DIGESTION OF CARBOHYDRATES

Dietary carbohydrates principally consist of the ***polysaccharides:*** Starch and glycogen. It also contains ***disaccharides:*** Sucrose (cane sugar), lactose (milk sugar) and maltose and in small amounts ***monosaccharides*** like fructose and pentoses. Liquid food materials like milk, soup, fruit juice escape digestion in mouth as they are swallowed, but solid foodstuffs are masticated thoroughly before they are swallowed.

Digestion in mouth: Digestion of carbohydrates startsat the mouth, where they come in contact with saliva during mastication. Saliva contains a carbohydrate splitting enzyme called ***salivary amylase (ptyalin)***.

1Action of *Ptyalin (Salivary Amylase)* It is α***-amylase,* requires Cl– ion for activation** and optimum pH 6.7 (range 6.6 to 6.8). ***The enzyme hydrolyzes* α*-1 → 4 glycosidic linkage at random deep inside polysaccharide*** molecule like starch, glycogen and dextrins, producing smaller molecules maltose, glucose and trisaccharide maltotriose. Ptyalin action stops in stomach when pH falls to 3.0.

2. Digestion in stomach: Practically no action. ***No carbohydrate splitting enzymes available in gastric juice.*** Some dietary sucrose may be hydrolysed to equimolar amounts of glucose and fructose by HCl.

3. Digestion in duodenum: Food bolus reaches the duodenum from stomach where it meets the pancreatic juice. Pancreatic juice contains a carbohydratesplitting enzyme ***pancreatic amylase*** (also called ***amylopsin)*** similar to salivary amylase.

Action of Pancreatic Amylase It is also an α*-amylase*, optimum pH 7.1. Like *ptyalin* ***it also requires Cl– for activity.*** The enzyme hydrolyses α- 1→4 glycosidic linkage situated well inside polysaccharide molecule. Other criteria and end products of action similar to ptyalin.

4. Digestion in Small Intestine *Action of Intestinal Juice*

Intestinal amylase: This hydrolyses terminal α-1→4, glycosidic linkage in polysaccharides and oligosaccharide molecules ***liberating free glucose molecule.***

• ***Lactase:*** It is a β***-galactosidase,*** its pH range is 5.4 to 6.0. Lactose is hydrolysed to equimolar amounts of glucose and galactose.

Isomaltase: It ***catalyses hydrolysis of* α*-1→ 6 glycosidic linkage,*** thus splitting α***-limit dextrin*** at the branching points and producing **maltose** and **glucose**.

• ***Maltase:*** The enzyme hydrolyses the α-1→4 glycosidic linkage between glucose units in maltose molecule liberating equimolar quantities of two glucose molecules. Its pH range is 5.8 to 6.2.

Five maltases have been identified in intestinal epithelial cells. ***Maltase V*** can act as *isomaltase* over and above its action on maltose.

• ***Sucrase:*** pH range 5.0 to 7.0. It hydrolyses sucrose molecule to form equimolar quantities of glucose and fructose. ***Maltase III and maltase IV also have sucrose activity***

ABSORPTION OF CARBOHYDRATES It is observed from above that carbohydrate digestion is complete when the food materials reach small intestine and all complex dietary carbohydrates like starch and glycogen and the disaccharides are ultimately converted to simpler monosaccharides. All monosaccharides, products of digestion of dietary carbohydrates, are practically completely absorbed almost entirely from the small intestine.

Rate of absorption diminishes from above downwards; proximal jejunum three times greater than that of distal ileum. It is also proved that some disaccharides, which escape digestion, may enter the cells lining the intestinal lumen may be by ***pinocytosis;*** and are hydrolysed within these cells. No carbohydrates higher than the monosaccharides can be absorbed directly into the bloodstream in normal health and if administered parenterally, they are eliminated as foreign bodies.

• **Cori** studied the rate of absorption of different sugars from small intestine in rat. Taking glucose absorption as 100, comparative rate of absorption of other sugars were found as follows: The above study proves that ***glucose*** and ***galactose are absorbed very fast;*** fructose and mannose intermediate rate and the pentoses are absorbed slowly. ***Galactose is absorbed more rapidly than glucose.***

Two mechanisms are suggested: **1. Simple diffusion:** This is dependent on sugar concentration gradients between the intestinal lumen, mucosal cells and blood plasma. All the monosaccharides are probably absorbed to some extent by simple 'passive' diffusion. **2. "Active" Transport Mechanisms** • Glucose and galactose are absorbed very rapidly and hence it has been suggested that they are ***absorbed actively and it requires energy.***• Fructose absorption is also rapid but not so much as compared to glucose and galactose, but it is definitely faster than pentoses. Hence fructose is not absorbed by simple diffusion alone and it is suggested that some mechanism facilitates its transport, called as ***facilitated transport.*** **Wilson and Crane's Hypothesis of Active Transport** ***Wilson and Crane*** have shown that sugars which are 'actively' transported have several chemical features in common. They suggested that to be actively transported sugar must have the following:

• They must have ***a six-membered ring,*** • Secondly, they must have ***one or more carbon atoms attached to C 5,*** and • Thirdly, they ***must have a –OH group at C-2*** with the same stereoconfiguration as occurs in D-glucose. ***–OH group and 5 hydroxymethyl or methyl group on the pyranose ring appear to be essential structural requirements for the active transport mechanism.***

Energy: It is provided by ATP, by the interaction of the sodium dependent sugar carrier and the sodium pumps, actively transported sugars are concentrated within the cell without any back leakage of the sugar into the lumen. ***It is believed that sodium binding by the carrier-protein is pre-requisite for glucose binding.*** Sodium binding changes the conformation of the protein molecule, enabling the binding of glucose to take place and thus the absorption to occur. It is presumed that analogous "carrier protein" exists for D-galactose also.

Glucose Transporters (GLUT) Several glucose transporters GLUT-1 to 7 have been described in various tissues.

Evidences in favour of the cotransport system of glucose absorption:
• The dependence of the active transport of glucose upon the presence of sodium ions has been demonstrated in isolated loops of rat intestine ***by replacing the sodium of bathing fluid by K+ and lithium. Under these circumstances, the rate of glucose transport is markedly reduced and ultimately stopped.***
• Drugs such as ***strophanthin*** and ***ouabain*** which inhibit sodium pump also inhibit active transport of sugars.

	Type of glucose transporter	*Tissue locations*	*Functions*
a.	**Sodium dependent transporter SGLT1**	Small intestine and kidney	Active uptake of glucose against a concentration gradient. Requires ATS for energy and Na^+
b.	**Facilitative bidirectional transporters:**		
	GLUT-1	Red blood cells, brain, kidney, colon, placenta	Glucose uptake independent of insulin.
	GLUT-2	Liver, kidney, small intestine, β-cells of pancreas	Rapid uptake or release of glucose.
	GLUT-3	Brain, kidney, placenta	Glucose uptake
	GLUT-4	Skeletal muscle, adipose tissue, heart	Insulin stimulated glucose uptake.
	GULT-5	Small intestine, testes sperms	Poor ability to transport glucose. Fructose transporter
	GLUT-7	Liver, endoplasmic reticulum	Transport glucose from ER to cytoplasm

Absorption of Other Sugars • Sugars like D-fructose and D-mannose are probably absorbed by ***facilitated transport*** which ***requires the presence of carrier protein but does not require energy.***
• Other sugars like ***pentoses*** and ***L-isomers*** of glucose and galactose are ***absorbed passively by simple diffusion.***

Facilitated Transport Vs Active Transport

Similarities
• Both appear to involve carrier proteins. • Both show specificity. • Both resemble a substrate-enzyme type of reaction. • Both have specific binding sites for solutes. • 'Carrier' is saturable so it has maximum rate of transport. • There is a binding constant for solute. • Structurally similar competitive inhibitors block transport.

Differences
• ***Facilitated transport can act bi-directionally, whereas active transport is unidirectional.*** • Active transport always occurs against an electrical or chemical gradient and hence requires energy.
Facilitated transport does not require energy. Mechanism of Facilitated Transport ***Ping-Pong' mechanism*** explains facilitated transport. • **Carrier Protein *exists in two principal conformations*** depending on the solute concentration. ***Two forms are:*** **– Pong *state,*** and **– Ping *state.***
• In the **Pong *state,*** it is exposed to high concentrations of solutes, and molecules of solutes bind to specific sites on the 'carrier protein'. This occurs in lipid bilayer of the cell with high solute concentration. • In inner side, a conformational change occurs to **Ping *state*** and the solute is discharged to the side favouring new equilibrium. • The empty carrier protein then reverts to the original conformation "Pong" state to complete the cycle.

Factors determining facilitated transport: Rate at which solutes enter a cell by facilitated transport is determined by the following factors: • Concentration gradient across the membrane. • The amount of "Carrier protein" available (key control system). • Rapidity of solute-carrier interaction. • Rapidity of conversion of conformation state from 'Pong' to 'Ping' and *vice versa.*

Factors Influencing Rate of Absorption

1. State of mucous membrane and length of time of contact: If mucous membrane is not healthy, absorption will suffer. Similarly in hurried bowel, length of contact is less and as such absorption will be less.

2. Hormones • ***Thyroid hormones:*** These increase absorption of hexoses and act directly on intestinal mucosa. • ***Adrenal cortex: Absorption decreases in adrenocortical deficiency,*** mainly due to decreased concentration of sodium in body fluids • ***Anterior pituitary:*** This affects absorption mainly through its influence on thyroids. Hyperpituitarism induces thyroid overactivity and *vice versa.* • ***Insulin:*** This ***has no effect on absorption of glucose.***

3. Vitamins: Absorption is diminished in states of deficiency of B-vitamins, viz, thiamine, pyridoxine and pantothenic acid. ***Inherited enzyme deficiencies:*** Inherited enzyme deficiencies like sucrase and lactase can interfere with hydrolysis of corresponding disaccharides and their absorption.

Lactase Deficiency Some infants may have deficiency of the enzyme lactase and they show intolerance to lactose, the sugar of milk. Symptoms and signs seen in affected infants include: • Diarrhoea and flatulence • Abdominal cramps • Distension. Explanation: The above features are explained as follows: • As lactose of milk cannot be hydrolysed due to deficiency of lactase enzyme, there occurs accumulation of lactose in intestinal tract, which is osmotically active and holds water, producing diarrhoea. • Accumulated lactose is also fermented by intestinal bacteria which produce gas and other products, producing flatulence, distension and abdominal cramps.

Sucrase Deficiency Inherited deficiency of sucrase and isomaltase have been reported. Symptoms occur in early childhood with ingestion of sugars (cane sugar and table sugar) sucrose, a disaccharide. Symptoms and signs as in lactase deficiency.

3. Disaccharidurıa An increase in the excretion of disaccharides may be observed in some patients with disaccharidase deficiency. As much as 300 mg or more of disaccharides may be excreted in those people and in patients with intestinal damage (e.g. sprue and celiac disease). 4. Monosaccharide Malabsorption Inherited disorders in which glucose and/or galactose are absorbed very slowly have been reported. The reason probably is absence of "carrier protein" necessary for absorption of glucose/galactose

Utilisation of Glucose in the Body General Outline After absorption of monosaccharides into the portal blood, it passes through the liver ***(the first 'filter')*** before entering the systemic circulation, a fact of considerable physiological and biochemical importance. ***In liver two mechanisms operate:***

• ***Withdrawal*** of carbohydrates from blood and • ***Release*** of glucose by liver to the blood. These two mechanisms are shown below in a tabular form in the box. All the above processes are finely regulated in the Liver cells, control exerted at substrate level, by the end products and by hormones. ***The amount of glucose reaching the systemic circulation at any instant, will be the resultant of operation of these two groups of opposing forces.*** Once glucose is in systemic circulation, it becomes available for its utilisation by "extrahepatic tissues". Thus extrahepatic tissues are presented with carbohydrates which have already been "picked over" by the liver in a selective manner.

Hence functional state of the liver will be of prime importance and will have a profound influence on the carbohydrate metabolism on the entire organism. Glucose is taken up by intestinal mucosal cells and kidney tubule cells by "active" transport. ***Hepatic cells are freely permeable to glucose.*** Insulin increases uptake of glucose by many extrahepatic tissues as skeletal muscle, heart muscle, diaphragm, adipose tissue, lactating mammary gland, etc.

GLYCOLYSIS

Definition: Oxidation of glucose or glycogen to pyruvate and lactate is called glycolysis. This was described by **Embden, Meyerhof** and **Parnas.** Hence, it is also called as **Embden Meyerhof pathway**. Process of fermentation in yeast cells was similar to breakdown of glycogen in muscles. ***It occurs virtually in all tissues. Erythrocytes and nervous tissues derive its energy mainly from glycolysis.*** This pathway is unique in the sense that it can utilise O2 if available (aerobic) and it can function in absence of O2 also (anaerobic).

Two Phases of Glycolysis • ***Aerobic phase:*** Oxidation is carried out by dehydrogenation and reducing equivalent is transferred to NAD+. Reduced NAD in presence of O2 is oxidized in electron-transport chain producing ATP. • ***Anaerobic phase:*** NADH cannot be oxidised in electron transport chain, so ***no ATP is produced in electron transport chain.*** But the NADH is oxidized to NAD+ by conversion of pyruvate to lactate, without producing ATP. ***Anaerobic phase limits the amount of energy per mol. of glucose oxidised. Hence, to provide a given amount of energy, more glucose must undergo glycolysis under anaerobic as compared to aerobic.***

Enzymes: Enzymes involved in glycolysis are ***extra mitochondrial.*** • This pathway is meant for provision of energy. • It has importance in skeletal muscle as glycolysis provides ATP even in absence of O2. Muscles can survive anoxic episodes. • Heart muscle: As compared to skeletal muscle, heart muscle is adapted for aerobic performance. It has relatively poor glycolytic activity and poor survival under conditions of ischaemia. • Role in cancer therapy: In fast-growing cancer cells, rate of glycolysis is very high. Produces more pyruvic acid (PA) than TCA cycle can handle. Accumulation of pyruvic acid leads to excessive formation of lactic acid producing local lactic acidosis. Local acid environment may be congenital for certain cancer therapy. • Haemolytic anaemias: Inherited enzyme deficiencies like hexokinase deficiency and pyruvate kinase deficiency in glycolytic pathway enzymes, can produce haemolytic anaemia. Series of reactions of glycolytic pathway which degrades glucose/glycogen to pyruvate/lactate are discussed below. For discussion and proper understanding, the various reactions can be ***arbitrarily*** divided into ***four stages.***

Stage I

This is a ***preparatory stage.*** Before the glucose molecule can be split, the ***rather asymmetric glucose molecule is converted to almost symmetrical form*** fructose 1,6- biphosphate by donation of 2 PO4 groups from ATP.

1. Uptake of glucose by cells and its phosphorylation: Glucose is freely permeable to Liver cells. Insulin facilitates the uptake of glucose in skeletal muscles, cardiac muscle, diaphragm and adipose tissue. Glucose is then phosphorylated to form glucose-6-P. The reaction is catalysed by the specific enzyme ***glucokinase*** in liver cells and by non-specific ***hexokinase*** in liver and extrahepatic tissues *(Refer second box in right hand side this page).*

Note

• **Reaction is *irreversible*** – ATP acts as PO 4 donor and it reacts as Mg-ATP complex. One high energy PO4 bond is utilised and ADP is produced. The reaction is accompanied by considerable loss of free energy as heat, and hence under physiologic conditions is regarded as irreversible. – Glucose-6-P formed is an important compound at the junction of several metabolic pathways like glycolysis, glycogenesis, glycogenolysis, gluconeogenesis, HMP-Shunt, uronic acid pathway. Thus it is a ***committed step*** in metabolic pathways.

2. Conversion of G-6-P to fructose-6-P: G-6-P after formation is converted to fructose-6-P by ***phosphohexose isomerase,*** which involves an aldose-ketose isomerisastion. The enzyme can act only on α-anomer of G-6-P.

3. Conversion of fructose-6-P to fructose-1, 6-bi-P: The above reaction is followed by another phosphorylation. Fructose-6-P is phosphorylated with ATP at 1-position catalysed by the enzyme ***phosphofructokinase-1*** to produce the **symmetrical molecule** fructose-1,6-bi-phosphate.

Note • The reaction is ***irreversible.*** • One ATP is utilised for phosphorylation. • ***Phosphofructokinase-1*** is the ***key enzyme*** in glycolysis which regulates breakdown of glucose. The enzyme is ***inducible,*** as well as ***allosterically modified.*** • *Phosphofructokinase-2* is an isoenzyme which catalyses the reaction to form fructose-2,6-bi-phosphate.

Energetic: Note that in this stage glucose oxidation does not yield any useful energy rather **there is expenditure of 2 ATP molecules for two phosphorylations (–2 ATP).**

Stage II ***Actual Splitting of Symmetrical Fructose-1-6-bi-P.*** Fructose-1,6-bi-P is split by the enzyme ***aldolase*** into two molecules of triose-phosphates, an aldotriose–glyceraldehyde-3-P and one ketotriose, Dihydroxy acetone-P.

Note

• The reaction is ***reversible*** and there is ***neither expenditure of energy nor formation of ATP*** • ***Aldolases*** are tetramers, containing 4 subunits. Two isoenzymes: ***Aldolase A:*** Occurs in most tissues, ***Aldolase B:*** Occurs in liver and kidney • The fructose-6-P exists in the cells in ***furanose*** formbut they react with isomerase, phosphofructokinase-1 and aldolase in the open-chain configuration • Both triose phosphates are interconvertible.

Inhibitors

• ***Bromohydroxyacetone-P:*** It **resembles structurally to dihydroxyacetone-P.** Hence it binds covalently with the γ-COOH group of a glutamate residue of the enzyme ***phosphotriose isomerase***

at the active site of the enzyme molecule. Thus the enzyme becomes inactive and cannot catalyse the reaction. It blocks glycolysis at the stage of dihydroxyacetone-P and leads to accumulation of dihydroxyacetone-P and fructose-1,6-bi-phosphate.

Hexokinase	Glucokinase
1. Non-specific, can phosphorylate any of the hexoses	1. Specific, can phosphorylate glucose only
2. More stable	2. Physiologically more labile
3. Found almost in all tissues	3. Found only in liver
4. Found in foetal as well as in adult liver	4. Found in adult liver, not in foetal liver
5. Allosteric inhibition by glucose-6-P	5. Not inhibited by Glucose-6-P
6. Km is low = 0.1 mM, hence high affinity for glucose	6. Km is high = 10 mM, low affinity for glucose
7. Not very much influenced by diabetic state/or fasting patients of DM, changes according to nutritional status	7. Depressed in fasting and in diabetes. Glucokinase is deficient in
8. No change with glucose feeding	8. Increased by feeding of glucose after fasting

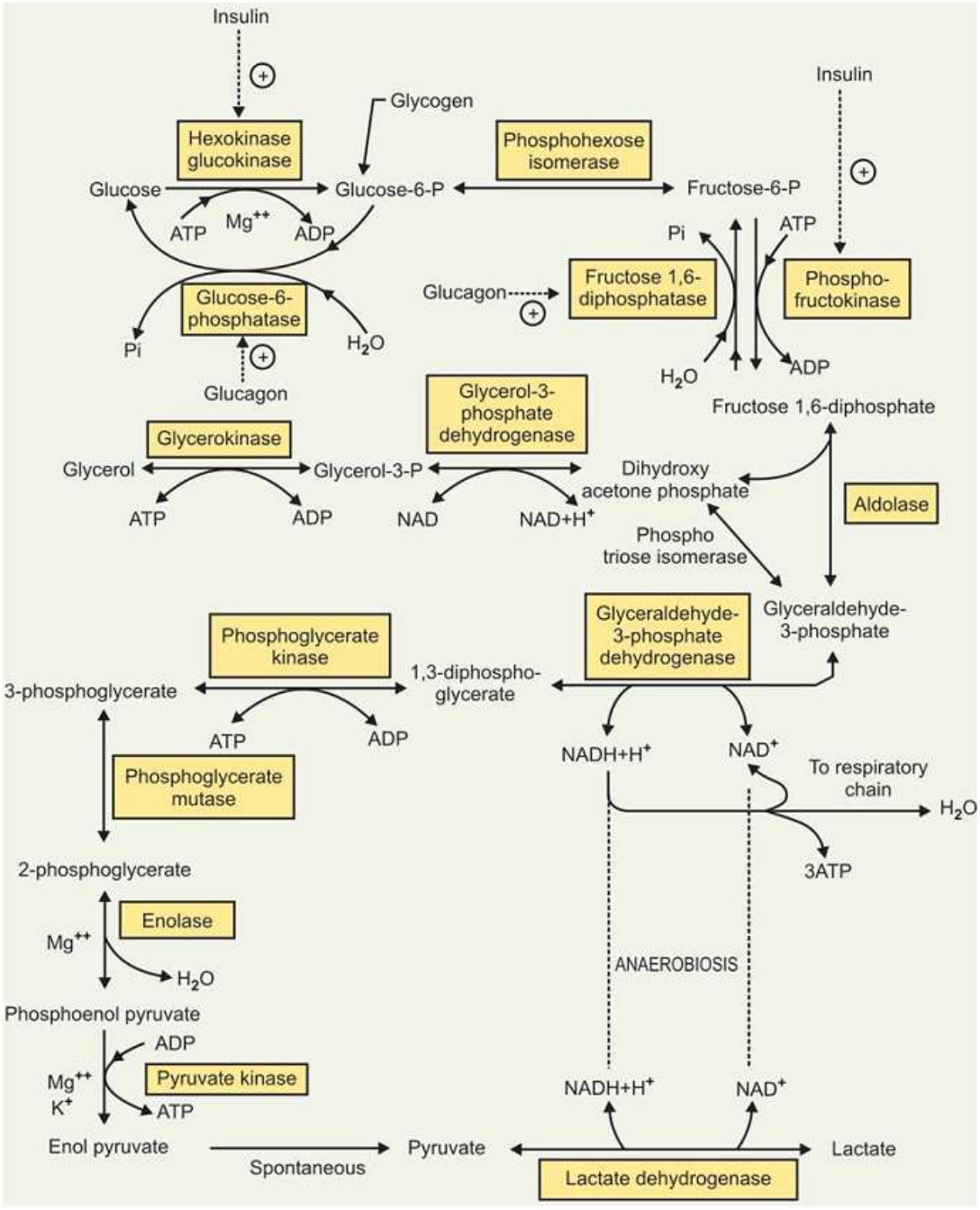

Fig. 23.1: Embden-Meyerhof pathway of glycolysis

Stage III

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

This stage consists of the following ***two reactions:***

1. Oxidation of glyceraldehyde-3-P to 1,3-bi-phosphoglycerate: Glycolysis proceeds by the oxidation of glyceraldehyde-3-P to form 1,3-bi-phosphoglycerate. Dihydroxyacetone-P also form 1,3-bi-phosphoglycerate via glyceraldehyde-3-P. Enzyme responsible is ***Glyceraldehyde-3-P***

dehydrogenase which is NAD+ dependant.

Characteristics of the Enzyme

• The enzyme is a ***tetramer,*** consisting of four identical polypeptides.
• Four –SH groups are present on each polypeptide derived from cysteine residue in the chain.
• One of the –SH group forms the "active site" of the enzyme molecule.

2. Conversion of 1,3-Biphosphoglycerate to 3-Phosphoglycerate The reaction is catalysed by the enzyme ***phosphoglycerate kinase***. The high energy PO4 bond at position-1 can donate the PO4 to ADP and forms ATP molecule.

Note: ***This is a unique example where ATP can be produced at substrate level*** without participating in electron transport chain. This type of reaction where ATP is formed at substrate level is called as ***Substrate level phosphorylation.***

Inhibitors

• ***Arsenite***

If present, it competes with inorganic Pi in the reaction of conversion of glyceraldehyde-3-P to 1,3 biphosphoglycerate and produces 1-arseno-3- phosphoglycerate, which hydrolyses spontaneously to yield 3-phosphoglycerate and heat. ***Thus in the next step no ATP is produced.*** This is an important example of the ability of arsenate to uncouple oxidation and phosphorylation.

• ***Iodoacetate and Iodoacetamide***

They bind covalently with –SH group and alkylate the –SH group of the enzyme *glyceraldehyde-3-P dehydrogenase.* They bind irreversibly with the enzyme and inhibits glycolysis. **This leads to accumulation of glyceraldehyde-3-P.**

Energetics

1. In first reaction of this stage —NADH produced in presence of O2 will be oxidised in electron transport chain to produce 3 ATP. Since two molecules of trioseP are formed per molecule of glucose oxidized 2 NADH will produce 6 ATP. +

2. The second reaction will produce one ATP. Two molecules of substrate will produce 2 ATP.
Net gain at this stage per molecule of glucose oxidised = + 8 ATP.

Stage IV

It is the ***recovery of the PO4 group*** from 3-Phosphoglycerate. The two molecules of 3 phosphoglycerate, the end-product of the previous stage, still retains the PO4 group originally derived from ATP in stage 1. Body wants back the two ATP spent in first stage for two phosphorylations. This is achieved by the following ***three reactions:***

1. Conversion of 3-Phosphoglycerate to 2-Phosphoglycerate 3-phosphoglycerate formed by the above reaction is converted to 2-phosphoglycerate, catalysed by the enzyme ***Phosphoglycerate mutase***. It is likely that 2, 3-bi-phosphoglycerate is an intermediate in the reaction and probably acts catalytically.

2. Conversion of 2-Phosphoglycerate to Phosphoenol Pyruvate The reaction is catalysed by the enzyme ***Enolase,*** the enzyme requires the presence of either Mg++ or Mn++ for activity. The reaction ***involves dehydration and redistribution of energy*** within the molecule raising the PO4 in position 2 to a "high-energy state".

3. Conversion of Phosphoenol Pyruvate to Pyruvate Phosphoenol pyruvate is converted to

'Enol' pyruvate, the reaction is catalysed by the enzyme ***Pyruvate kinase.*** The high energy PO4 of phosphoenol pyruvate is directly transferred to ADP producing ATP (Refer box).

Note

• Reaction is ***irreversible.*** • ATP is formed at the substrate level without electron transport chain. This is another example of ***substrate level phosphorylation*** in glycolytic pathway • "Enol" pyruvate is converted to `keto' pyruvate spontaneously.

Inhibitors Fluoride inhibits the enzyme enolase.

Sodium fluoride is used along with K-oxalate for collection of blood for glucose estimation. If K-oxalate is used alone, then in vitro glycolysis will reduce the glucose value in the sample.

Functions of Fluoride • Inhibits in vitro glycolysis by inhibiting enzyme enolase • Also acts as anticoagulant, and • Act. as an antiseptic.

B. In Glycolysis—in Absence of O2 (Anaerobic Phase)

• In absence of O2, reoxidation of NADH at glyceraldehyde-3-P-dehydrogenase stage cannot take place in electron-transport chain. • But the cells have limited coenzyme. Hence to continue the glycolytic cycle NADH must be oxidised to NAD+. This is achieved by reoxidation of NADH by conversion of pyruvate to lactate **(without producing ATP)** by the enzyme lactate dehydrogenase.

• It is to be noted that in the reaction catalysed by glyceraldehyde-3-P-dehydrogenase, therefore, no ATP is produced. ***In anaerobic phase per molecule of glucose oxidation 4 – 2 = 2 ATP will be produced.***

• Tissues that function under hypoxic circumstances will produce lactic acid from glucose oxidation, producing local acidosis. If lactate production is more it can produce metabolic acidosis. • Vigorously contracting skeletal muscle will produce relative anaerobiosis and glycolysis will produce lactic acid. • Whether O 2 is present or not, glycolysis in erythrocytes always terminate in pyruvate and lactate. • When there is relative anaerobiosis, glycolysis will stop as cells will exhaust NAD+. • Inhibitor of Lactate Dehydrogenase (LDH) is Oxamate: It competitively inhibits lactate dehydrogenase and prevents the reoxidation of NADH.

REGULATION OF GLYCOLYSIS

Regulation of glycolysis achieved by **three types of mechanisms**

(a) Changes in the rate of enzyme synthesis, Induction/ repression.
(b) Covalent modification by reversible phosphorylation.
(c) Allosteric modification.

(a) Induction and repression of key enzymes: This is not rapid and takes several hours to come into operation.

• ***Glucose:*** When there is increased substrate, i.e. glucose, the enzymes involved in utilisation of glucose are activated. On the other hand, enzymes responsible for producing glucose (gluconeogenesis) are inhibited. Glucose also increases the activity of the key enzymes *glucokinase, phosphofructokinase-1* and *pyruvate kinase.*

• ***Insulin:*** The secretion of insulin which is responsive to blood glucose concentration enhances the synthesis of the key enzymes responsible for glycolysis. On the other hand, it antagonises the effects of glucocorticoids and glucagon-stimulated c-AMP in stimulating the key enzymes

responsible for gluconeogenesis.

(b) Covalent modification by reversible phosphorylation: Hormones like epinephrine and glucagon which increase cAMP level activate cAMP-dependant *Protein kinase* which can phosphorylate and inactivate the Key enzyme *Pyruvate kinase* and, thus, inhibit glycolysis. ***This is a rapid process and occurs quickly.***

(c) Allosteric modification: *Phosphofructokinase-1* is the Key regulatory enzyme and is subject to *"feedback"* control.

• ***Inhibition of the enzyme:*** The enzyme is inhibited by citrate and by ATP.

• ***Activator of the enzyme:*** The enzyme is activated by AMP.

• ***AMP acts as the indicator of energy status of the cell:*** When ATP is used in energy requiring processes resulting in formation of ADP, the concentration of AMP increases. Normally ATP concentration may be fifty times that of AMP concentration at equilibrium, a small decrease in ATP concentration will cause a several fold rise in AMP concentration. Thus a large change in AMP concentration acts as a metabolic amplifier of a small change in ATP concentration. The above mechanism allows the activity of the enzyme *phosphofructokinase-1* to be highly sensitive to even small changes of energy status of the cell and hence it controls the amount of glucose that should undergo
glycolysis prior to its entry as acetyl-CoA in TCA cycle.

• ***In hypoxia:*** The concentration of ATP in the cells decreases and there is increase in concentration of AMP which explains why glycolysis should increase in absence of O2.

PECULIARITIES OF GLUCOSE OXIDATION BY RB CELLS AND RAPOPORT-LUEBERING SHUNT

RB cells are structurally and metabolically unique as compared to other cells.

A. Structural Peculiarities: Structurally mature erythrocytes ***do not possess*** **nucleus nor cytoplasmic subcellular structures.**

B. Metabolic peculiarities: Metabolically mature erythrocytes:

• Entirely ***depends on glucose for its energy***, i.e. glycolysis. More than > 90 per cent of total energy is met by glycolysis.

• Glucose is ***freely permeable*** to erythrocytes like Liver cells.

• Glucose oxidation always ends in formation of pyruvic acid (PA) and lactic acid (LA), whether O2 is available or not.

• The enzyme ***pyruvate dehydrogenase complex is absent*** hence Pyruvic acid is not converted to "acetyl-CoA".

RAPOPORT-LUEBERING SHUNT OR CYCLE

Maintains a high steady state concentration of **2,3-biphosphoglycerate (2,3-BPG)**, produced by a diversion in glycolytic pathway. This diversion is called as **Rapoport-Luebering cycle or shunt (RLC or RLS).** A supplementary to glycolysis.

Functional Significance of this Shunt Pathway (a) ***Factors which waste energy are not present in RB Cells***

• Energy demanding ***endergonic*** reactions utilizing ATP is not present in mature human red blood cells. • *ATPase* activity which controls ATP/ADP ratio is not active in mature RB Cells. RB cells utilise more glucose than it requires to maintain cellular integrity, resulting in accumulation of ATP and 1,3-BPG, causing cessation of glycolysis. ***RLC or RLS provides a***

mechanism to dissipate the excess energy. RL shunt/cycle is shown schematically in the box below.

(b) *Role in Hb*

• **Adult Hb-A**

1: 2,3-BPG concentration is high, affinity to O2 less and unloading/dissociation is more.

• **Hb-F:** 2,3-BPG concentration is low, affinity to O2 is more, and unloading/dissociation is less.

(c) Role in hypoxia: Tissue hypoxia has an important effect on the level of Red cells BPG. Pulmonary hypoxic hypoxia, stagnant hypoxia either as a result of CV failure or shock, and anaemic hypoxia, as in a deficit of red cells mass, ***all favour an increase in red cells BPG level, thus enhancing unloading of O2 in tissues.*** When normal individuals ascend to a height of 450 metres, there is a rise in red cells BPG level. The maximum rise occurs by 48 hours and returns to normal within a similar period after descent to sealevel.

(d) Inherited enzyme deficiency: Several hereditary defects in enzyme of red-cell glycolysis that affect red cell BPG concentration such as rare ***hexokinase deficiency*** and much more commonly occurring ***pyruvate kinase (PK) deficiency,*** also exhibit alterations of red cells BPG concentration. In a patient with red cell **hexokinase *deficiency*** a decrease in BPG concentration to about 2/3 of normal has been reported. In ***PK deficiency*** (pyruvate kinase) BPG is more than twice normal. As a result, affinity for oxygen of Hb is greater than normal in 'hexokinase' deficiency and less than normal in 'pyruvate kinase' deficiency.

	Hexokinase deficient red cells	*Pyruvate kinase deficient red cells*
• 2,3-BPG	↓	↑
• Affinity to O_2	↑	↓
• Unloading of O_2	↓	↑

FORMATION AND FATE OF PYRUVIC ACID

• From oxidation of glucose (Glycolysis)
• From lactic acid by oxidation
• Deamination of Alanine
• Glucogenic amino acids-pyruvate forming
• Decarboxylation of oxaloacetic acid (OAA)

Pyruvic acid is a key substance in phase-II metabolism.

1. Principally it is formed from **oxidation of glucose** (glycolysis) by EM Pathway. In addition to that pyruvic acid can be formed in the body from various other sources. They are:

2. Conversion of lactic acid to pyruvic acid (see below).

3. Also formed from **deamination** of amino acid **alanine**.

4. Certain other amino acids during their catabolism produces pyruvic acid, e.g. ***glycine, serine, cysteine/ and cystine*** and ***threonine (Glucogenic a-a).***

5. Pyruvic acid can also be formed from ***decarboxylation*** of dicarboxylic ketoacid **oxaloacetic**

acid, which can be ***spontaneous decarboxylation*** or can be ***catalyzed by the enzyme*** *oxaloacetate decarboxylase.*
6. Lastly pyruvic acid can be formed in the body from **malic acid** by *malic enzyme.*

• Forms acetyl-CoA by oxidative decarboxylation (in presence of O2) • Forms lactic acid by reduction (in absence of O2) • Forms alanine by amination • Forms glucose (gluconeogenesis) • Forms malic acid →□□□□□□□

Fate of pyruvic acid depends on the redox state of the tissues: • ***In Presence of O2:*** Pyruvic acid is oxidatively decarboxylated to two-carbon unit "Acetyl-CoA" • ***In absence of O2:*** Pyruvic acid is converted to Lactic acid (LA) Other fates of Pyruvic acid can be summed up as follows: • Pyruvic acid can be **aminated** to form the amino acid alanine • Pyruvic acid can be **converted to form glucose** in the body • Pyruvic acid can be converted to malic acid, which in turn can form oxaloacetic acid (OAA) • Pyruvic acid can be converted directly to oxaloacetic acid in the body by ***CO2-fixation (CO2-assimilation) reaction.*** Last two reactions are important in the body as OAA can be formed and supplied to TCA cycle in case of relative deficiency (see "anaplerotic" reactions below).
1. Conversion of Pyruvic Acid to Lactic Acid It is an important reaction, because it occurs in skeletal muscles working under conditions of absolute or relative lack of O 2. In anaerobic glycolysis, Pyruvate acts as a temporary H-store. It dehydrogenates (oxidises), the reduced NADH + H+ back to oxidized NAD+, so that glycolysis can continue even in absence of O2. ***Pyruvate is thus reduced to Lactic acid. In presence of O2, Lactic acid can be oxidised to pyruvic acid again.***
Characteristics of this reaction:
• ***Reversible*** reaction
• Oxidation-reduction
• Same enzyme and co-enzyme required.

Anaplerotic Reactions

A sudden influx of Pyruvic acid (PA) or of "acetyl-CoA" to the TCA cycle might seriously deplete the supplies of OAA required for the citrate synthase reaction. Two reactions that are auxiliary to the TCA cycle operate to prevent this situation. These are known as ***anaplerotic (Filling up)*** reactions.

These **two reactions** are:
2. Conversion of PA to OAA (by CO2-Fixation Reaction) Pyruvic acid can be converted to oxaloacetate by the enzyme ***Pyruvate carboxylase***. The enzyme requires:
• 'Biotin' as a prosthetic group which brings CO2
• ATP and Mg++
• Requires 'acetyl-CoA'
Acetyl-CoA does not enter into the reaction but may by combination with the enzyme maintains it in "active" conformation ***(+ve modifier).*** The generation of "acetylCoA" in metabolic reactions activates the enzyme and promote the formation of oxaloacetic acid (OAA) required for oxidation of acetyl-CoA in the TCA Cycle.
3. Conversion of PA to OAA Through Malic Acid Formation The other anaplerotic reaction is

formation of malic acid by *Malic enzyme* in presence of CO2 and NADPH. The 'malate' is converted to OAA by dehydrogenation by the enzyme ***Malate dehydrogenase*** in presence of NAD+.

4. Conversion of PA to Acetyl-CoA In presence of O2, Pyruvate undergoes oxidative decarboxylation to form 2-C compound 'acetyl-CoA'

Pyruvate formed in cytosol is transported to mitochondrion by a 'transport' protein. Since the overall reaction involves both oxidation and loss of CO2 (decarboxylation) it is termed oxidative decarboxylation.The mechanism of the reaction is one of the most complex involved in metabolism of carbohydrates. The reaction is catalysed by a ***multienzyme complex*** called ***pyruvate dehydrogenase complex***, which can exist both as "inactive form" and the "active" form (see regulation below):

The enzyme complex consists of: ***29 molecules of Pyruvate dehydrogenase (PD) + 8 molecules of Flavoprotein containing dihydrolipoyl dehydrogenase, and + 1 molecule of dihydrolipoyl transacetylase. The enzyme complex for its activity requires at least six coenzymes/cofactors:*** **• Thiamine pyrophosphate (TPP) • Lipoic acid • CoA–SH • FAD • NAD+ and• Mg++**

The overall reaction can be represented as follows:

1. 'Acetyl' moiety of PA is transferred to CoA –SH.

2. Carbon of COOH group is liberated as CO2 (decarboxylation).

3. Remaining two H atoms: One from –COOH group of PA and another from CoA –SH (–SH group) are transferred to NAD+, by way of a mechanism involving Lipoic acid and FAD.

Details of reactions: Reaction is not so simple as shown in left hand side column. The reaction is catalysed by several different enzymes working sequentially in multienzyme complex. • Pyruvate is decarboxylated to a hydroxyethyl derivative of the thiazole ring of enzyme bound TPP. • Which in turn, reacts with oxidised lipoamide to form *acetyl lipoamide*. In the presence of ***dihydrolipoyl transacetylase***, acetyl lipoamide reacts with CoA-SH to form "Acetyl-CoA" and reduced lipoamide. • The latter is reoxidised by a FP in the presence of ***dihydrolipoyl dehydrogenase.*** • Finally, the reduced FP is oxidised by NAD+, which in turn transfers the reducing equivalent to the 'electron transport chain'. The sequence of events that occur is shown schematically