synthase enzyme activity is composed of:

GT = galactosyltransferase

α-LA = α-lactalbumin

NDPase = nucleotide diphosphatase





Sources of blood glucose

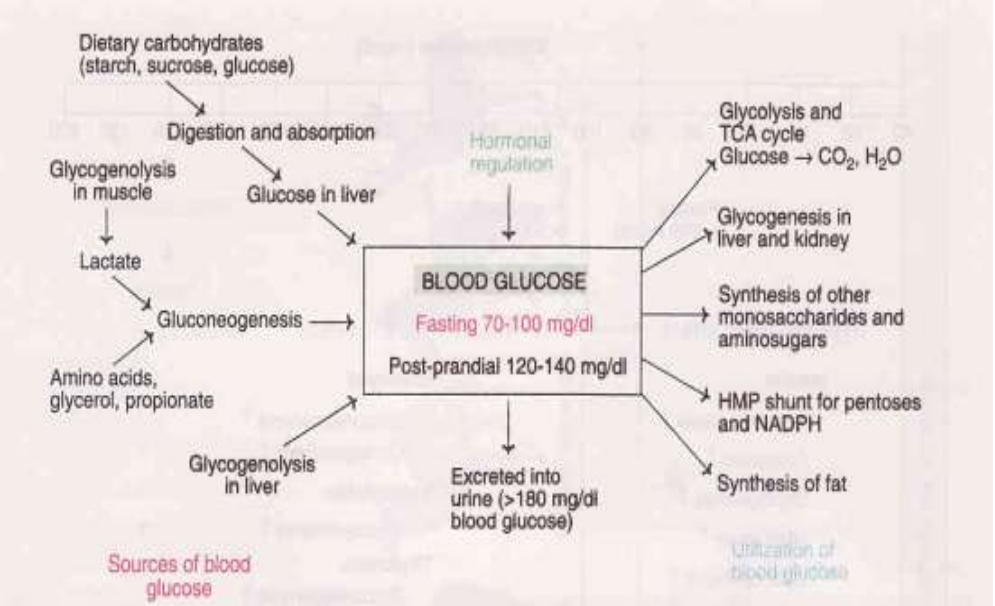
1. **Dietary sources :** The dietary carbohydrates are digested and ***absorbed as monosaccharides*** (glucose, fructose, galactose etc.). The liver is capable of ***converting*** fructose and galactose ***into glucose***, which can readily enter blood.

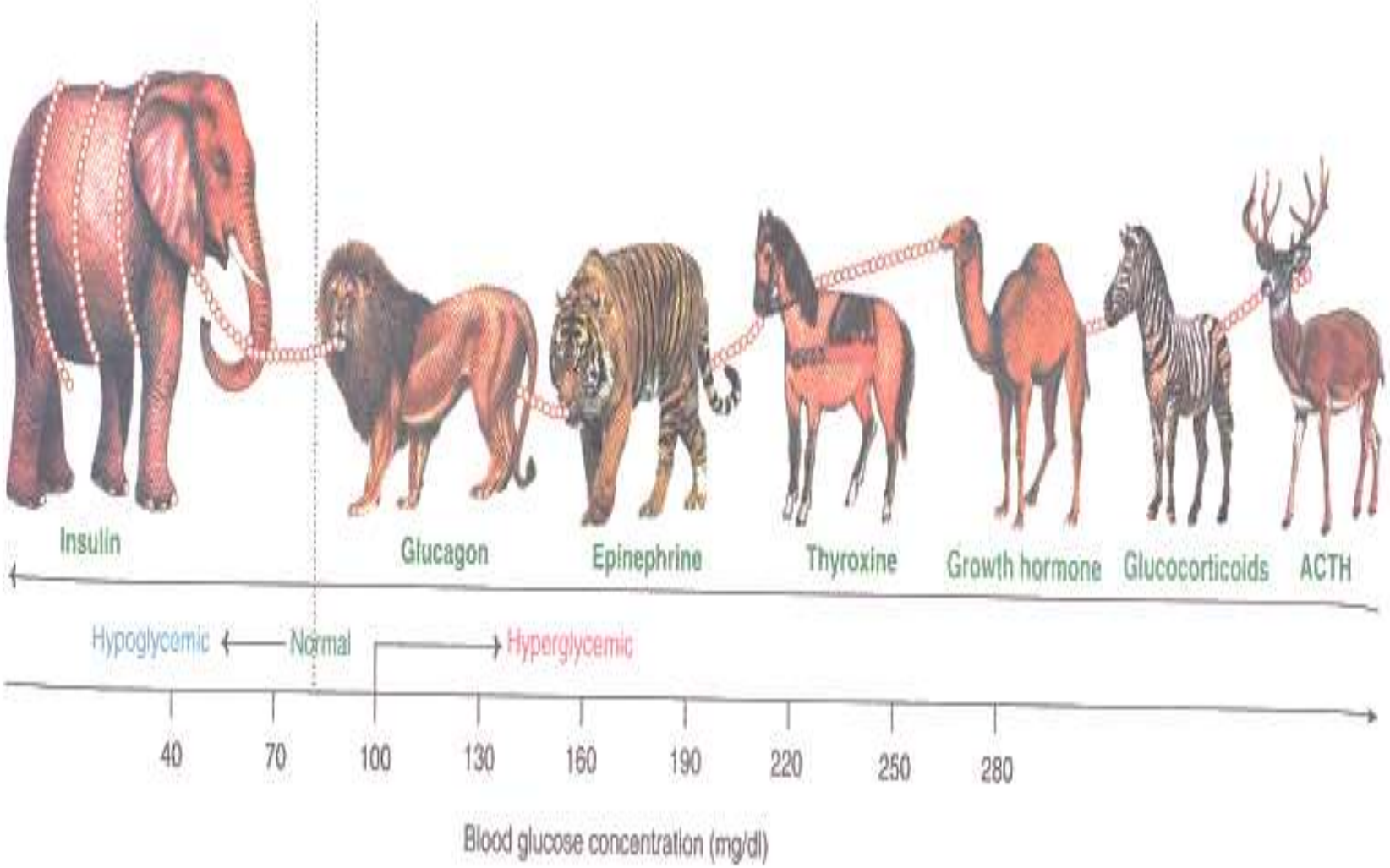
2. **Gluconeogenesis :** The degradation of glycogen in muscle results in the formation of lactate. Breakdown of fat in adipose tissue will produce free glycerol and propionate. Lactate, glycerol, propionate and some amino acids are good precursors for ***glucose synthesis*** (gluconeogenesis) that actively occurs in liver and kidney. Gluconeogenesis continuously adds glucose to the blood. Cori cycle is responsible for the conversion of muscle lactate to glucose in liver.

3. **Glycogenolysis :** Degradation of glycogen in liver produces free glucose. This is in contrast to muscle glycogenolysis where glucose is not formed in sufficient amount due to lack of the enzyme glucose 6-phosphatase. However, the contribution of liver glycogenolysis to blood glucose is rather limited and can meet only the short intervals of emergency. This is due to the limited presence of glycogen in liver. An adult liver (weighing about 1.5 kg) can provide only 40-50 g of blood glucose from glycogen, that can last only for a few hours to meet the body requirements.

Hormonal Regulation of Carbohydrates Metabolism

Hormones play a significant role in the regulation of blood glucose concentration (***Figs.36.6*** and ***36.7***). Primarily, ***insulin lowers blood glucose*** level (hypoglycemic) while the ***rest of the hormones oppose*** the actions of insulin (hyperglycemia).




Insulin
Glucagon
Epinephrine
Thyroxine
Growth hormone
Glucocorticoids
ACTH
Hypoglycemic
Normal
Hyperglycemic
40
70
100
130
160
190
220
250
280
Blood glucose concentration (mg/dl)

Insulin : Insulin is produced by β-cells of the islets of Langerhans in response to hyperglycemia (elevated blood glucose level). Some amino acids, free fatty acids, ketone bodies, drugs such as tolbutamide also cause the secretion of insulin.

Insulin is basically a hypoglycemic hormone that ***lowers*** in ***blood glucose level*** through various means. It is an anti-diabetogenic hormone. For details of insulin action on glucose homeostasis refer metabolic effects of insulin (carbohydrate metabolism) in this chapter.

Glucagon : Glucagon is synthesized by α-cells of the islets of Langerhans of the pancreas. Hypoglycemia (low blood glucose level) stimulates its production. Glucagon is basically involved in ***elevating blood glucose*** concentration. It enhances gluconeogenesis and glycogenolysis.

Epinephrine : This hormone is secreted by adrenal medulla. It acts both on muscle and liver to bring about glycogenolysis by increasing phosphorylase activity. The end product is glucose in liver and lactate in muscle. The net outcome is that epinephrine ***increases blood glucose level.***

Thyroxine : It is a hormone of thyroid gland. It elevates blood glucose level by stimulating hepatic glycogenolysis and gluconeogenesis.

Glucocorticoids : These hormones are produced by adrenal cortex. Glucocorticoids stimulate protein metabolism and increase gluconeogenesis (increase the activities of enzymes—glucose 6-phosphatase and fructose 1,6-bisphosphatase). The glucose utilization by extrahepatic tissues is inhibited by glucocorticoids. The overall effect of glucocorticoids is to ***elevate blood glucose concentration***.

Growth hormone and adrenocorticotropic hormone (ACTH) : The anterior pituitary gland secretes growth hormone and ACTH. The uptake of glucose by certain tissues (muscle, adipose tissue etc.) is decreased by growth hormone. ACTH decreases glucose utilization. The net effect of both these hormones is ***hyperglycemic***.

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3. David L. Nelson; Michael M. Cox “Lehninger Principles of Biochemistry” Seventh Edition, Macmillan 2017, ISBN:9781464187957

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METABOLISM OF CARBOHYDRATES - 4

TEJASVI NAVADHITAMASTU

"Let our (the teacher and the taught) learning be radiant"

Let our efforts at learning be luminous and filled with joy, and endowed with the force of purpose

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E – Content

Course: M.Sc.
Subject: Biochemistry; Biotechnology
Topic: Metabolism of Carbohydrates
Subtopic: Gluconeogenesis, HMP-Shunt, Lactose Metabolism
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GLUCONEOGENESIS

The synthesis of glucose from non-carbohydrate compounds is known as gluconeogenesis. The major ***substrates***/precursors for gluconeogenesis are ***lactate***, ***pyruvate***, ***glucogenic amino acids***, ***propionate*** and ***glycerol.***

Importance of gluconeogenesis

Glucose occupies a key position in the metabolism and its continuous supply is absolutely essential to the body for a variety of functions

1. Brain and central nervous system, erythrocytes, testes and kidney medulla are dependent on glucose for continuous supply of energy. Human brain alone requires about 120 g of glucose per day, out of about 160 g needed by the entire body.

2. Glucose is the only source that supplies energy to the skeletal muscle, under anaerobic conditions.

3. In fasting even more than a day, gluconeogenesis must occur to meet the basal requirements of the body for glucose and to maintain the intermediates of citric acid cycle. This is essential for the survival of humans and other animals.

4. Certain metabolites produced in the tissues accumulate in the blood, e.g. lactate, glycerol, propionate etc. Gluconeogenesis effectively clears them from the blood.

Gluconeogenesis from amino acids

The carbon skeleton of glucogenic amino acids (all except leucine and lysine) results in the formation of pyruvate or the intermediates of citric acid cycle (***Fig.13.11***) which, ultimately, result in the synthesis of glucose.

Gluconeogenesis from glycerol

Glycerol is liberated mostly in the adipose tissue by the hydrolysis of fats (triacylglycerols). The enzyme glycerokinase (found in liver and kidney, absent in adipose tissue) activates glycerol to glycerol 3-phosphate. The latter is converted to dihydroxyacetone phosphate by glycerol 3-phosphate dehydrogenase. Dihydroxyacetone phosphate is an intermediate in glycolysis which can be conveniently used for glucose production.

Gluconeogenesis from propionate

Oxidation of odd chain fatty acids and the breakdown of some amino acids (methionine, isoleucine) yields a three carbon propionyl CoA. Propionyl CoA carboxylase acts on this in presence of ATP and biotin and converts to methyl malonyl CoA which is then converted to succinyl CoA in presence of B_{12} coenzyme (***Refer Fig.7.38***). Succinyl CoA formed from propionyl CoA enters gluconeogenesis via citric acid cycle.

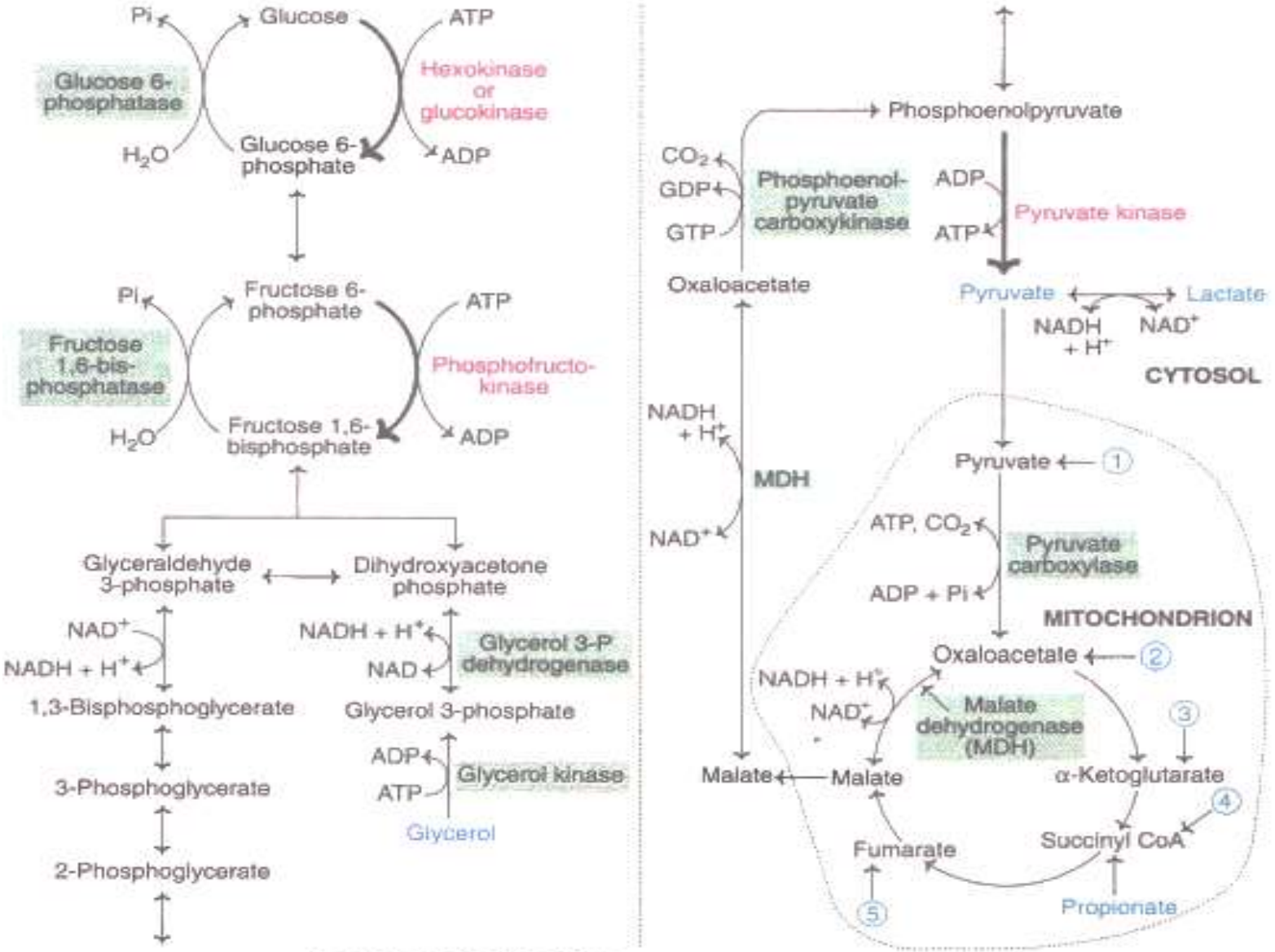


Fig. 13.11 contd. next column

Fig. 13.11 : *The pathway of gluconeogenesis. [The enzymes catalysing irreversible steps in glycolysis are shown in red. The important enzymes participating in gluconeogenesis are shown in shaded green. The substrates for gluconeogenesis are in blue. The numbers represent the entry of glucogenic amino acids : (1) Alanine, glycine, serine, cysteine, threonine and tryptophan; (2) Aspartate and asparagine; (3) Arginine, glutamate, glutamine, histidine, proline; (4) Isoleucine, methionine, valine; (5) Phenylalanine, tyrosine].*

Gluconeogenesis from lactate (Cori cycle)

Lactate produced by active skeletal muscle is a major precursor for gluconeogenesis. Under anaerobic conditions, pyruvate is reduced to lactate by lactate dehydrogenase (LDH)

$$\text{Pyruvate} + \text{NADH} + \text{H}^+ \overset{\text{LDH}}{\rightleftharpoons} \text{Lactate} + \text{NAD}^+$$

Lactate is a dead end in glycolysis, since it must be reconverted to pyruvate for its further metabolism. The very purpose of lactate production is to regenerate NADH so that glycolysis proceeds uninterrupted in skeletal muscle. Lactate or pyruvate produced in the muscle cannot be utilized for the synthesis of glucose due to the absence of the key enzymes of gluconeogenesis (glucose 6-phosphatase and fructose 1,6-bisphosphatase).

The plasma membrane is freely permeable to lactate. Lactate is carried from the skeletal muscle through blood and handed over to liver, where it is oxidized to pyruvate. Pyruvate, so produced, is converted to glucose by gluconeogenesis, which is then transported to the skeletal muscle.

The cycle involving the ***synthesis of glucose in liver from the skeletal muscle lactate*** and the reuse of glucose thus synthesized by the muscle for energy purpose is known as Cori cycle (***Fig.13.13***).

Glucose-alanine cycle

There is a continuous transport of amino acids from muscle to liver, which predominantly occurs during starvation. Alanine dominates among the transported amino acids. It is postulated that pyruvate in skeletal muscle undergoes transamination to produce alanine. Alanine is transported to liver and used for gluconeogenesis. This cycle is referred to as glucose-alanine cycle (***Fig.13.13***).

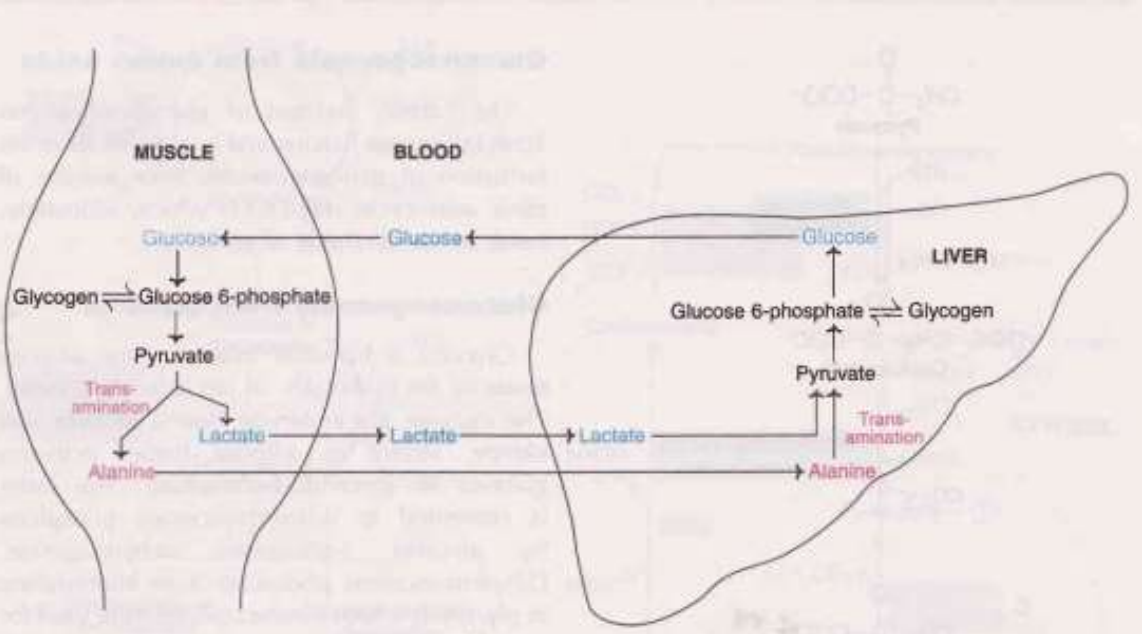


Fig. 13.13 : *The Cori cycle (blue) and glucose-alanine (red) cycle (other reactions common for both cycles).*

Why store glycogen as a fuel reserve?

As such, fat is the fuel reserve of the body. However, fat is not preferred, instead glycogen is chosen for a routine, and day to day use of energy for the following reasons

- Glycogen can be rapidly mobilized
- Glycogen can generate energy in the absence of oxygen
- Brain depends on continuous glucose supply (which mostly comes from glycogen.)

On the other hand, fat mobilization is slow, needs O_2 for energy production and cannot produce glucose (to a significant extent). Thus, fat may be considered as a fixed deposit while glycogen is in the current/saving account in a bank!

Alcohol inhibits gluconeogenesis

Ethanol oxidation in the liver to acetaldehyde by the enzyme alcohol dehydrogenase utilizes NAD^+. The excess NADH produced in the liver interferes with gluconeogenesis as illustrated below.

$$\text{Ethanol} + NAD^+ \longrightarrow \text{Acetaldehyde} + NADH + H^+$$

$$\text{Pyruvate} + NADH + H^+ \leftrightarrow \text{Lactate} + NAD^+$$

$$\text{Oxaloacetate} + NADH + H^+ \leftrightarrow \text{Malate} + NAD^+$$

Regulation of gluconeogenesis

The hormone ***glucagon*** and the availability of substrates mainly regulate gluconeogenesis, as discussed hereunder.

Influence of glucagon : This is a hormone, secreted by α-cells of the pancreatic islets. Glucagon stimulates gluconeogenesis by two mechanisms

1. Active form of pyruvate kinase is converted to inactive form through the mediation of cyclic AMP, brought about by glucagon. ***Decreased pyruvate kinase*** results in the reduced conversion of phosphoenol pyruvate to pyruvate and the former is diverted for the synthesis of glucose.

2. Glucagon reduces the concentration of fructose 2,6-bisphosphate. This compound allosterically inhibits phosphofructokinase and activates fructose 1,6-bisphosphatase, both favour increased gluconeogenesis.

Availability of substrates : Among the various substrates, glucogenic amino acids have stimulating influence on gluconeogenesis. This is particularly important in a condition like diabetes mellitus (decreased insulin level) where amino acids are mobilized from muscle protein for the purpose of gluconeogenesis.

Acetyl CoA promotes gluconeogenesis : During starvation—due to excessive lipolysis in adipose tissue—acetyl CoA accumulates in the liver. Acetyl CoA allosterically activates pyruvate carboxylase resulting in enhanced glucose production.

THE PENTOSE PHOSPHATE PATHWAY

Hexose monophosphate pathway or ***HMP shunt*** is also called ***pentose phosphate pathway*** or phosphogluconate pathway. ***This is an alternative pathway to glycolysis and TCA cycle for the oxidation of glucose.*** However, HMP shunt is more anabolic in nature, since it is concerned with the biosynthesis of NADPH and pentoses.

The enzymes of HMP shunt are located in the ***cytosol***. The tissues such as ***liver, adipose tissue, adrenal gland, erythrocytes, testes*** and ***lactating mammary gland***, are highly active in HMP shunt. Most of these tissues are involved in the biosynthesis of fatty acids and steroids which are dependent on the supply of NADPH.

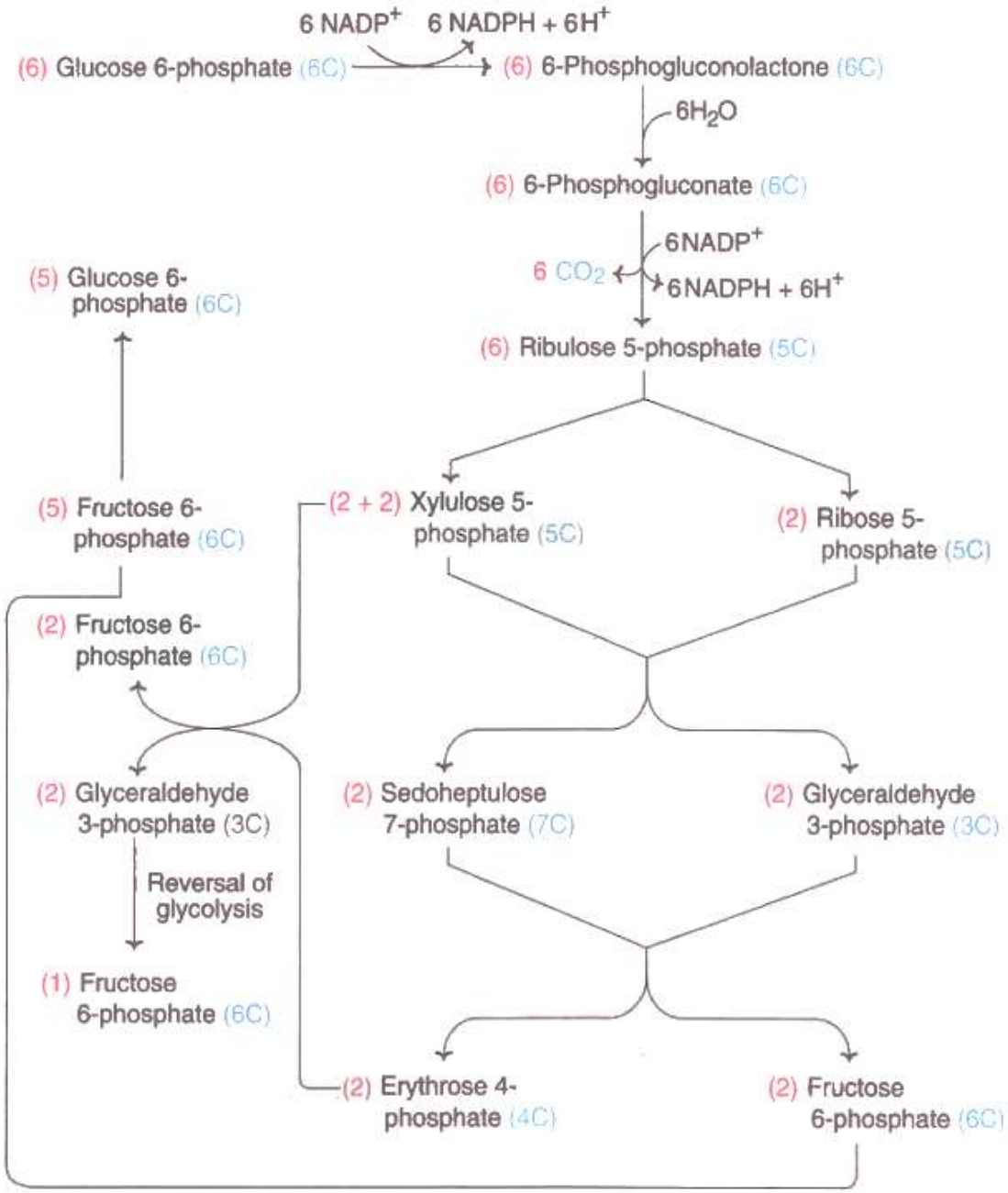
The overall reaction may be represented as

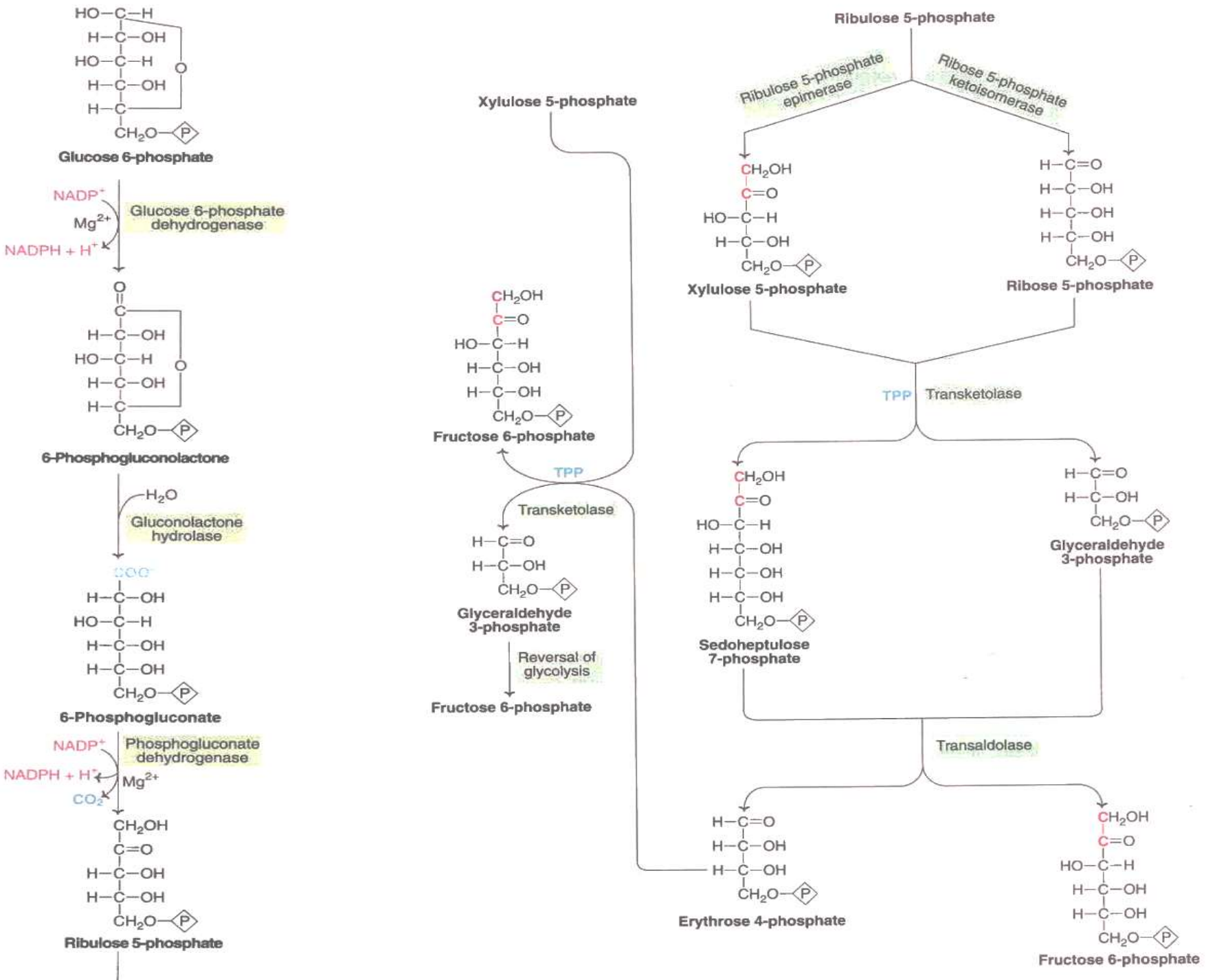
6 Glucose 6-phosphate + 12 $NADP^+$ + $6H_2O$ $\longrightarrow$ $6CO_2$ + 12 NADPH + $12H^+$ + 5 Glucose 6-phosphate.

1. **Oxidative phase :** Glucose 6-phosphate dehydrogenase (G6PD) is an NADP-dependent enzyme that converts glucose 6-phosphate to 6-phosphogluconolactone. The latter is then hydrolysed by the gluconolactone hydrolase to 6-phosphogluconate. The next reaction involving the synthesis of NADPH is catalysed by 6-phosphogluconate dehydrogenase to produce 3 keto 6-phosphogluconate which then undergoes decarboxylation to give ribulose 5-phosphate.

2. **Non-oxidative phase :** The non-oxidative reactions are concerned with the interconversion of three, four, five and seven carbon monosaccharides. Ribulose 5-phosphate is acted upon by an epimerase to produce xylulose 5-phosphate while ribose 5-phosphate ketoisomerase converts ribulose 5-phosphate to ribose 5-phosphate.

G6PD regulates HMP shunt : The first reaction catalysed by G6PD is most regulatory in HMP shunt. This enzyme catalyses an irreversible reaction. NADPH competitively inhibits G6PD. It is the ratio of NADPH/NAD^+ that ultimately determines the flux of this cycle.


6 NADP+
6 NADPH + 6H+
(6) Glucose 6-phosphate (6C)
(6) 6-Phosphogluconolactone (6C)
6H2O
(6) 6-Phosphogluconate (6C)
6NADP+
6 CO2
6NADPH + 6H+
(6) Ribulose 5-phosphate (5C)
(5) Glucose 6-phosphate (6C)
(5) Fructose 6-phosphate (6C)
(2 + 2) Xylulose 5-phosphate (5C)
(2) Ribose 5-phosphate (5C)
(2) Fructose 6-phosphate (6C)
(2) Glyceraldehyde 3-phosphate (3C)
(2) Sedoheptulose 7-phosphate (7C)
(2) Glyceraldehyde 3-phosphate (3C)
Reversal of glycolysis
(1) Fructose 6-phosphate (6C)
(2) Erythrose 4-phosphate (4C)
(2) Fructose 6-phosphate (6C)

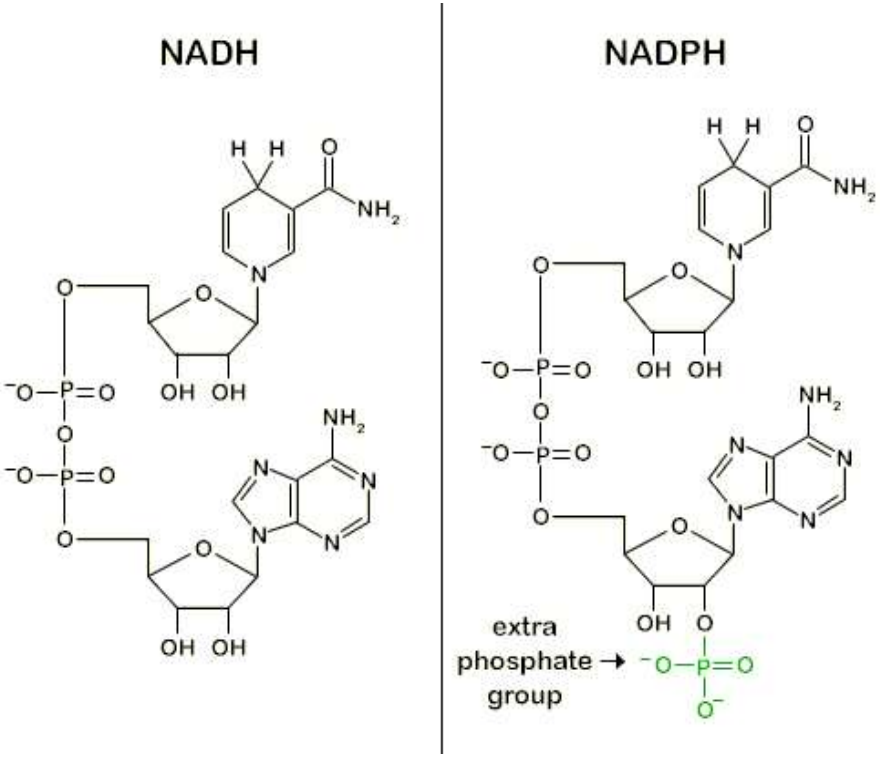

Glucose 6-phosphate
NADP+
Mg2+
NADPH + H+
Glucose 6-phosphate dehydrogenase
6-Phosphogluconolactone
H2O
Gluconolactone hydrolase
6-Phosphogluconate
NADP+
NADPH + H+
Mg2+
CO2
Phosphogluconate dehydrogenase
Ribulose 5-phosphate
Xylulose 5-phosphate
Fructose 6-phosphate
TPP
Transketolase
Glyceraldehyde 3-phosphate
Reversal of glycolysis
Fructose 6-phosphate
Ribulose 5-phosphate
Ribulose 5-phosphate epimerase
Ribose 5-phosphate ketoisomerase
Xylulose 5-phosphate
Ribose 5-phosphate
TPP
Transketolase
Sedoheptulose 7-phosphate
Glyceraldehyde 3-phosphate
Transaldolase
Erythrose 4-phosphate
Fructose 6-phosphate

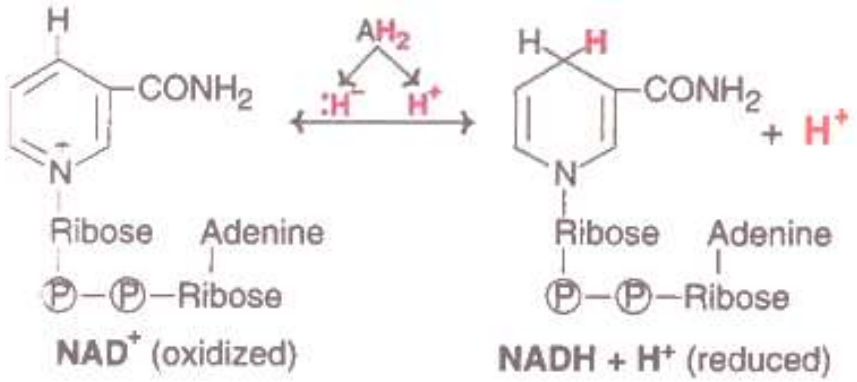
NADH vs. NADPH

NADH provides energy for Catabolic reactions and as for NADPH it provides energy for anabolic reactions

The phosphate group in NADPH doesn't affect the redox abilities of the molecule, it is too far away from the part of the molecule involved in the electron transfer. What the phosphate group does is to allow enzymes to discriminate between NADH and NADPH, which allows the cell to regulate both independently.

The ratio of NAD^+ to NADH inside the cell is high, while the ratio of $NADP^+$ to NADPH is kept low. The role of NADPH is mostly anabolic reactions, where NADPH is needed as a reducing agent, the role of NADH is mostly in catabolic reactions, where NAD^+ is needed as a oxidizing agent.





Significance of HMP shunt

Importance of pentoses

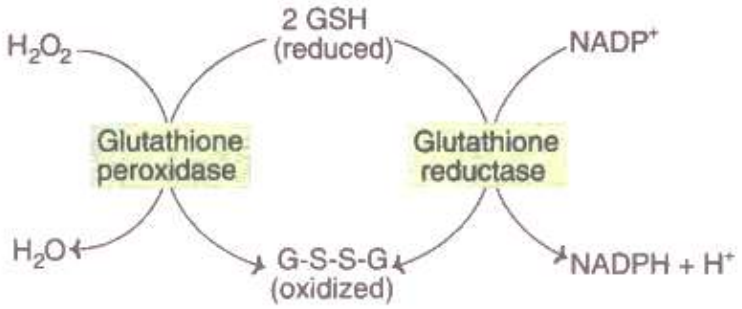
In the HMP shunt, hexoses are converted into pentoses, the most important being ribose 5-phosphate. This pentose or its derivatives are useful for the ***synthesis of nucleic acids*** (RNA and DNA) and many ***nucleotides*** such as ATP, NAD^+, FAD and CoA.

Importance of NADPH

1. NADPH is required for the reductive ***biosynthesis of fatty acids and steroids***, hence HMP shunt is more active in the tissues concerned with lipogenesis, e.g. adipose tissue, liver etc.

2. NADPH is used in the synthesis of certain amino acids involving the enzyme ***glutamate dehydrogenase.***

3. There is a continuous production of H_2O_2 in the living cells which can chemically damage unsaturated lipids, proteins and DNA. This is, however, prevented to a large extent through ***antioxidant reactions*** involving NADPH. Glutathione mediated reduction of H_2O_2 is given in the next column.



Glutathione (reduced, GSH) detoxifies H_2O_2, peroxidase catalyses this reaction. NADPH is responsible for the regeneration of reduced glutathione from the oxidized one.

4. Microsomal cytochrome P_{450} system (in liver) brings about the ***detoxification of drugs*** and foreign compounds by hydroxylation reactions involving NADPH.

5. **Phagocytosis** is the engulfment of foreign particles, including microorganisms, carried out by white blood cells. The process requires the supply of NADPH.

6. **Special functions of NADPH in RBC :** NADPH produced in erythrocytes has special functions to perform. It maintains the concentration of reduced glutathione (reaction explained in 3) which is essentially required to preserve the ***integrity of RBC membrane.*** NADPH is also necessary to keep the ferrous iron (Fe^{2+}) of hemoglobin in the reduced state so that accumulation of methemoglobin (Fe^{3+}) is prevented.

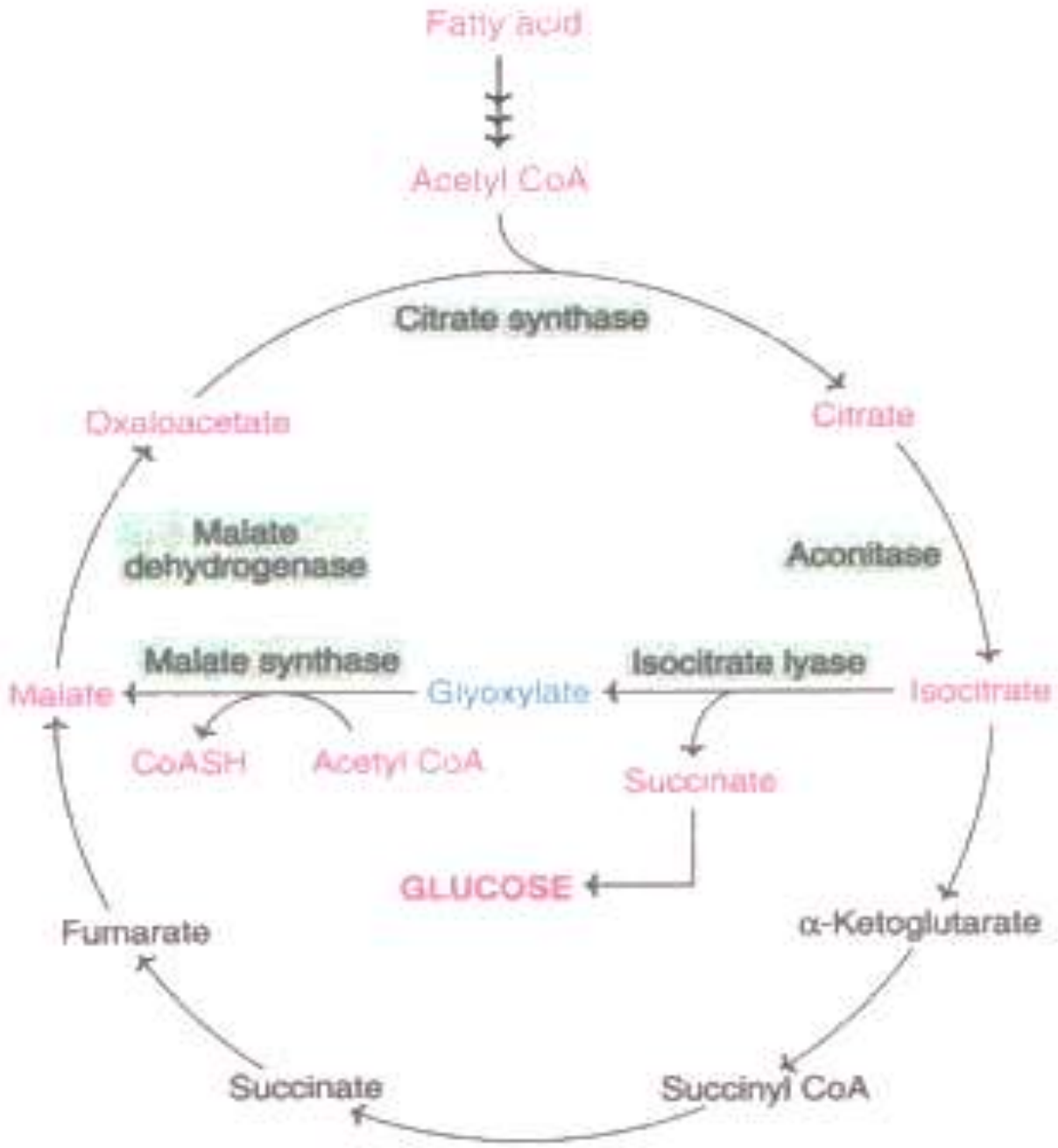
GLYOXYLATE CYCLE

The animals, including man, cannot carry out the net synthesis of carbohydrate from fat. However, the ***plants*** and many ***microorganisms*** are equipped with the metabolic machinery—namely the glyoxylate cycle—***to convert fat into carbohydrates***. This pathway is very significant in the germinating seeds where the stored triacylglycerol (fat) is converted to sugars to meet the energy needs.

Location of the cycle : The glyoxylate cycle occurs in ***glyoxysomes***, specialized cellular organelles, where fatty acid oxidation is also operative.

Reactions of the cycle : The glyoxylate cycle (***Fig.13.26***) is regarded as an anabolic variant of citric acid cycle. Acetyl CoA produced from fatty acid oxidation condenses with oxaloacetate to give citrate which is then converted to isocitrate. At this stage, isocitrate bypasses the citric acid cycle and is cleaved by isocitrate lyase to succinate and glyoxylate. Another molecule of acetyl CoA is now utilized to combine with glyoxylate to form malate. This reaction is catalysed by malate synthase and the malate so formed enters citric acid cycle.

The glyoxylate cycle is a cyclic pathway that results in the conversion of two 2-carbon fragments of acetyl CoA to 4-carbon compound, succinate. The succinate is converted to oxaloacetate and then to glucose involving the reactions of gluconeogenesis.


Fatty acid
Acetyl CoA
Citrate synthase
Oxaloacetate
Citrate
Malate dehydrogenase
Aconitase
Malate synthase
Isocitrate lyase
Malate
Glyoxylate
Isocitrate
CoASH
Acetyl CoA
Succinate
GLUCOSE
α-Ketoglutarate
Fumarate
Succinate
Succinyl CoA

URONIC ACID PATHWAY

This is an alternative oxidative pathway for glucose and is also known as ***glucuronic acid pathway*** (***Fig.13.22***). It is concerned with the synthesis of glucuronic acid, pentoses and vitamin, ascorbic acid (except in primates and guinea pigs). Dietary xylulose enters uronic acid pathway through which it can participate in other metabolisms. In most of the pathways of carbohydrate metabolism, phosphate esters participate, whereas, in uronic acid pathway, the free sugars or sugar acids are involved.

1. **Formation and importance of UDP-glucuronate :** Glucose 6-phosphate is first converted to glucose 1-phosphate. UDP-glucose is then synthesized by the enzyme UDP-glucose pyrophosphorylase. Till this step, the reactions are the same as described in glycogenesis (***Fig.13.14***). UDP-glucose dehydrogenase oxidizes UDP-glucose to UDP-glucuronate.

UDP-glucuronate is the metabolically active form of glucuronate which is utilized for conjugation with many substances like bilirubin, steroid hormones and certain drugs. Several insoluble compounds are converted to soluble ones through conjugation and, further, the drugs are detoxified. UDP-glucuronate is also required for the synthesis of glycosaminoglycans and proteoglycans.

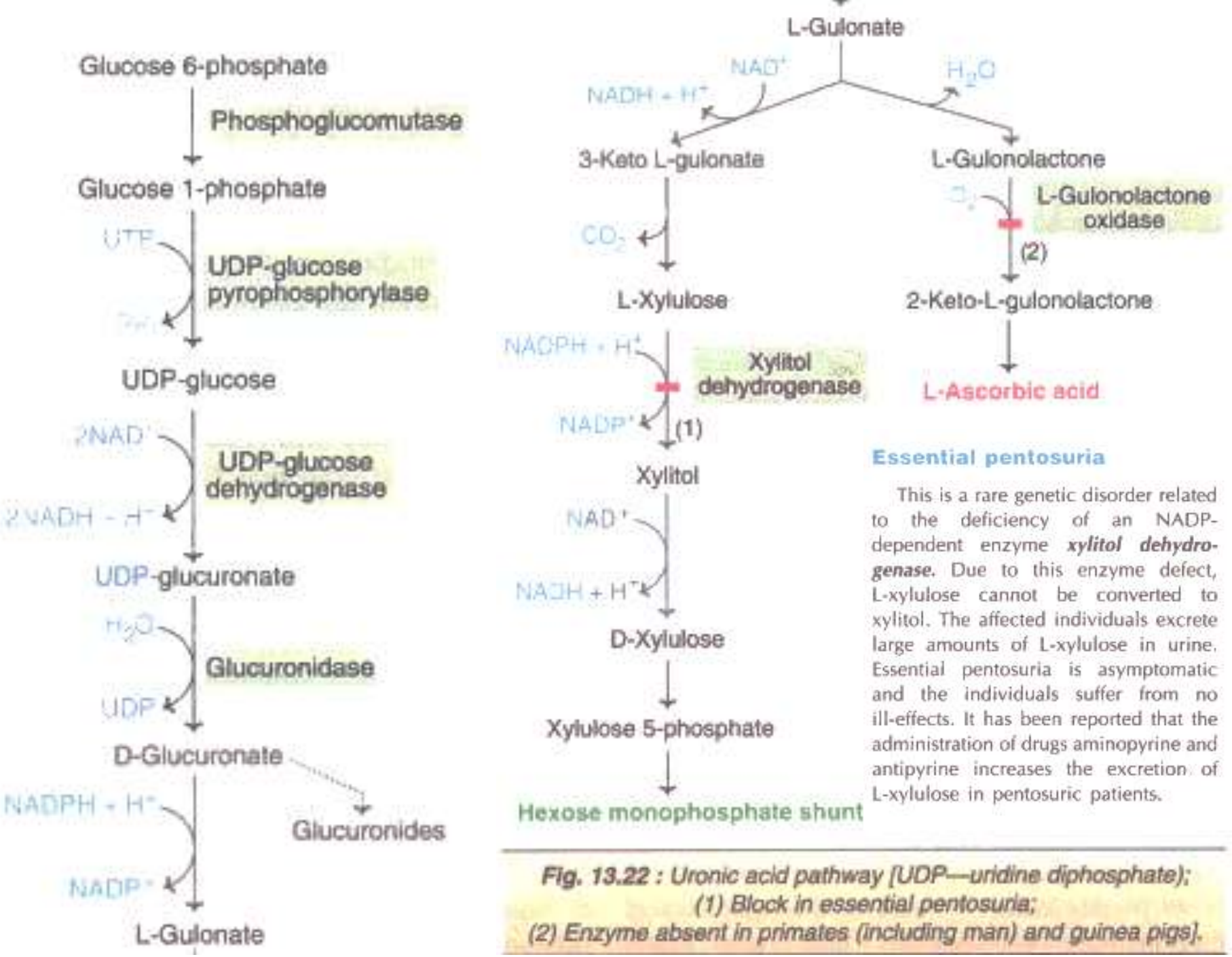
2. **Conversion of UDP-glucuronate to L-gulonate :** UDP-glucuronate loses its UDP moiety in a hydrolytic reaction and releases D-glucuronate which is reduced to L-gulonate by an NADPH-dependent reaction.

3. **Synthesis of ascorbic acid in some animals :** L-Gulonate is the precursor for the synthesis of ascorbic acid (vitamin C) in many animals. The enzyme ***L-gulonolactone oxidase***—which converts gulonate to ascorbic acid—is absent in man, other primates and guinea pigs. Therefore, vitamin C has to be supplemented in the diet for these animals.

4. **Oxidation of L-gulonate :** L-Gulonate is oxidized to 3-ketogulonate and then decarboxylated to a pentose, L-xylulose. L-Xylulose is converted to D-xylulose via xylitol by a reduction (NADPH-dependent) followed by an oxidation (NAD^+-dependent) reaction. This is necessary since the D-xylulose (and not L-form)—after getting phosphorylated—can enter the hexose monophosphate shunt, for further metabolism.

Effect of drugs on uronic acid pathway

Administration of drugs (barbital, chlorobutanol etc.) significantly increases the uronic acid pathway to achieve more synthesis of glucuronate from glucose. Certain drugs (aminopyrine, antipyrine) were found to enhance the synthesis of ascorbic acid in rats.



Essential pentosuria

This is a rare genetic disorder related to the deficiency of an NADP-dependent enzyme ***xylitol dehydrogenase.*** Due to this enzyme defect, L-xylulose cannot be converted to xylitol. The affected individuals excrete large amounts of L-xylulose in urine. Essential pentosuria is asymptomatic and the individuals suffer from no ill-effects. It has been reported that the administration of drugs aminopyrine and antipyrine increases the excretion of L-xylulose in pentosuric patients.

Fig. 13.22 : Uronic acid pathway [UDP—uridine diphosphate); (1) Block in essential pentosuria; (2) Enzyme absent in primates (including man) and guinea pigs].

Metabolism of Disaccharides: Lactose Synthesis

The lactose synthesis pathway is shown in the figure below. The following points are relevant to this figure (as indicated by the numbers on the figure; see below the figure for the legend of abbreviations):

1.One glucose is converted to UDP-glucose, which in turn is converted to one UDP-galactose. Another glucose is used for lactose synthesis without modification. Therefore, 2 glucoses are required for each lactose molecule synthesized.

2.Glucose passes across the Golgi membrane into the Golgi lumen by a glucose transporter (GLUT 1). **The presence of GLUT 1 on the Golgi membrane apparently is specific to the mammary epithelial cell, as most cells do not have this glucose transporter on the Golgi membrane.** The transport of glucose is not active (not requiring energy), and is apparently not rate limiting. But it is affected by glucose levels in the cytoplasm. The Golgi is shown in the image below, along with a secretory vesicle (SV) that contains a casein micelle (arrow).

3.UDP-galactose is actively transported into the Golgi lumen, and transport of UDP-galactose into the Golgi lumen may be rate limiting to lactose synthesis. UDP-glucose is not transported into the Golgi.

4.Lactose is a nonpermeable disaccharide which can not diffuse out of the Golgi membrane or out of secretory vesicles' membrane. This characteristic is important for milk synthesis because it is the synthesis of the nondiffusible lactose which results in water being drawn into the Golgi.

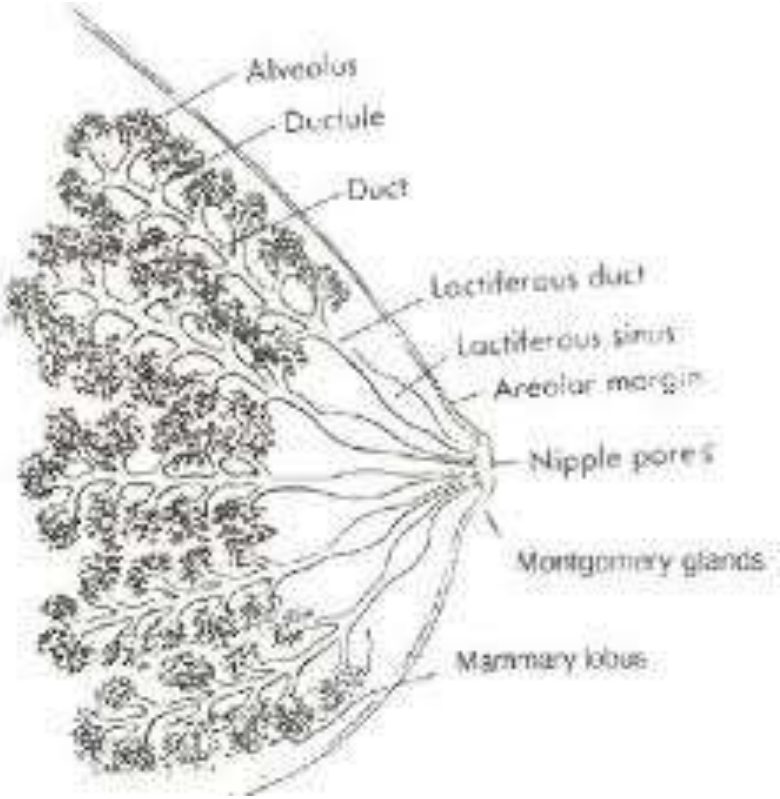
5.The UDP generated from lactose synthesis could be inhibitory to lactose synthesis if it accumulated in the Golgi lumen. However, UDP is rapidly hydrolyzed into UMP and inorganic P by nucleoside diphosphatase (NDPase). UMP is actively removed from the Golgi, while the inorganic P diffuses out of the Golgi.

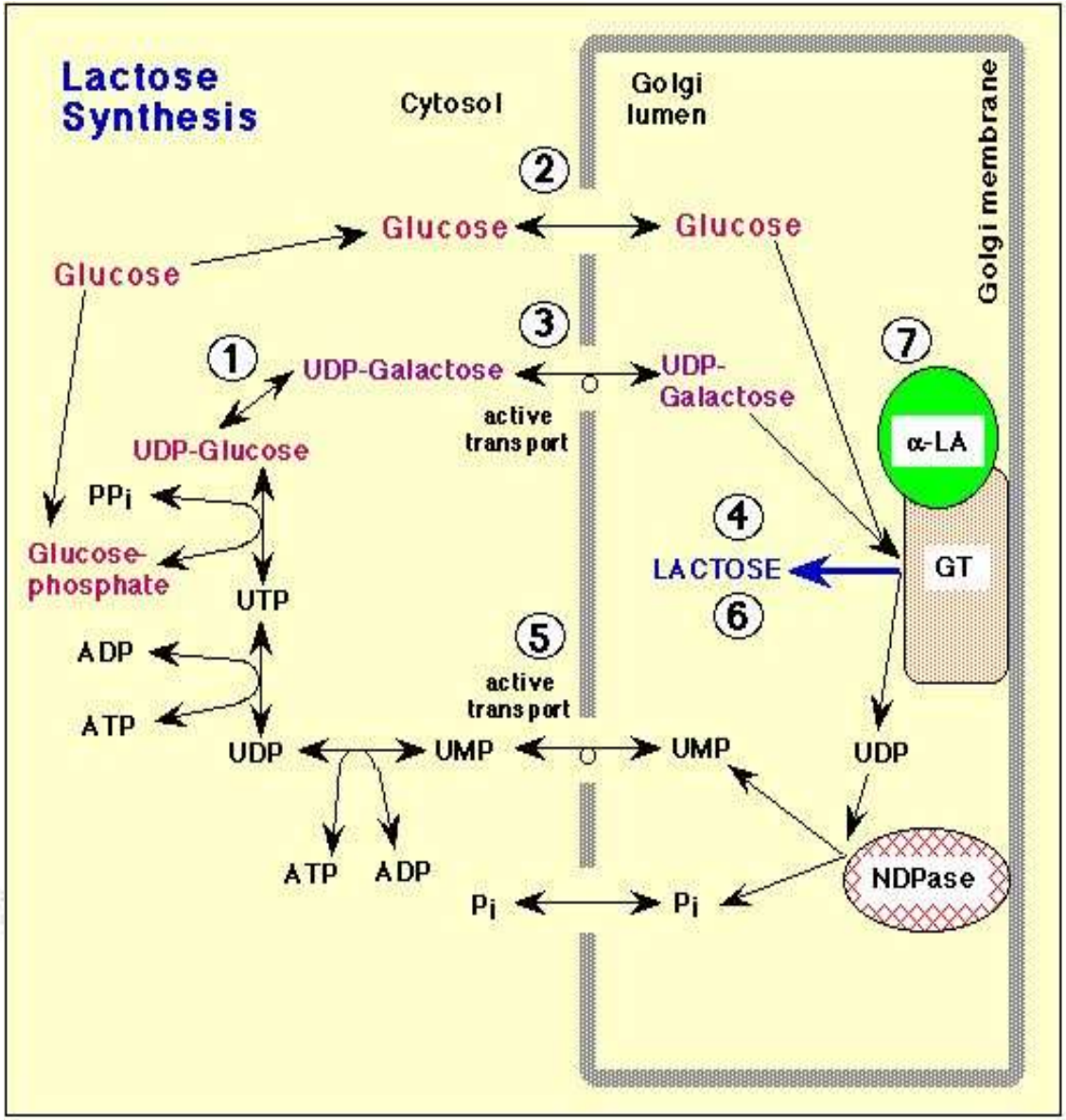
6.The lactose synthesis reaction is essentially one-way, that is, lactose is not hydrolyzed to form glucose and galactose. The very high levels of lactose do not inhibit its own synthesis.
7.The lactose synthase enzyme activity is composed of:

GT = galactosyltransferase

α-LA = α-lactalbumin

NDPase = nucleotide diphosphatase





Sources of blood glucose

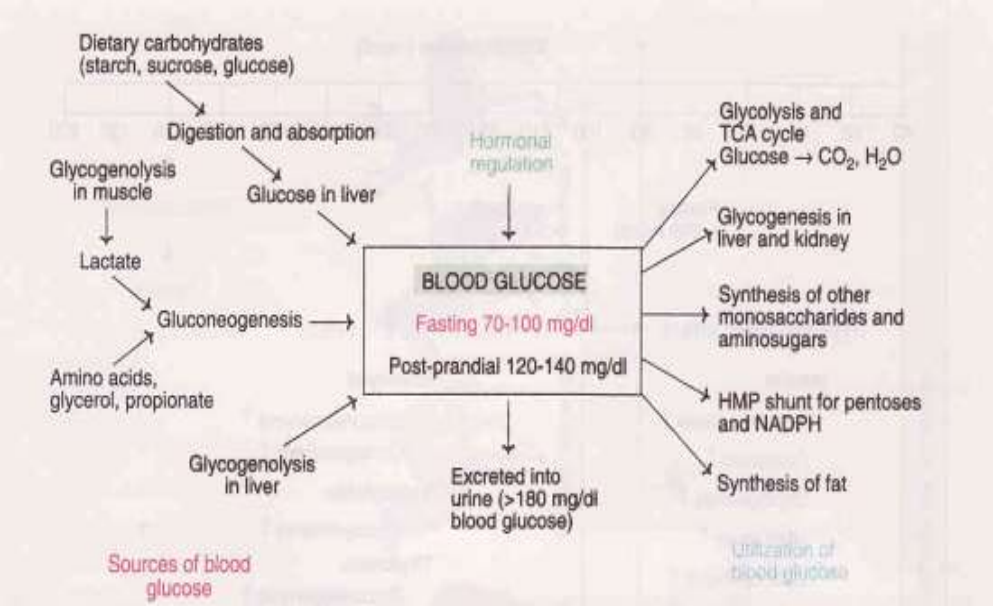
1. **Dietary sources :** The dietary carbohydrates are digested and ***absorbed as monosaccharides*** (glucose, fructose, galactose etc.). The liver is capable of ***converting*** fructose and galactose ***into glucose***, which can readily enter blood.

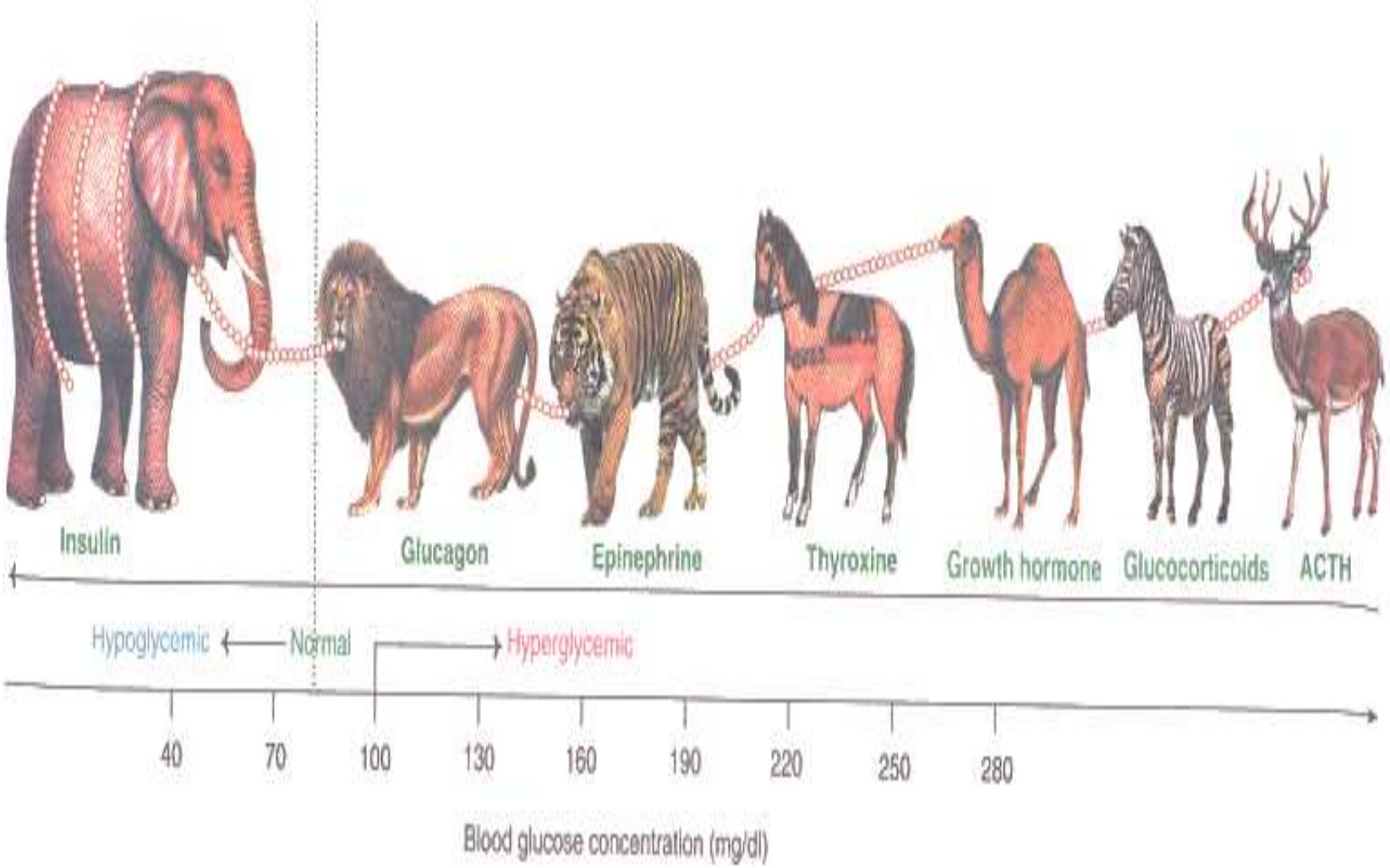
2. **Gluconeogenesis :** The degradation of glycogen in muscle results in the formation of lactate. Breakdown of fat in adipose tissue will produce free glycerol and propionate. Lactate, glycerol, propionate and some amino acids are good precursors for ***glucose synthesis*** (gluconeogenesis) that actively occurs in liver and kidney. Gluconeogenesis continuously adds glucose to the blood. Cori cycle is responsible for the conversion of muscle lactate to glucose in liver.

3. **Glycogenolysis :** Degradation of glycogen in liver produces free glucose. This is in contrast to muscle glycogenolysis where glucose is not formed in sufficient amount due to lack of the enzyme glucose 6-phosphatase. However, the contribution of liver glycogenolysis to blood glucose is rather limited and can meet only the short intervals of emergency. This is due to the limited presence of glycogen in liver. An adult liver (weighing about 1.5 kg) can provide only 40-50 g of blood glucose from glycogen, that can last only for a few hours to meet the body requirements.

Hormonal Regulation of Carbohydrates Metabolism

Hormones play a significant role in the regulation of blood glucose concentration (***Figs.36.6*** and ***36.7***). Primarily, ***insulin lowers blood glucose*** level (hypoglycemic) while the ***rest of the hormones oppose*** the actions of insulin (hyperglycemia).




Insulin
Glucagon
Epinephrine
Thyroxine
Growth hormone
Glucocorticoids
ACTH
Hypoglycemic
Normal
Hyperglycemic
40
70
100
130
160
190
220
250
280
Blood glucose concentration (mg/dl)

Insulin : Insulin is produced by β-cells of the islets of Langerhans in response to hyperglycemia (elevated blood glucose level). Some amino acids, free fatty acids, ketone bodies, drugs such as tolbutamide also cause the secretion of insulin.

Insulin is basically a hypoglycemic hormone that ***lowers*** in ***blood glucose level*** through various means. It is an anti-diabetogenic hormone. For details of insulin action on glucose homeostasis refer metabolic effects of insulin (carbohydrate metabolism) in this chapter.

Glucagon : Glucagon is synthesized by α-cells of the islets of Langerhans of the pancreas. Hypoglycemia (low blood glucose level) stimulates its production. Glucagon is basically involved in ***elevating blood glucose*** concentration. It enhances gluconeogenesis and glycogenolysis.

Epinephrine : This hormone is secreted by adrenal medulla. It acts both on muscle and liver to bring about glycogenolysis by increasing phosphorylase activity. The end product is glucose in liver and lactate in muscle. The net outcome is that epinephrine ***increases blood glucose level.***

Thyroxine : It is a hormone of thyroid gland. It elevates blood glucose level by stimulating hepatic glycogenolysis and gluconeogenesis.

Glucocorticoids : These hormones are produced by adrenal cortex. Glucocorticoids stimulate protein metabolism and increase gluconeogenesis (increase the activities of enzymes—glucose 6-phosphatase and fructose 1,6-bisphosphatase). The glucose utilization by extrahepatic tissues is inhibited by glucocorticoids. The overall effect of glucocorticoids is to ***elevate blood glucose concentration***.

Growth hormone and adrenocorticotropic hormone (ACTH) : The anterior pituitary gland secretes growth hormone and ACTH. The uptake of glucose by certain tissues (muscle, adipose tissue etc.) is decreased by growth hormone. ACTH decreases glucose utilization. The net effect of both these hormones is ***hyperglycemic***.

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