erythrocytes whereas GLUT-4 is abundant in skeletal muscle and adipose tissue (***Table 13.1***).

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.

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-Parnas pathway (EMP pathway)*** in honour of the 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.

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 points 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).

Reactions of glycolysis

An overview of glycolysis is depicted in ***Fig.13.3***, while detailed sequence of reactions is given in ***Fig.13.3***. The pathway can be divided into three distinct phases

A. Energy investment phase or priming stage

B. Splitting phase

C. Energy generation phase.

Table 13.1 Major glucose transporters along with tissue location and functions

Transporter	Location	Major function(s)
GLUT–1	Brain, kidney, RBC, retina, colon, placenta	Glucose uptake
GLUT–2	Liver, kidney, small intestine, β-cells of pancreas	Quick uptake and release of glucose
GLUT–3	Brain, placenta, kidney	Glucose uptake
GLUT–4	Heart, muscle, skeletal muscle, adipose tissue	Insulin mediated glucose uptake
GLUT–5	Small intestine, testis, sperm, kidney	Absorption of fructose
GLUT–7	Liver	Release of glucose from endoplasmic reticulum to cytosol
SGLUT–1	Intestine, kidney	Sodium dependent active uptake of glucose against concentration gradient (cotransport of Na and glucose)

The sequence of reactions are discussed below.

A. **Energy investment phase (preparatory 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 committed*** 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 (pay off 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 5 ATP (2 × 2.5 ATP) are synthesized by oxidative phosphorylation.

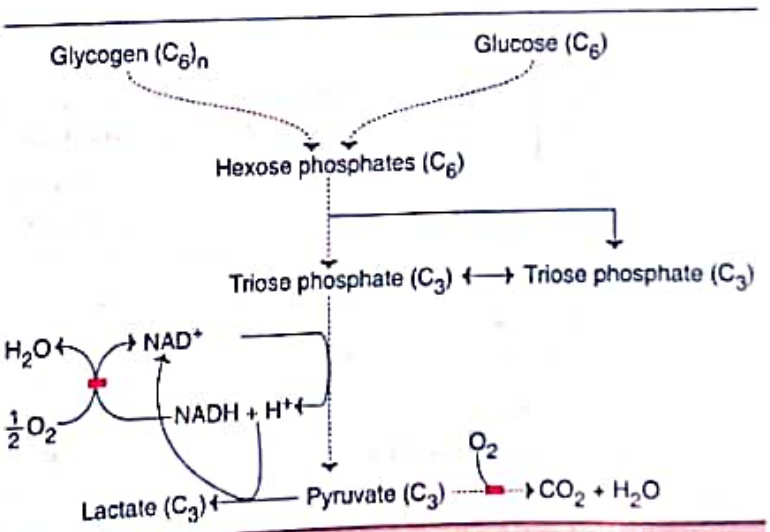
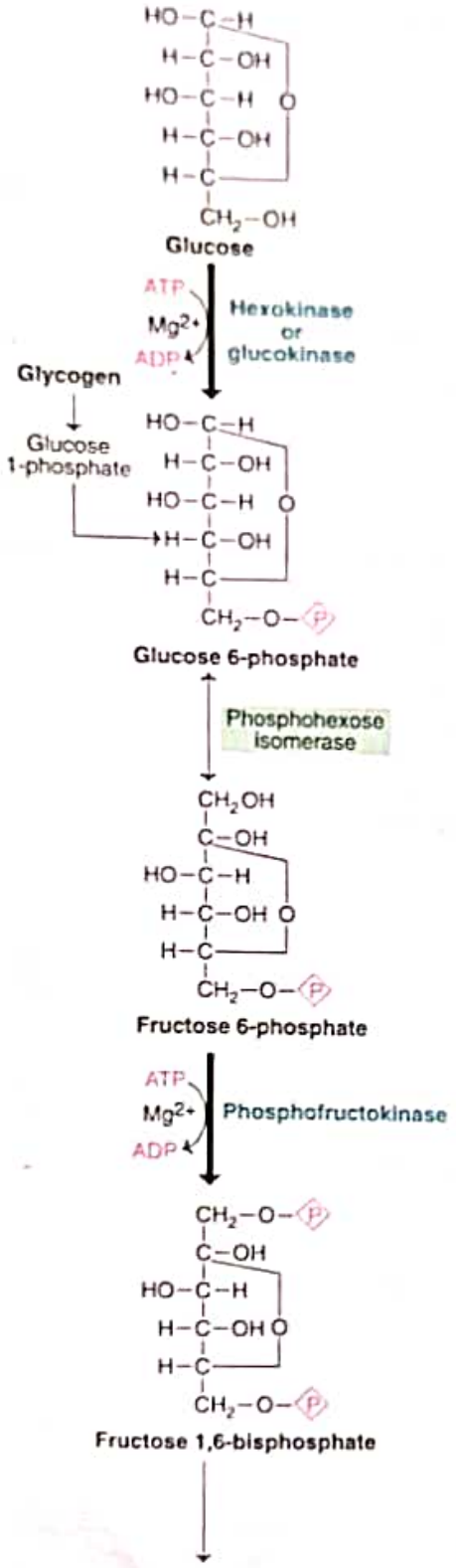


Fig. 13.3 : An overview of glycolysis (= Reactions blocked under anaerobic conditions; In RBC, these two reactions do not occur due to lack of mitochondria)



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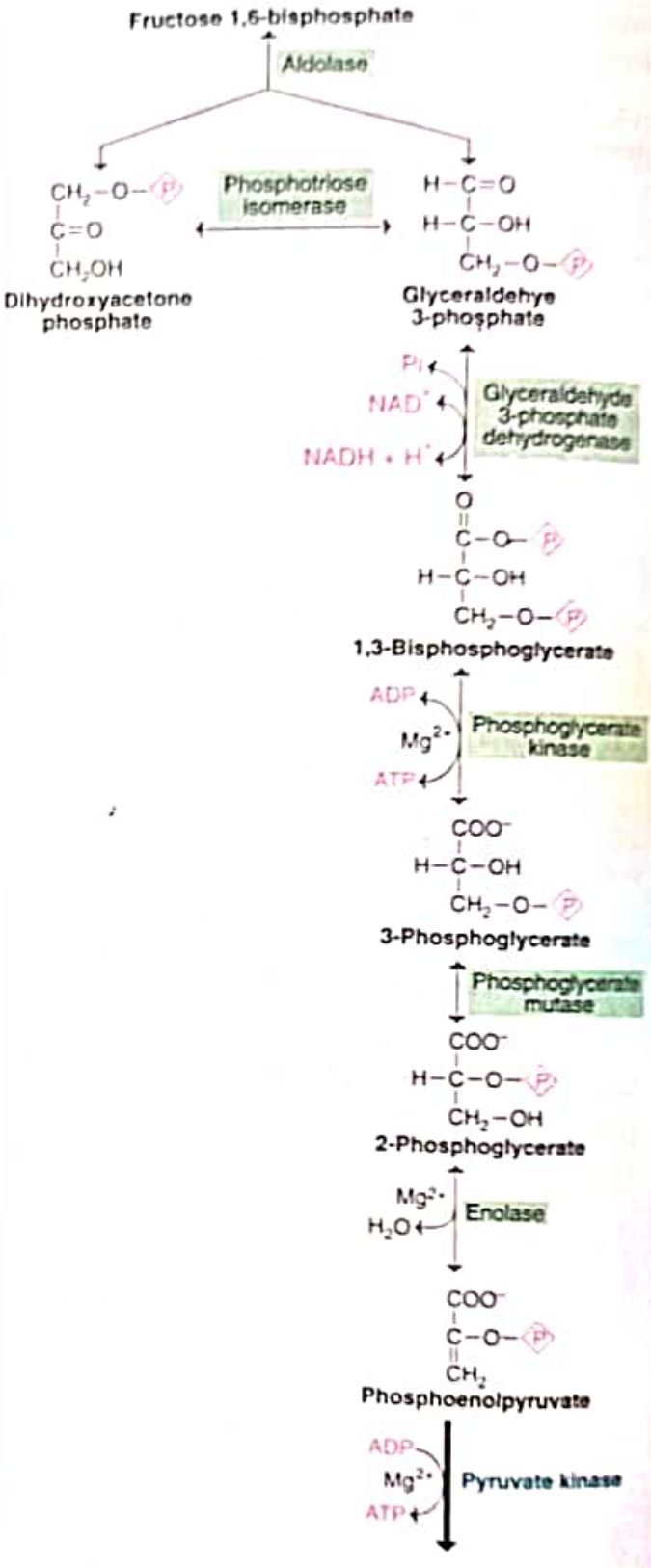

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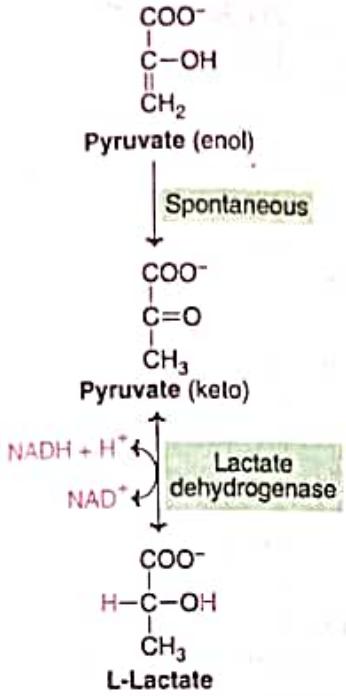

Fig. 13.4 : The reactions in the pathway of glycolysis (The three steps catalysed by hexokinase, phosphofructokinase and pyruvate kinase, shown in thick lines are irreversible).

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. (Fluoride combines with Mg^{2+} and phosphate to form a complex that binds with active site of enolase and blocks access of substrate. Thus, fluoride is an ***unusual competitive inhibitor***).
10. The enzyme pyruvate kinase catalyses the transfer of high energy phosphate from phosphoenol pyruvate to ADP, leading to the formation of ATP. This step also is a ***substrate level phosphorylation.*** This reaction is irreversible.

Conversion of pyruvate to lactate—significance

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, thiamine deficiency, alcoholism, von Gierke's disease etc.

Severe forms of lactic acidosis are observed due to impairment/collapse of circulatory system which is often encountered in ***myocardial infarction, pulmonary embolism, uncontrolled hemorrhage and severe shock***. This type of lactic

acidosis is due to inadequate supply of O_2 to the tissues with a drastic reduction in ATP synthesis (since the cells have to survive in anaerobic conditions) which may even lead to death. The term ***oxygen debt*** refers to the excess amount of O_2 required to recover. In clinical practice, measurement of plasma lactic acid is useful to know about the oxygen debt, and monitor the patient's recovery, and save the patient from morbidity and mortality.

Production of ATP in glycolysis

The details of ATP generation in glycolysis (from glucose) are given in ***Table 13.2***. Under ***anaerobic conditions, 2 ATP*** are synthesized while, under ***aerobic conditions, 7 ATP*** are synthesized, if malate the shuttle pathway operates.

When the glycolysis occurs from glycogen, one more ATP is generated. This is because no ATP is consumed for the activation of glucose (glycogen directly produces glucose 1-phosphate which forms glucose 6-phosphate). Thus, in ***anaerobic glycolysis, 3 ATP are produced from glycogen*** and this is more advantageous than from glucose.

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, 2.5 ATP are generated from each molecule of NADH. This is in contrast to glycerolphosphate shuttle that produces only 1.5 ATP.

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 cancer cells get adapted to hypoxic glycolysis through the involvement of a transcription factor

Table 13.2 Generation of ATP in glucose metabolism

Pathway	Enzyme (method of ATP synthesis)	Number of ATP synthesized
Glycolysis	Glyceraldehyde 3-phosphate dehydrogenase (2 NADH, ETC, oxidative phosphorylation)	5
	Phosphoglycerate kinase (substrate level phosphorylation)	2
	Pyruvate kinase (substrate level phosphorylation)	2
	Two ATP are consumed in the reactions catalysed by hexokinase and phosphofructokinase	–2
	Net ATP synthesis in glycolysis in aerobic condition	***7***
	Pyruvate dehydrogenase (2 NADH, ETC, oxidative phosphorylation)	5
Citric acid cycle	Isocitrate dehydrogenase (2 NADH, ETC, oxidative phosphorylation)	5
	α-Ketoglutarate dehydrogenase (2 NADH, ETC, oxidative phosphorylation)	5
	Succinate thiokinase (substrate level phosphorylation)	2
	Succinate dehydrogenase (2 $FADH_2$, ETC, oxidative phosphorylation)	3
	Malate dehydrogenase (2 NADH, ETC, oxidative phosphorylation)	5
	Total ATP per mole of glucose under aerobic condition	***32***
	Total ATP per mole of glucose under anaerobic condition	***2***

Note : *Values represent ATP synthesized as per the new P:O ratios of 2.5 for NADH and 1.5 for $FADH_2$.*

Medical Concepts/ Clinical Correlates

- Glycolysis is an important source of energy supply to brain, retina, skin and renal medulla.
- The crucial significance of glycolysis is its ability to generate ATP in the absence of oxygen.
- Skeletal muscle, during strenuous exercise, requires the occurrence of uninterrupted glycolysis. This is due to the limited supply of oxygen.
- The cardiac muscle cannot survive for long in the absence of oxygen since it is not well adapted for glycolysis under anaerobic conditions.
- The occurrence of glycolysis is very much elevated in rapidly growing cancer cells.
- Glycolysis in erythrocytes is associated with 2,3-bisphosphoglycerate (2,3-BPG) production. In the presence of 2,3-BPG, oxyhemoglobin unloads more oxygen to the tissues.

namely ***hypoxia-inducible transcription factor (HIF)***. HIF increases the synthesis of glycolytic enzymes and the glucose transporters. However, the cancer cells cannot grow and survive without proper vascularization. One of the modalities of ***cancer treatment is to use drugs that can inhibit vascularization of tumors***.

Irreversible steps in glycolysis

Most of the reactions of glycolysis are reversible. However, the three steps catalysed by the enzymes ***hexokinase*** (or glucokinase), ***phosphofructokinase*** and ***pyruvate kinase***, are irreversible. These three stages mainly regulate glycolysis. The ***reversal of glycolysis***, with ***alternate arrangements made at the three irreversible stages***, leads to the synthesis of glucose from pyruvate (***gluconeogenesis***).

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.

Role of fructose 2,6-bisphosphate in glycolysis

Fructose 2,6-bisphosphate (F2,6-BP) is considered to be the most important ***regulatory factor*** (activator) for controlling PFK and, ultimately, ***glycolysis*** in the liver. F2,6-BP is synthesized from fructose 6-phosphate by the enzyme phosphofructokinase called PFK-2 (PFK-1 is the glycolytic enzyme). F2,6-BP is hydrolysed by fructose 2,6-bisphosphatase. The function of synthesis and degradation of F2,6-BP is brought

Table 13.3 Regulation of glycolysis

Enzyme	Activation	Inhibition
Hexokinase	—	Glucose 6-phosphate
Glucokinase	Insulin	Glucagon
Phosphofructokinase	Insulin, fructose 2,6-bisphosphate, ADP, AMP, Pi	Glucagon, ATP, citrate
Pyruvate kinase	Insulin, fructose 1,6-bisphosphate	Glucagon, ATP, cyclic AMP

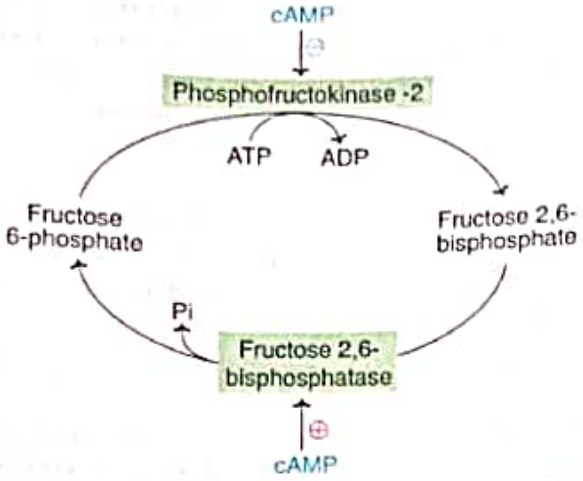

Fig. 13.5 : *Regulation of fructose 2,6-bisphosphatase.*

out by a ***single enzyme*** (same polypeptide with two active sites) which is referred to as ***bifunctional enzyme (Fig.13.5)***. In fact, the combined name of phosphofructokinase-2/fructose 2,6-bisphosphatase is used to refer to the enzyme that synthesizes and degrades F2,6-BP.

The activity of PFK-2 and fructose 2,6-bisphosphatase is controlled by covalent modification which, in turn, is regulated by cyclic AMP (cAMP is the second messenger for certain hormones). Cyclic AMP brings about dephosphorylation of the bifunctional enzyme, resulting in inactivation of active site responsible for the synthesis of F2,6-BP but activation of the active site responsible for the hydrolysis of F2,6-BP.

Pyruvate kinase also regulates glycolysis. This enzyme is inhibited by ATP and activated by F1,6-BP. Pyruvate kinase is active (a) in dephosphorylated state and inactive (b) in phosphorylated state. Inactivation of pyruvate kinase by phosphorylation is brought about by cAMP-dependent protein kinase. The hormone—***glucagon inhibits*** hepatic glycolysis by this mechanism (***Fig.13.6***).

Pasteur effect

The ***inhibition of glycolysis by oxygen*** (aerobic condition) is known as Pasteur effect. It was discovered by Louis Pasteur, more than a century ago, while studying fermentation by yeast. He observed that when anaerobic yeast cultures 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.

Glycolysis and dental caries

Dental caries refers to the ***destruction or decalcification of hard teeth due to organic acids*** released by bacterial infections. The anaerobic bacteria (e.g. ***Streptococcus mutans, Lactobacillus*** sps) that colonize the oral cavity contribute to the development of dental caries. These bacteria grow optimally on refined and fermentable sugars (e.g. sucrose of chocolates,

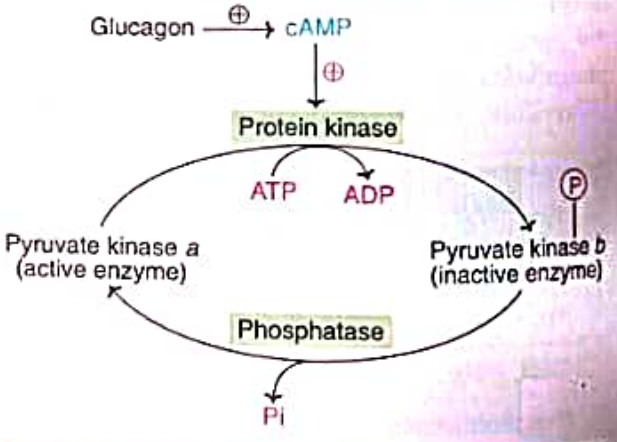

Fig. 13.6 : *Regulation of pyruvate kinase.*