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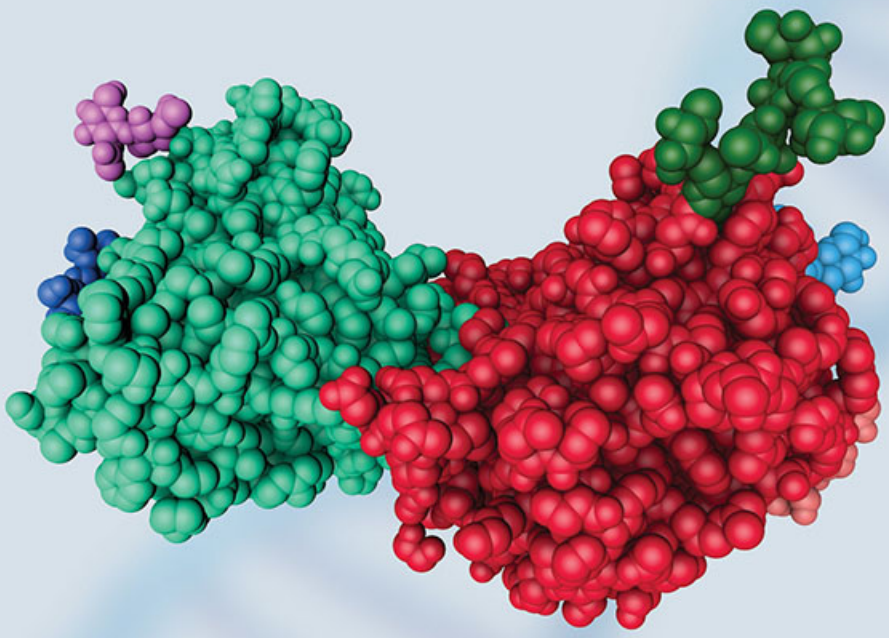


BIOCHEMISTRY

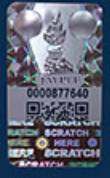
A Clinically Integrated Approach with Case Scenarios and Clinical Applications

Updated self-assessment



As per the Revised Competency-based Medical Education Curriculum (NMC)

Pankaja Naik



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Contents

1. Cell, its Organelles and Transport Across Cell Membranes 1

- Cell Types and Subcellular Components *1*
- Structure and Functions of Cell Membrane *3*
- Structure and Functions of Subcellular Organelles *6*
- Cytoskeleton *11*
- Cell Fractionation and Marker Enzymes for Different Organelles *12*
- Membrane Transport *13*

2. Chemistry of Carbohydrates 19

- Definition, Functions and Classification of Carbohydrates *19*
- Monosaccharide: Structure and Isomerism *22*
- Biologically Important Monosaccharide Derivatives *24*
- Glycoside and its Importance *27*
- Disaccharides *27*
- Polysaccharides (Glycans) *29*
- Mucopolysaccharidoses *33*

3. Chemistry of Lipids 40

- Lipids: Definition, Classification and Functions *40*
- Fatty Acids: Definition, Functions, and Classification *42*
- Essential Fatty Acids: Importance and Deficiency Manifestations *45*
- Triglycerides: Composition and Importance *46*
- Phospholipids: Composition, Types and Clinical Importance *47*
- Sphingolipids: Composition, Types and Functions *51*
- Lipoproteins: Composition, Types and Importance *53*
- Cholesterol: Structure and Importance *56*

4. Chemistry of Amino Acids and Proteins 60

- Amino Acids: General Characteristics and Classification *60*
- Properties of Amino Acids *67*
- Biologically Important Peptides *70*
- Protein: Definition, Classification, and Functions *72*
- Structural Organization of Proteins *75*
- Denaturation of Proteins *83*
- Structure-Function Relationship of Proteins *84*

5. Plasma Proteins 87

- Plasma Proteins and their Functions *87*
- Major Classes of Plasma Proteins and Clinical Importance *89*
- Acute Phase Proteins *93*
- Diagnostic Importance of Electrophoretic Pattern of Serum Proteins *94*

6. Enzymes 99

- Enzymes—Definition and General Properties *99*
- Iubmb Classification and Nomenclature of Enzymes *100*
- Cofactors and Coenzymes *103*
- Mechanism of Enzyme Activity and Enzyme Specificity *104*
- Enzyme Kinetics: Factors Affecting the Enzyme Activity *106*
- Enzyme Inhibition *112*
- Isoenzyme: Definition, Examples and Diagnostic Importance *118*
- Diagnostically Important Serum Enzymes *120*

7. Chemistry of Hemoglobin and Hemoglobinopathies 131

- Structure and Functions of Hemoglobin *131*
- Binding Sites for Oxygen, Hydrogen (H^+) and Carbon Dioxide (CO_2) with Hemoglobin *132*
- Cooperative Oxygen Binding of Hemoglobin *133*
- Effect of 2,3-Bisphosphoglycerate on Binding of Oxygen to Hemoglobin *134*
- Types of Normal Human Hemoglobin *135*
- Derivatives of Hemoglobin *136*
- Hemoglobinopathies *140*

8. Biological Oxidation 149

- Biochemical Processes Involved in Generation of ATP in Cells *149*
- Electron Transport Chain *150*
- Oxidative Phosphorylation *152*
- Inhibitors of Electron Transport Chain and Oxidative Phosphorylation *153*
- Shuttle Systems for Oxidation of Extra Mitochondrial NADH *155*
- Disorders of Mitochondrial Electron Transport and Oxidative Phosphorylation *156*

9. Vitamins 163

- Classification of Vitamins *163*
- Water-Soluble Vitamins and their Deficiency Manifestations *165*
- Fat Soluble Vitamins and their Deficiency Manifestations *189*

10. Metabolism of Carbohydrates 206

- Digestion, Absorption, Transport of Carbohydrates *206*
- Disorders of Digestion and Absorption of Carbohydrates *210*
- Metabolic Fate of Carbohydrates *212*
- Glycolysis *213*
- Metabolic Fates of Pyruvate *223*

- Citric Acid Cycle 226
- Gluconeogenesis 231
- Glycogen Metabolism 236
- Hexose Monophosphate Pathway 250
- Uronic Acid Pathway 255
- Galactose Metabolism 257
- Fructose Metabolism 259
- Regulation of Blood Glucose Level in Fed and Fasting State 260
- Diabetes Mellitus 263
- Laboratory Investigation for Diagnosis of Diabetes 269

11. Metabolism of Lipids 279

- Digestion, Absorption, and Transport of Dietary Lipids 279
- Lipoprotein Metabolism and Associated Disorders 284
- Triacylglycerol Metabolism 299
- Fatty Acid Oxidation 301
- Ketone Bodies Metabolism 309
- Biosynthesis of Fatty Acids 314
- Cholesterol Metabolism 319
- Atherosclerosis, Fatty Liver, Cholelithiasis and Obesity 323
- Eicosanoids 328
- Lipid Profile Tests and Interpretations with Various Disorders 332

12. Metabolism of Proteins 340

- Digestion and Absorption of Proteins 340
- Amino Acid Pool 343
- Stages of Catabolism of Amino Acids 345
- Formation and Transport of Ammonia 347
- Urea Cycle 350
- Metabolism of Amino Acids 355
- Biogenic Amines and Polyamines 386
- Newborn Screening 389

13. Integration of Metabolism 394

- Definition and Significance of Integration of Metabolism 394
- Integration of Metabolism in Well Fed and Fasting States 395

14. Metabolism of Hemoglobin 405

- Biosynthesis of Heme 405
- Disorders of Heme Biosynthesis 408
- Catabolism of Heme 415
- Hyperbilirubinemia 416

15. Minerals 424

- Sodium and Potassium 424
- Calcium 426

- Phosphorus 432
- Magnesium 433
- Copper 433
- Fluorine 438
- Iodine 438
- Iron 439
- Molybdenum 444
- Selenium 445
- Zinc 445

16. Water and Electrolyte Balance 450

- Total Body Water and its Distribution 450
- Electrolytes and their Distribution in ICF and ECF 451
- Regulation of Water and Electrolyte Balance 452
- Disorders of Water and Electrolyte Balance 454

17. Acid-base Balance 463

- Normal pH of Body Fluids 463
- Maintenance of Normal Blood pH 464
- Disorders Associated with Acid-base Balance 469
- Use of Anion Gap to Diagnose Acid-base Disorders 475
- Arterial Blood Gas Analysis in Acid-base Imbalance 477

18. Chemistry of Nucleic Acids and Purine and Pyrimidine Nucleotide Metabolism 481

- Structure and Functions of Nucleotides 481
- Biologically Important Free Nucleotides 483
- Synthetic Analogs of Nucleotides or Antimetabolites 486
- DNA Structure and Function 486
- RNA Structure and Function 491
- Biosynthesis of Purine Nucleotides 494
- Catabolism of Purine Nucleotides 500
- Disorders of Purine Metabolism 500
- De Novo Synthesis of Pyrimidine Nucleotides 507
- Catabolism of Pyrimidine Nucleotides 508
- Disorders Associated with Pyrimidine Nucleotide Metabolism 508

19. DNA Replication and Repair 514

- Replication in Prokaryotes 514
- Replication in Eukaryotes 519
- End-replication Problem 521
- Inhibitors of Replication and their Clinical Importance 523
- DNA Repair Mechanisms 523
- Cell Cycle 529

20. Transcription and RNA Processing 533

- Processes Involved in Transcription 533
- Transcription in Prokaryotes 533
- Transcription in Eukaryotes 536
- Post-transcriptional Modification 538
- Inhibitors of Transcription and their Clinical Significance 543

21. Genetic Code and Translation 546

- Genetic Code and its Characteristics 546
- Wobble Hypothesis for Codon-anticodon Interactions 548
- Eukaryotic Translation, Folding and Processing of Proteins 549
- Translation in Eukaryotes 549
- Post-translational Modifications 555
- Inhibitors of Translation and their Clinical Importance in Medicine 556
- Protein Targeting and Sorting 556

22. Regulation of Gene Expression and Gene Mutation 560

- Gene Expression 561
- Regulation of Gene Expression in Prokaryotes 561
- Regulation of Gene Expression in Eukaryotes 563
- Mutations 567

23. Molecular Techniques and Applications 573

- Recombinant DNA 573
- Cloning of DNA 575
- Applications of Recombinant DNA Technology 576
- Genomic and C-DNA Libraries 579
- DNA Probes 579
- Blot Transfer Techniques and their Applications 580
- Restriction Fragment Length Polymorphism 581
- Polymerase Chain Reaction: Technique and its Applications in Medicine 581
- Microarray, FISH and CRISPR Techniques 583

24. Nutrition in Health and Disease 590

- Dietary Components 590
- Importance and Requirement of Carbohydrates, Dietary Fibers, Fats, Proteins, Vitamins and Minerals 591
- Basal Metabolic Rate 596
- Thermogenic Effect of Food or Specific Dynamic Action 597
- Balanced Diet for Optimal Health 597
- Protein Energy Malnutrition 598
- Obesity 602
- Dietary Advice in Adult, Childhood, and in Pregnancy for Optimal Health 602
- Dietary Advice for Optimal Health in Diabetes Mellitus and Coronary Heart Disease 604
- Nutritional Importance of Commonly Used Food Items 606

- Energy Content of Different Food Items 608
- Glycemic Index 608

25. Organ Functions, Disorders and Function Tests 612

- Functions, Disorders and Tests to Assess the Functions of Liver 612
- Functions, Disorders and Tests to Assess the Functions of Kidney 620
- Functions, Disorders and Tests to Assess the Functions of Thyroid 634
- Functions, Disorders and Tests to Assess the Functions of Adrenal Glands 642
- Hormones as Biomarkers of Reproductive Function 647
- Prenatal Screening 652

26. Extracellular Matrix 655

- Types, Functions, and Components of Extracellular Matrix 655
- Structure and Functions of Major Proteins of ECM 657
- Proteoglycans and Glycoproteins 659
- Disorders of Components of ECM 660

27. Immunoglobulins and Immune System 667

- Classification of Immune System 667
- Organs and Cells Involved in Immune System 668
- Components of Innate Immune System 670
- Components of Adaptive Immune System 671
- Immunoglobulins 671
- Innate Immune Responses 675
- Adaptive Immune Responses 677

28. Biochemistry of Cancer and HIV 681

- Characteristics of Cancer Cell 681
- Molecular Basis of Cancer (Carcinogenesis) 682
- Carcinogens 683
- Oncogenes and Proto-oncogenes 685
- Tumor Suppressor Genes 685
- Apoptosis 686
- Tumor Biomarkers 686
- Biochemical Basis of Cancer Therapy 689
- HIV and AIDS 690

29. Xenobiotics and Detoxification 694

- Biotransformation and Detoxification 694
- Phase-I Reactions 695
- Phase-II Reactions 696
- Role of Xenobiotics in Disease 698

30. Free Radicals and Antioxidant Defense Systems 701

- Free Radical and Reactive Oxygen Species *701*
- Biological and External Sources of Free Radicals *702*
- Oxidative Damage *703*
- Antioxidant Defense Systems *704*
- Oxidative Stress and its Role in Pathogenesis *706*

31. Metabolism of Alcohol 709

- Absorption and Transport of Alcohol *709*
- Metabolism of Ethanol *709*
- Acute Biochemical Changes in Alcoholism *711*
- Chronic Effects of Alcoholism *711*

32. Instrumentation and Techniques 714

- Commonly Used Techniques *714*
- Commonly Used Instruments *726*

33. Artificial Intelligence in Healthcare Industry 732

- Artificial Intelligence *732*
- Role of Artificial Intelligence in Healthcare *732*
- Advantages and Disadvantages of AI in Healthcare *734*

34. AETCOM Module 1.1 736

- Who is a Physician? *736*
- Qualities of a Physician *736*
- Roles of a Physician *737*
- Lifelong Learning for Physician *738*
- Role of Physicians in Healthcare System *740*
- Physicians' Role and Responsibility to Society and Community *741*

Index ***743***

10

CHAPTER

Metabolism of Carbohydrates

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Learning Objectives

On completion of the chapter, the students should be able to:

- Describe digestion, absorption, transport of carbohydrates and associated disorders.
- Describe glycolysis: Pathway, energetics, significance, regulation and associated disorders.
- Describe metabolic fates of pyruvate and associated disorders.
- Describe TCA cycle: Pathway, energetics, regulation, and significance.
- Describe gluconeogenesis: Pathway, regulation and significance.
- Describe glycogen metabolism: Pathway, significance, regulation and associated disorders.
- Describe hexose monophosphate pathway (HMP): Regulation, significance and associated disorders.
- Describe uronic acid pathway and its significance.
- Describe galactose metabolism: Pathway, significance and associated disorders.
- Describe fructose metabolism: Pathway, significance and associated disorders.
- Describe regulation of blood glucose level in fed and fasting state.
- Describe diabetes mellitus: Types, symptoms, clinical features, and metabolic changes occur in diabetes mellitus.
- Describe laboratory investigations for diagnosis of diabetes mellitus.

Scan me for NMC Competencies

OVERVIEW

Carbohydrates occupy an important place in metabolism because of their roles in energy production and various biosynthetic pathways. Carbohydrates are an important source of energy for most living organisms. The major source of carbohydrates is found in plants. This chapter focuses on the chemical reactions involved in the:

- Digestion of dietary carbohydrate
- The storage of temporary excess of carbohydrate as glycogen
- Conversion of glucose to other molecules and control of these metabolic pathways
- Disorders related to carbohydrate metabolism

DIGESTION, ABSORPTION, TRANSPORT OF CARBOHYDRATES

Digestion of Carbohydrates

The principal sites of carbohydrate digestion are the **mouth** and **small intestine**. The dietary carbohydrate consists of:

- **Polysaccharides:** Starch, glycogen and cellulose
- **Disaccharides:** Sucrose and lactose
- **Monosaccharides:** Mainly glucose and fructose

Monosaccharides need no digestion prior to absorption, whereas disaccharides and polysaccharides must be hydrolyzed to simple sugars before their absorption **(Fig. 10.1)**.

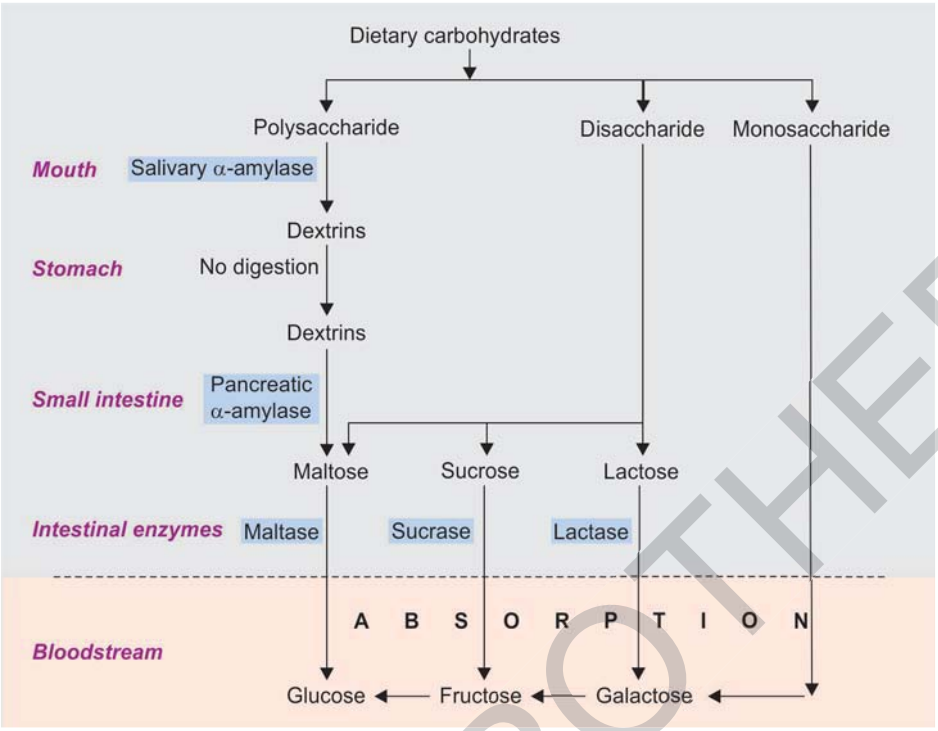

FIG. 10.1: Flow sheet of digestion of carbohydrates.

Digestion in Mouth

Digestion of carbohydrates begins in the mouth. Salivary gland secretes α-**amylase (ptyalin)**, which initiates the hydrolysis of a **starch** and **glycogen**. This enzyme is endoglycosidase that hydrolyzes random internal α-(1 → 4) glycosidic bonds. It cannot catalyze the hydrolysis of α-(1 → 6) glycosidic bonds, found at the branch point of glycogen and starch.

During mastication salivary α-amylase acts briefly on dietary starch and glycogen in random manner breaking some α-(1 → 4) bonds. α-amylase hydrolyzes starch into **(Fig. 10.2)**:

- *Disaccharides*, maltose
- *Trisaccharides*, maltotriose (trimer of glucose linked by α-(1 → 4) glycosidic bond)
- *Oligosaccharides* (with one or more α-(1 → 6) glycosidic bonds of about 8 glucose residues in length called dextrins).

Digestion in Stomach

Carbohydrate digestion halts temporarily in the stomach because the high acidity inactivates the salivary α-amylase.

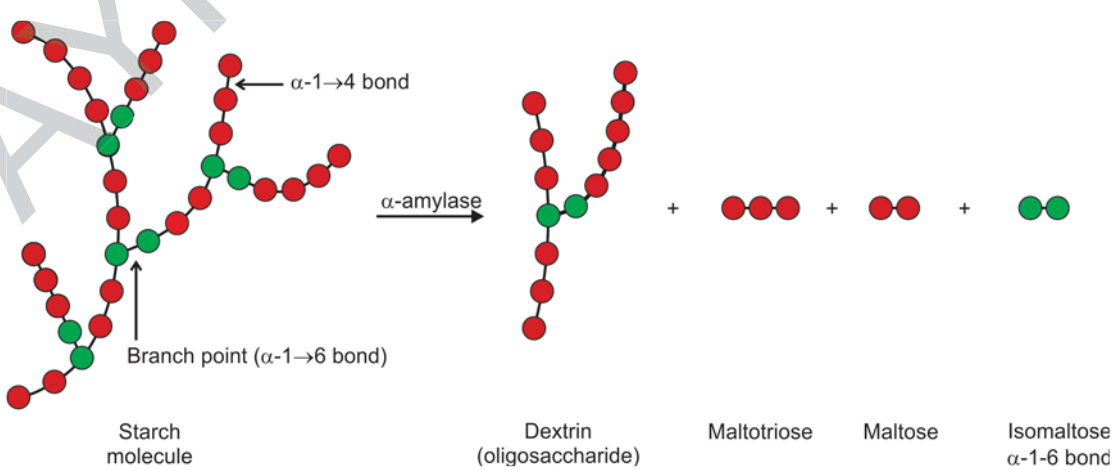

FIG. 10.2: Action of α-amylase on starch and glycogen.

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Digestion in Intestine

Further digestion of carbohydrates occurs in the small intestine by **pancreatic enzymes**. When acidic stomach contents (chyme) reach the small intestine, they stimulate mucosal cells of the duodenum to release **secretin** and **cholecystokinin (CCK)**, the two local peptide hormones that stimulate the pancreas to release pancreatic juice into the intestinal lumen.

- **Secretin** stimulates the release of **bicarbonate** whose function is to neutralize the acidic chyme.
- **CCK** stimulates the release of digestive enzymes including pancreatic α-**amylase**.

There are two phases of intestinal digestion:

1. Digestion due to pancreatic α-amylase.
2. Digestion due to intestinal brush border membrane enzymes.

Digestion due to Pancreatic α-amylase

- The function of pancreatic α-amylase is to degrade dextrins further. This degradation results in a mixture of **maltose**, **isomaltose** and **α-limit dextrin**. The α-limit dextrins are smaller oligosaccharides containing 3–5 glucose units. α-limit dextrin is formed because α-amylase cannot hydrolyze the (1 → 6) glycosidic linkages present.
- Humans are unable to digest cellulose a polysaccharide of plant origin containing β-(1, 4) glycosidic bonds between glucose residues, due to absence of β-(1, 4) glucosidase enzyme in digestive juice. Undigested cellulose called **roughage** or **fiber**. Fiber aids intestinal motility and acts as a stool softer and prevents constipation.

INSIGHT 10.1

Isoenzymes of α-amylase

- Salivary α-amylase and pancreatic α-amylase are isoenzyme forms.
- The pancreatic isoenzyme is not glycosylated, but the salivary isoenzyme may exist in both glycosylated and deglycosylated forms.

CLINICAL APPROACH 10.1

Dietary Fiber: Implications for Reducing Chronic Disease Risk

- Dietary fiber is a type of carbohydrate that cannot be digested by our enzymes.
- It is found in edible plant foods such as cereals, fruits, vegetables, dried peas, nuts, lentils and grains.
- Fiber is grouped by its physical properties and is called **soluble**, **insoluble** or **resistant starch**.
- Most sources of dietary fiber tend to have a combination of both soluble and insoluble fiber in varying proportions.
- **Role of dietary fibers:**
  - Dietary fiber helps to reduce the risk of diseases such as **diabetes**, **coronary heart disease** and **bowel cancer**.
  - Fiber reaches the large bowel undigested where it is fermented by bacteria. The by-products of this fermentation are **butyrate** and **short chain fatty acids (SCFAs)**. Butyrate has **anti-proliferative** activity and reduces risk of colorectal cancer.
  - Soluble fibers help to:
    - Support the growth of probiotic bacteria needed to maintain a healthy gut.
    - Reduce cholesterol absorption by binding to it in the gut.
    - Slow down the time for food to pass through the stomach into the small intestine. This helps slow down the absorption of glucose into the bloodstream and has the benefits of feeling fuller for longer, helping to control blood sugar levels.
- Insoluble fiber does not dissolve in water and is found in foods like whole meal bread, wheat bran, vegetables and nuts. Insoluble fiber adds bulk to stools by absorbing water, and helps to prevent constipation.



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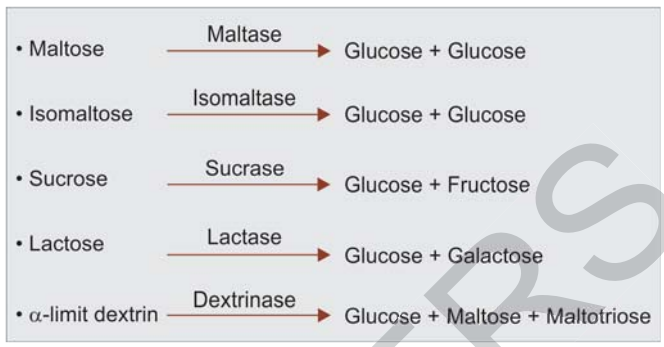
INSIGHT 10.2

What is Resistant Starch (RS)?

- Resistant starch (RS) is a type of starch that **resists digestion** in the small intestine and ferments in the large intestine. Thus, resistant starch is so named because it resists digestion.
- All starches are composed of two types of polysaccharides: **amylose** and **amylopectin**. The proportion of amylose and amylopectin can vary from one variety of starch to the other.
- Amylose predominates in resistant starch.
- Most starches are broken down by **amylase** enzyme in small intestine into glucose, which is then absorbed into the blood.
- RS is a type of starch that is not fully broken down and absorbed in the small intestine. Instead, RS makes its way to the large intestine (colon), where intestinal bacteria ferment it to **short-chain fatty acids (SCFA)** such as **acetate**, **butyrate**, and **propionate**, along with gases are produced.
- The extent to which starch in foods is hydrolyzed by **α-amylase** is determined by its structure. The amount of **amylose** and **amylopectin** basically determines the digestibility of starch.
  - **Amylopectin** of starch is highly branched, leaving more surface area available for digestion. It is broken down quickly, and produces a larger rise in blood glucose and has higher glycemic index which subsequently raises insulin.
  - **Amylose** is a straight chain, which limits the amount of surface area exposed for digestion and is digested more slowly. They raise less blood glucose and have low glycemic index subsequently less rise in insulin.
- Starches with **lower amylose** contain will have **higher glycemic index**; inversely starches with a **higher amylose** content will have **low glycemic indexes**. Foods that have a low glycemic index are considered to be more beneficial since they cause less fluctuation in insulin secretion.
- Foods that contain resistant starch include:
  - Plantains and green bananas (as a banana ripens the starch changes to a regular starch)
  - Beans, peas, and lentils (white beans and lentils are the highest in resistant starch)
  - Whole grains including oats and barley
  - Cooked and cooled rice

Digestion due to Intestinal Brush Border Membrane Enzymes

Enzymes responsible for the final phase of carbohydrate digestion are located in the brush border membrane. The enzymes and the reactions they catalyze are as follows:



- All the above enzymes in the brush border membrane are disaccharidases except **dextrinase** which hydrolyzes α-(1 → 6) bonds at the branch points of the limit dextrins.
- The end products of carbohydrate digestion are **glucose**, **fructose** and **galactose** which are readily absorbed through the intestinal mucosal cells into the bloodstream.

Absorption of Carbohydrates

Carbohydrates are absorbed as monosaccharides from the intestinal lumen through the mucosal epithelial cells into the bloodstream of the portal venous system. Two mechanisms are responsible for the absorption of monosaccharides:

- **Active transport** against a concentration gradient (uphill process, i.e., from a low glucose concentration outside the cell to a higher concentration within the cell).
- **Facilitative transport**, with concentration gradient (downhill process, i.e., from a higher concentration to a lower one).

Active Transport against Concentration Gradient

The transport of glucose and galactose across the brush border membrane of mucosal cells occurs by an active transport, energy requiring process that requires a specific transport protein and the presence of sodium ions **(Fig. 10.3)**.

A **sodium/glucose linked transport-1 (SGLT-1)** binds both glucose and Na^+ at separate sites and transports them both through the plasma membrane of the intestinal cell. The Na^+ is transported down its concentration gradient (higher concentration to lower concentration) and at the same time glucose is transported against its concentration gradient. The free energy required for this active transport is obtained from the hydrolysis of ATP linked to a **sodium pump** that expels Na^+ from the cell in exchange of K^+ **(Fig. 10.3)**.

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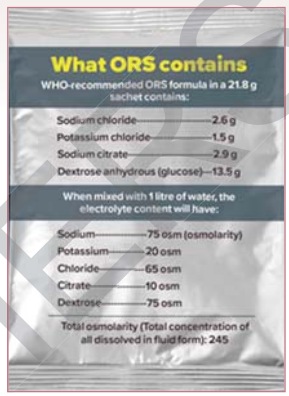
CLINICAL APPROACH 10.2

Formulation of ORS (Oral Rehydration Solution/Salt) based on the Sodium-glucose Cotransport Mechanism



- **Oral rehydration solution (ORS)** is the key treatment of acute diarrhea in children, to restore the electrolyte balance.
- Oral rehydration solution (ORS) is a balanced mixture of electrolytes and glucose.
- ORSs include both **sodium** and **glucose** because, without glucose, intestinal sodium is not actively absorbed.
- As well as Na^+ absorption from GIT lumen simultaneously requires glucose molecules. Sodium and glucose transport are coupled in the small intestine **(Fig. 10.3)**.
- Dehydrated patients with diarrhea do not absorb oral sodium chloride alone, as cellular transport of sodium and glucose is linked. Water and electrolyte sodium absorption will occur only in presence of glucose.
- ***A pack of ORS consists of 4 constituents:***
  1. *Sodium chloride*: 2.6 grams
  2. *Sodium citrate*: 2.9 grams
  3. *Potassium chloride*: 1.5 grams
  4. *Glucose*: 13.5 grams
- These are to be dissolved in 1 liter of clean drinking water. ORS is generally provided pre-packaged in a dry form to be reconstituted when required.

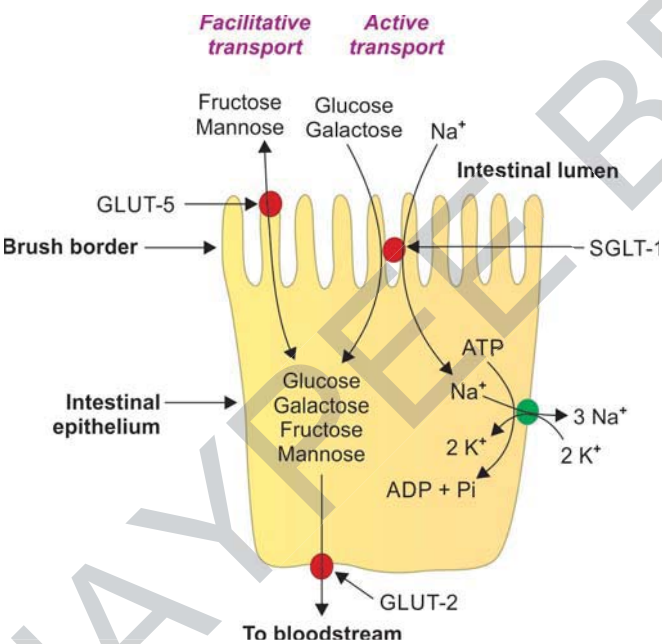


FIG. 10.3: Transport of glucose, fructose, galactose and mannose.
(SGLT: Sodium dependent glucose transporter)

Facilitative Transport (with the Concentration Gradient)

Fructose and **mannose** are transported across the brush border by a **Na^+ independent facilitative diffusion** process, involving another specific glucose transporter, **GLUT-5**. Movement of sugar in facilitative diffusion is strictly downhill, going from a higher concentration to a lower one until it reaches equilibrium. The same transport can also be used by glucose and galactose if the concentration gradient is favorable.

Transport of Carbohydrates

The sodium-independent transporter, **GLUT-2** facilitates transport of sugars out of the mucosal cells and transported to the liver through portal circulation. Several glucose transporter proteins have been described in various tissues. The role of these glucose transporter proteins is shown in **Table 10.1**.

DISORDERS OF DIGESTION AND ABSORPTION OF CARBOHYDRATES

Lactose Intolerance

Lactose intolerance is a digestive disorder caused by the inability to digest **lactose**. Lactose is the disaccharide found in milk and dairy products. Lactase secreted in the small intestinal brush border, breaks down lactose into glucose and galactose, which can be easily absorbed into the bloodstream. Lactose intolerance affects about 65% of the world's population, and it gets worse as individuals get older. Both children and adults can have lactose intolerance.

Causes

- Lactose intolerance is a clinical condition, resulting from acquired **deficiency of lactase** and becoming intolerant to lactate present in milk and milk products.

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TABLE 10.1: Glucose transporters.

Transporters	*Occurrence*	*Function*
Facilitative bidirectional transporters		
GLUT-1	Brain, kidney, colon, placenta and erythrocyte	Uptake of glucose
GLUT-2	Liver, pancreatic β-cell, small intestine, kidney	Rapid uptake and release of glucose
GLUT-3	Brain, kidney, placenta	Uptake of glucose
GLUT-4	Heart and skeletal muscle, adipose tissue	Insulin stimulated uptake of glucose
GLUT-5	Small intestine	Absorption of fructose
Sodium-dependent unidirectional transporter		
SGLT-1	Small intestine and kidney	Active uptake of glucose from lumen of intestine and reabsorption of glucose in proximal tubule of kidney against concentration gradient

- Lactose intolerance can be present at birth but usually occurs later in life due to decrease in the production of lactase with increasing age.
- There are four leading causes of lactase deficiency:
  1. ***Primary lactase deficiency:*** It is the most common cause of lactase deficiency, also known as **lactase non-persistence**. There is a gradual decline in lactase enzyme activity with increasing age. Enzyme activity begins to decline in infancy, and symptoms manifest in adolescence or early adulthood.
  2. ***Secondary lactase deficiency:*** Due to several infectious, inflammatory, or other diseases, injury to intestinal mucosa. Common causes include: gastroenteritis, celiac disease, Crohn's disease (inflammatory bowel disease), chemotherapy, antibiotics.
  3. ***Congenital lactase deficiency:*** There is a decrease or absence of lactase enzyme activity since birth due to **autosomal recessive** inheritance. It manifests in the newborn after ingestion of milk. It is a rare cause of the deficiency.
  4. ***Developmental lactase deficiency:*** It is seen in premature infants born at 28 to 37 weeks of gestation. The infant's intestine is underdeveloped, resulting in an inability to hydrolyze lactose. This condition improves with increasing age due to the maturation of the intestine, which results in adequate lactase activity.

Signs and Symptoms

Signs and symptoms of lactose intolerance manifest 30 minutes to 1 to 2 hours after ingesting milk (dairy) products. Common signs and symptoms may include the following:

- Diarrhea
- Abdominal bloating
- Abdominal pain
- Nausea and vomiting
- Fullness
- Flatulence

Biochemical basis of Signs and Symptoms of Lactose Intolerance

- In lactose intolerance, lactose stays in the digestive system where it's fermented by bacteria. This leads to the production of various gases, which cause the symptoms associated with lactose intolerance.
- The lactose is a good energy source for microorganisms in the colon and ferment it to lactic acid and toxic products such as **methane CH_4** and **hydrogen gas H_2** that cause **abdominal cramps** and **diarrhea**.
- The problem is further complicated because undigested lactose and its metabolites such as low molecular weight acids like **acetic acid**, **propionic acid** and **butyric acid** which are **osmotically active** increase the osmolarity of the intestinal contents, and draws water into the intestine resulting in osmotic diarrhea.

Diagnostic Tests

There are three major tests that help diagnose lactose intolerance. They include:

1. **Hydrogen breath test:** This test measures the amount of hydrogen gas in breath. Normally, a small amount of hydrogen gas is found in breath. In lactose malabsorption, undigested lactose causes high levels of hydrogen in breath. The test is positive for lactose malabsorption if the post-lactose breath hydrogen value rises >20 ppm compared with the baseline.
2. **Stool acidity test:** This test diagnoses lactose intolerance in infants and children. Undigested lactose gets fermented and produces lactic acid which lowers the stool pH. The stool pH in infants with lactose intolerance is typically below 5.5 to 6.0.
3. **Lactose tolerance blood test:** This test involves the measure the level of glucose in blood before and after consuming a high-lactose diet. The glucose levels must ideally rise. Unchanged glucose levels indicate that the body is unable to digest lactose.

Treatment/Management

Management of lactose intolerance consists of **dietary modification**; avoid the consumption of products containing much lactose, and lactase supplementation.

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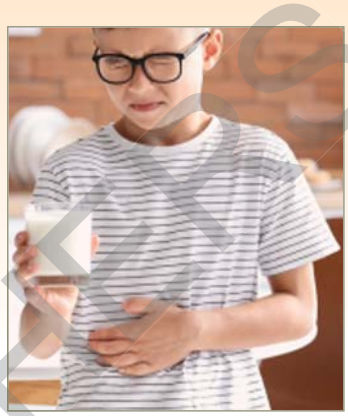
CASE STUDY 10.1: LACTOSE INTOLERANCE

Case Scenario

A 15-year-old African American boy came to India on an exchange visit for 2 months. After 2 weeks in India, he complained of abdominal discomfort, a feeling of being bloated, increased passage of urine, and more recently, the development of diarrhea. His only change in diet noted at the time was the introduction of milk. He was consuming about 1 to 1.5 liters per day. A diagnosis of lactose intolerance was made.



Questions

1. What is lactose intolerance?
2. What are the symptoms of lactose intolerance?
3. What are the causes of lactose intolerance?
4. How is lactose intolerance diagnosed?
5. How is lactose intolerance treated?

Discussion

1. Lactose intolerance is a clinical syndrome that manifests with characteristic signs and symptoms upon consuming food substances containing lactose, a disaccharide. Normally lactose is hydrolyzed into glucose and galactose by the **lactase enzyme**, which is found in the small intestinal brush border. Deficiency of lactase results in clinical symptoms.
2. Lactose intolerance presents with abdominal bloating and pain, loose stools, nausea, flatulence, and borborygmi (intestinal rumbling sounds caused by passing gas).

Answers to questions 3, 4 and 5: Refer given text under heading lactose intolerance.

Congenital Sucrase–Isomaltase Deficiency (CSID)

- **Sucrase–isomaltase enzyme** is a heterodimer composed of two subunits: **sucrase** and **isomaltase**.
- Congenital sucrase-isomaltase deficiency is due to mutation in the sucrase isomaltase gene that affects the digestion of sucrose and maltose. Mutations cause the enzyme activity to be greatly reduced or completely absent.
- Congenital sucrase–isomaltase deficiency is a rare **autosomal recessive** disorder.
- The disease begins to show signs in late infancy or early childhood after weaning when sucrose-containing foods are ingested for the first time such as various fruits and grains.
- Symptoms of CSID include osmotic diarrhea, abdominal pain, bloating, flatulence, vomiting and cramps when sugar is ingested.

METABOLIC FATE OF CARBOHYDRATES

After being absorbed from the intestinal tract, the monosaccharides are carried by the portal circulation directly to the liver. In the liver, most of the free D-glucose entering is phosphorylated to **glucose-6-phosphate** and sugar is trapped within the cell and it cannot diffuse back out of the cell because its plasma membrane is impermeable to the glucose-6-phosphate. The rest of the glucose passes into the systemic blood supply.

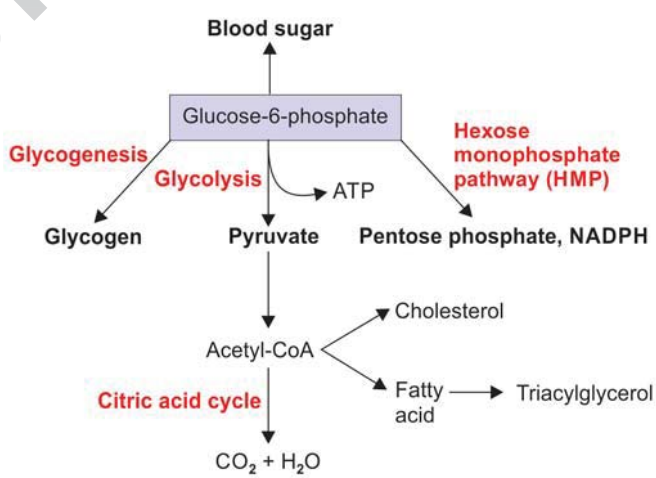


FIG. 10.4: Metabolic pathways for glucose-6-phosphate in the liver.

Other dietary monosaccharides **D-fructose** and **D-galactose** are phosphorylated and may be converted into glucose in the liver. Glucose-6-phosphate is an intermediate in several metabolic pathways that uses glucose in the liver **(Fig. 10.4)** depending upon the supply and demand includes:

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- Glycolysis
- Hexose monophosphate pathway (HMP)
- Glycogenesis

GLYCOLYSIS

Glycolysis (from the Greek **glykys**, "sweet or sugar", and lysis "splitting"), is the sequence of reactions that metabolizes one molecule of **glucose** to two molecules of **pyruvate** in the presence of **oxygen**. During the sequential reactions of glycolysis, some of the free energy released from glucose is conserved in the form of ATP and NADH. Pyruvate can be further processed **anaerobically** to **lactate**. Under aerobic conditions, pyruvate can be completely oxidized to CO_2, generating much more ATP. This pathway is also called **Embden-Meyerhof pathway**, after two pioneers of research on glycolysis.

Reactions of Glycolysis

In eukaryotic cells, glycolysis takes place in the **cytoplasm**. The breakdown of six carbon glucose into two molecules of three carbon pyruvate occurs in 10 **steps** which can be divided into two phases **(Fig. 10.5)**.

1. **Ist phase**, first 5 steps constitute the preparative phase (energy requiring phase). No ATP is generated in this phase.
2. **IInd phase**, energy generating phase.

Ist Phase, Energy Requiring Phase or Preparative Phase

The first five reactions of glycolysis correspond to this phase where phosphorylated form of glucose and fructose are synthesized at the expense of **two moles of ATP** per glucose molecule. The hexose glucose is cleaved into two triose phosphates (glyceraldehyde-3-phosphate).

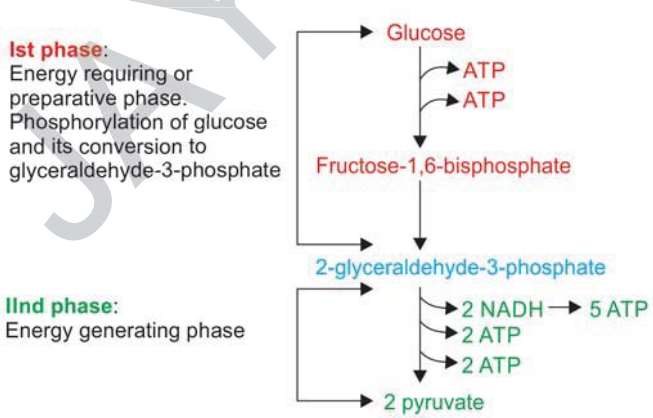

FIG. 10.5: Phases of the glycolytic pathway.

Reactions of Ist Phase

1. **Phosphorylation of glucose** to **glucose-6-phosphate** to trap the glucose in the cell **(Fig. 10.6)**: Glucose enters cells through specific transport proteins. In the first step of glycolysis, glucose is activated for subsequent reactions by its phosphorylation at C-6 to yield **glucose-6-phosphate** catalyzed by the enzyme **hexokinase**. ATP is required as a phosphate donor. The reaction is accompanied by a considerable loss of free energy as heat and therefore under physiologic conditions is regarded as **irreversible**. Hexokinase is a **regulatory enzyme** in glycolysis.

INSIGHT 10.3

Isoenzyme Forms of Hexokinase

The human genome encodes **four different hexokinases, I to IV** all of which catalyze the same reaction. They differ with respect to their kinetic properties.

- Brain and kidney have chiefly the type I isoenzyme
- Skeletal muscle has type II
- Adipose tissue has both I and II
- Liver possesses all four isoenzyme of hexokinase but the principal form of hexokinase is type IV, commonly known as **glucokinase**.

The difference between muscle hexokinase and liver hexokinase, i.e., glucokinase is given in **Table 10.2**.

TABLE 10.2: Differences between muscle hexokinase and glucokinase (liver hexokinase).

Muscle hexokinase	*Glucokinase (liver hexokinase)*
Distributed in extrahepatic tissue	Present in liver and β-cells of pancreas
High affinity for its substrate glucose (low K_m)	Low affinity for its substrate glucose (high K_m)
Inhibited by its product glucose-6-phosphate, in an allosteric manner	No inhibition by its product glucose-6-phosphate
Its function is to ensure supply of glucose for the tissues irrespective of blood glucose concentration	Its function is to remove glucose from the blood, when the blood glucose level increases (following meal)
Catalyze the phosphorylation of other hexoses like fructose, galactose also	Specific for glucose
Its activity is not affected by insulin	It is an inducible enzyme that increases its synthesis in response to insulin

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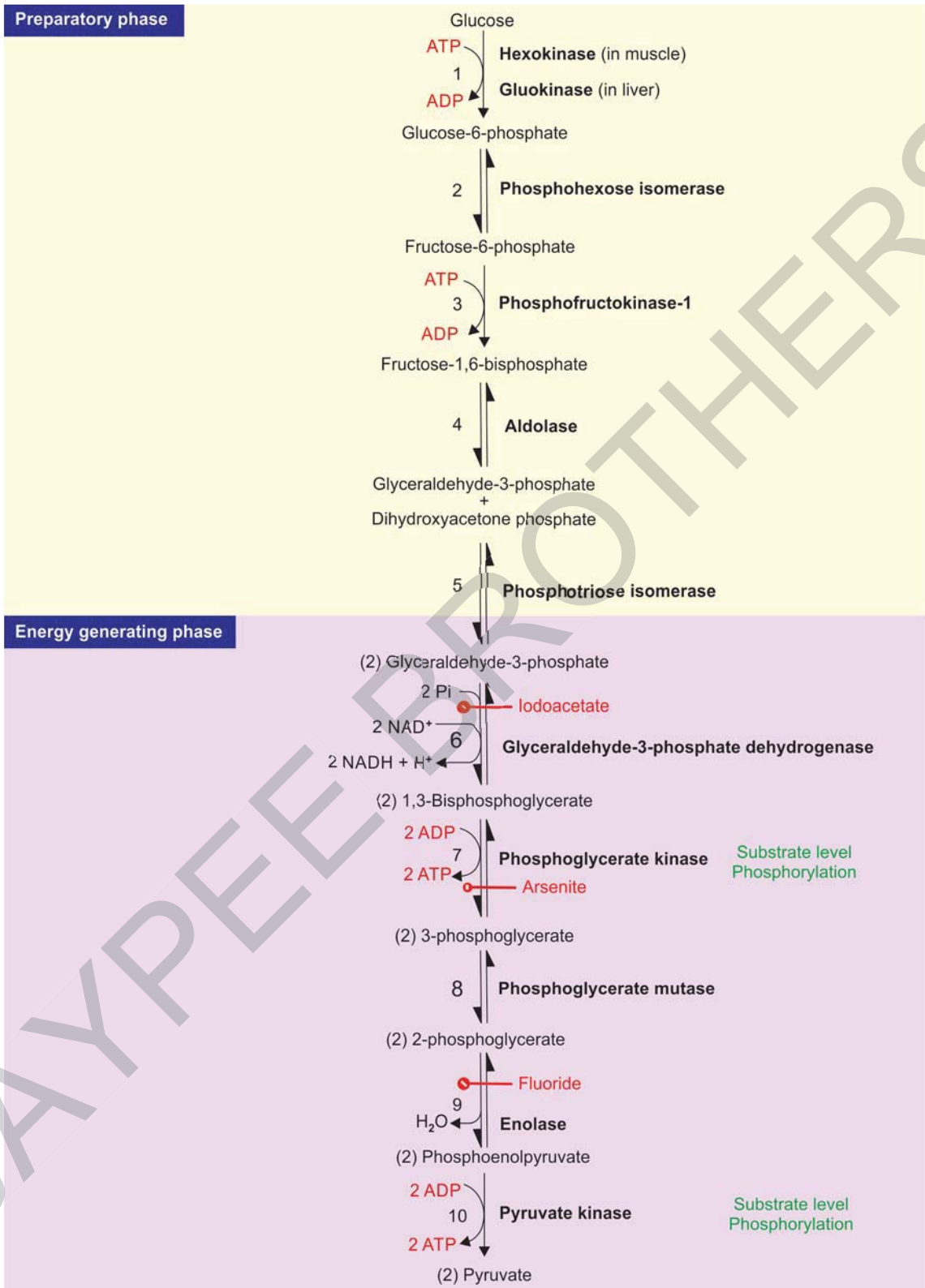

FIG. 10.6: The reactions of glycolysis.

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2. **Conversion of glucose-6-phosphate to fructose-6-phosphate:** The enzyme **phosphohexose isomerase** catalyzes the reversible of isomerization of glucose-6-phosphate an aldose, to **fructose-6-phosphate** a ketose.
3. **Phosphorylation of fructose-6-phosphate to fructose 1,6-bisphosphate:** Fructose-6-phosphate to fructose 1,6-bisphosphate is a **second phosphorylation reaction of glycolysis**. Phosphofructokinase-1 (PFK-1) catalyzes the transfer of a phosphoryl group from ATP to fructose-6-phosphate to yield **fructose 1,6-bisphosphate**. This step is **irreversible committed step** under physiological conditions.
   - **Phosphofructokinase-1** is both **allosteric** and an **inducible** enzyme which is the **rate limiting, regulatory enzyme of glycolysis**.
   - Both hexokinase and PFK-1 are important regulatory enzymes in glycolysis, but PFK-1 is the **committed** step. This reaction directs glucose to glycolysis only.
   - Its activity is increased whenever the cell's ATP supply is depleted or when the ATP breakdown products, ADP and AMP accumulate.
   - The enzyme is inhibited whenever the cell has ample ATP. **Fructose 2,6-bisphosphate** is potent allosteric activator of PFK-1.

Fructose is more rapidly glycolyzed by the liver than glucose, because it bypasses the step in glucose metabolism catalyzed by phosphofructokinase, at which point metabolic control is exerted on the rate of catabolism of glucose.

4. **Cleavage of fructose-1,6-bisphosphate: Fructose-1,6-bisphosphate** is cleaved by **aldolase** to two triose phosphates, glyceraldehyde-3-phosphate an aldose, and dihydroxyacetone phosphate (DHAP), a ketose. Several tissue-specific isoenzymes of aldolase exist. Aldolase-A occurs in most tissues and aldolase-B occurs in liver and kidney.
5. **Interconversion of triose phosphate:** Dihydroxyacetone phosphate (DHAP) is isomerized to glyceraldehyde-3-phosphate by the enzyme **phosphotriose isomerase**, so that for every molecule of glucose entering glycolysis, 2 moles of glyceraldehyde-3-phosphate are formed. This reaction completes the preparative phase of glycolysis.

IInd Phase, Energy Generating Phase

In IInd phase of glycolysis, the chemical energy of glucose molecule is conserved in the form of ATP and NADH **(Fig. 10.6)**.

6. **Oxidation of glyceraldehyde-3-phosphate to 1,3-bisphosphoglycerate:** The first step in the energy generating phase is the oxidation of glyceraldehyde-3-phosphate to **1,3-bisphosphoglycerate**, catalyzed by **glyceraldehyde-3-phosphate dehydrogenase**. It is a reversible reaction. The reducing equivalents NADH + H^+ formed are reoxidized by electron transport chain, to generate **2.5 ATP** molecules per NADH + H^+.
7. **Phosphoryl transfer from 1,3-bisphosphoglycerate to ADP:** The **phosphoglycerate kinase** transfers the high energy phosphoryl group from 1,3-bisphosphoglycerate to ADP forming **ATP** and **3-phosphoglycerate**. This is the first step in glycolysis that generates **ATP**, an example of **substrate level phosphorylation**. Since two molecules of triose phosphate are formed per molecule of glucose undergoing glycolysis two molecules of ATP are generated at this stage per molecule of glucose.
8. **Conversion of 3-phosphoglycerate to 2-phosphoglycerate:** 3-phosphoglycerate to 2-phosphoglycerate is a reversible reaction catalyzed by **phosphoglycerate mutase**. It catalyzes reversible shift of the phosphoryl group between C-2 and C-3 of glycerate; Mg^+ is essential for this reaction.
9. **Dehydration of 2-phosphoglycerate to phosphoenolpyruvate:** 2-phosphoglycerate to phosphoenolpyruvate (PEP) is catalyzed by **enolase**. Enolase promotes reversible removal of a molecule of water from 2-phosphoglycerate, raising the phosphate on position 2 to high energy state, thus forming phosphoenolpyruvate.
10. **Transfer of phosphoryl group from phosphoenolpyruvate to ADP:** The last step in glycolysis is the transfer of the phosphoryl group from phosphoenolpyruvate to ADP, catalyzed by **pyruvate kinase** which requires K^+ and either Mg^{2+} or Mn^{2+}. This is an **irreversible** reaction. The high energy phosphate of phosphoenolpyruvate is transferred to ADP by the enzyme **pyruvate kinase** to generate ATP, **another example of substrate level phosphorylation**. **Pyruvate kinase** is an allosteric enzyme. Enol pyruvate formed in this reaction is converted spontaneously to the keto form of **pyruvate**. Under aerobic conditions, pyruvate is transported into mitochondria and undergoes oxidative decarboxylation to acetyl-CoA then oxidation to CO_2 in the citric acid cycle.

Pyruvate kinase (PK) deficiency is the most common defect of the glycolytic pathway leading to **congenital hemolytic anemia**.

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INSIGHT 10.4

Poisons that Inhibit Enzymes of Glycolysis

- **Iodoacetate:** Inhibit glyceraldehyde-3-phosphate dehydrogenase.
- **Fluoride:** Inhibit enolase, a property that can be made use in the estimation of glucose. Glycolysis is inhibited by taking a sample into test tubes containing fluoride.
- **Arsenate:** Arsenate is toxic to phosphoglycerate kinase reaction. Arsenate competes with inorganic phosphate (Pi) forming 1-arseno-3-phosphoglycerate, which undergoes spontaneous hydrolysis to 3-phosphoglycerate without forming ATP.
- **Arsenate and mercuric ions** react with the –SH groups of lipoic acid (one component of pyruvate dehydrogenase) and inhibit pyruvate dehydrogenase and develops lactic acidosis.

CLINICAL APPROACH 10.3

Clinical Implication of Inhibition of Enolase Enzyme by Fluoride

- Measurements of blood-glucose concentration are used for the diagnosis and management of diabetes. Frequently these measurements are made in the clinical laboratory more than 1 h after the collection of the blood sample.
- Because RBCs can metabolize anaerobically **glucose to lactate**; glucose in the blood will be consumed, and lactate will be produced, which will lead to acidification of the blood sample.
- These reactions proceed in RBCs, even at room temperature, so that both blood glucose concentration and pH will decrease during standing, possibly leading to a false diagnosis of hypoglycemia and/or acidemia.
- Anaerobic metabolism of glucose can be prevented by adding an inhibitor of glycolysis (by inhibiting glycolytic enzyme **enolase**) to the blood collection tube (vacutainer tube).
- Blood samples for glucose estimation are collected in **grey colored** vacutainer tube containing sodium fluoride.
- Fluoride is a strong competitive inhibitor of **enolase**, blocking glycolysis and lactate production in the RBC.



Vacutainer containing sodium fluoride for blood glucose

CASE STUDY 10.2: BLOOD SAMPLE COLLECTION FAULT

Case Scenario

A 54-year-old man was admitted to the hospital for preoperative evaluation and elective knee surgery. On the morning of surgery, the patient was awakened by the phlebotomist who drew his blood for cross-matching using special pink-top (EDTA) vacutainer and for basic laboratories test including blood sugar he collected blood in a plain vacutainer at 7 AM. The sample was sent to the laboratory for analysis.

The sample of laboratory investigations was performed late at 11AM. All laboratory results were normal except blood glucose which was 45 mg/dL. Later that morning, a laboratory technician noticed the blunder done by phlebotomist while collecting blood sample specifically for blood glucose. Consultant doctor requested for repeat blood sample in a correct vacutainer. After processing the repeat sample the blood glucose level was found 85 mg/dL.

Questions

- What is the mistake done by phlebotomist? Which vacutainer is supposed to use by him?
- Which is the preferred sample for blood glucose?
- What is the reason of low blood glucose values in the blood sample collected in a plain vacutainer?

(Contd...)

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(Contd...)

Discussion

- Phlebotomist collected the blood sample for glucose in plain vacutainer instead of grey colored vacutainer tube containing potassium oxalate and sodium fluoride.
- ***Plasma is the preferred sample compared to serum for glucose estimation***. Measurement of glucose in plasma allows samples to be centrifuged promptly without waiting for the blood to clot, and values can be measured immediately to avoid glycolysis.
- RBCs can metabolize glucose to lactate by glycolysis and lowers the glucose in blood collected in plain vacutainer. Blood samples for glucose estimation should be collected in grey colored vacutainer tube containing sodium fluoride, antiglycolytic agent which prevents loss of glucose by spontaneous glycolysis in collected blood. The rate of loss of blood glucose is 10 mg/dL per hour if blood is collected in plain vacutainer. Four-hour gap since the time of collection and actual analysis make loss of 40 mg/dL in the above case.

Anaerobic Glycolysis

The availability of oxygen now determines which of the two pathways is followed:

1. If anaerobic conditions prevail, the reoxidation of NADH to NAD^+ by transfer of reducing equivalents through the respiratory chain to oxygen is prevented. Under these conditions, **pyruvate** is reduced to **lactate,** by **lactate dehydrogenase** accepting electrons from NADH and thereby regenerating the NAD^+ necessary for glycolysis to continue **(Fig. 10.7)**.
2. Tissues that function under low oxygen conditions (hypoxia) produce lactate, e.g., vigorously contracting skeletal muscle. Certain tissues and cell types (retina and erythrocytes) converts glucose to lactate even under aerobic conditions because of absence of mitochondria.

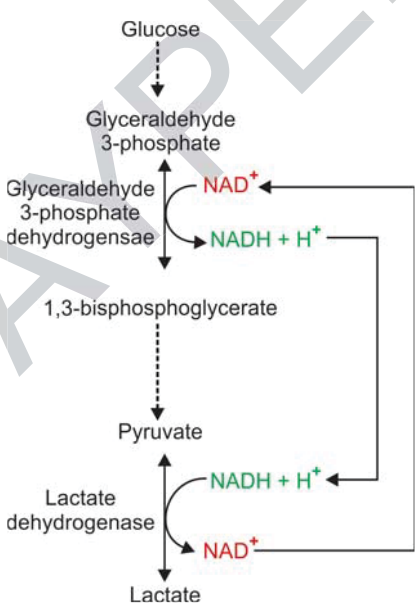

FIG. 10.7: Anaerobic glycolysis. Conversion of pyruvate to lactate.

CLINICAL APPROACH 10.4

Lactate and its Clinical Implications

- Lactate production is increased in **septic shock** and many **cancers**.
- Septic shock is a severe complication of sepsis (infection) that can include insufficient circulation, very low blood pressure (hypotension), an altered mental state, and organ dysfunction.
- Inadequate supply of oxygen to the tissues leads to **anaerobic** metabolism and increased **lactate** production which reflects shock status.
- Measurement of blood lactate is useful to assess the presence and severity of **septic shock** and to monitor the **patient's recovery**.
- In many disorders, levels of blood lactate provide rapid and early detection of **oxygen debt** (temporary oxygen shortage) in patients.
- The amount of oxygen required to remove the lactic acid (created from anaerobic cellular respiration in anoxic episodes), and replace the body's reserves of oxygen, is called the **oxygen debt**.

Rapoport-Luebering Cycle (2,3-Bisphosphoglycerate Pathway in Erythrocytes)

- In erythrocytes, the reaction of glycolysis catalyzed by **phosphoglycerate kinase** may be bypassed to some extent and 1,3-bisphosphoglycerate is converted to **2,3-bisphosphoglycerate** by an enzyme **bisphosphoglycerate mutase**.
- Then 2,3-bisphosphoglycerate converted to 3-phosphoglycerate and inorganic phosphate (P_i) by 2,3-bisphosphoglycerate phosphatase **(Fig. 10.8)**.
- This pathway is also called **Rapoport-Luebering cycle** or **bisphosphoglycerate shunt**.

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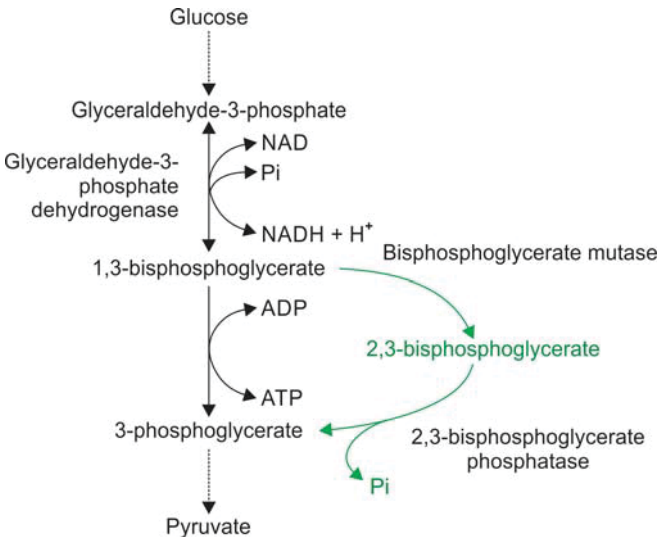


FIG. 10.8: Rapoport-Luebering cycle of erythrocytes.

- This alternative pathway involves **no net yield of ATP** from glycolysis as due to lack of mitochondria in erythrocytes glycolysis occur anaerobically to lactate.
- Fetal Hb (HbF) is less sensitive than HbA to the effects of 2,3-BPG; the higher oxygen affinity of HbF, even in the presence of 2,3-BPG, promotes efficient transfer of O_2 across the placenta from HbA to HbF.
- In the RBC, **10%–20%** of the glycolytic intermediate 1,3-bisphosphoglycerate is diverted to the synthesis of 2,3-bisphosphoglycerate (2,3-BPG).

Significance of Rapoport-Luebering Cycle

- It supplies 2,3-bisphosphoglycerate for the **hemoglobin function** in oxygen transport. 2,3-bisphosphoglycerate regulates the binding and release of oxygen from hemoglobin.
- 2,3-bisphosphoglycerate present in erythrocytes acts as a **buffer**. Organic phosphate in the form of **2,3-bisphosphoglycerate** accounts for about 16% of the **non-carbonate buffer** value of erythrocyte fluid.
- 2,3-BPG concentration increases in the RBC during adaptation to high altitude, in chronic obstructive pulmonary disease, and in anemia, promoting the release of O_2 to tissues when the O_2 tension and saturation of hemoglobin is decreased in the lung.

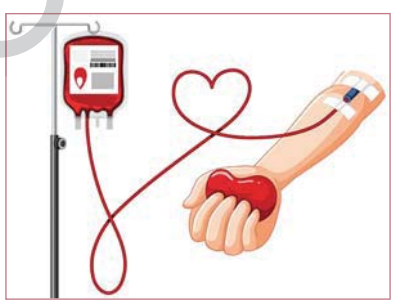
Significance of Glycolysis

- Glycolysis is the principal route for glucose metabolism for the production of ATP molecules.
- It also provide pathway for the metabolism of fructose and galactose derived from diet.
- Glycolysis has the ability to provide ATP in the absence of oxygen and allows tissues to survive anoxic episodes.

CLINICAL APPROACH 10.5

The Significance of 2,3-BPG in Blood Transfusions

- 2,3-BPG plays an important role in blood that has been stored for transfusion.
- The concentration of 2,3-BPG in the stored red blood cells can fall to one tenth of its concentration within a few days, resulting in a decreased ability of transfused blood to deliver O_2 to tissues.
- The problem cannot be solved by incorporating 2,3-BPG into the medium because this polar molecule cannot be transferred across the cell membrane.
- The decrease can be prevented by storing blood in the presence of inosine, (purine nucleoside containing ribose sugar) that is converted to BPG by RBC.
- Inosine can enter RBC, where its ribose moiety is released from purine base and ribose then phosphorylated and enter the hexose monophosphate pathway and converted to 2,3-BPG via glycolysis.



- It generates precursors for biosynthetic pathways, for example:
  - Pyruvate may be transaminated to amino acid **alanine**.
  - In the liver, pyruvate provides substrate, acetyl-CoA for **fatty acid** and **cholesterol** biosynthesis.
  - Glycerol-3-phosphate derived from glycolytic pathway forms the backbone of **triacylglycerol** and **phospholipids**.
- In erythrocytes glycolysis supplies 2,3-BPG which is required for the hemoglobin function in the transport of oxygen.
- The reoxidation of NADH via lactate formation (anaerobic glycolysis) allows glycolysis to proceed in the absence of oxygen by regenerating sufficient NAD^+ necessary for glycolysis to continue.
- Anaerobic glycolysis is an emergency source of ATP.
- In mammals, glucose is the only fuel that the brain uses under nonstarvation conditions and the only fuel that red blood cells can use at all.

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Regulation of Glycolysis

- The glycolytic pathway has dual role. It degrades glucose to:
  - Generate ATP
  - Provide precursors for synthesis of fatty acids, cholesterol, triglyceride **(Fig. 10.4)** and alanine.
- The rate of conversion of glucose into pyruvate is regulated to meet these two major cellular needs.
- Glycolysis is regulated at **three irreversible** steps. These reactions are catalyzed by:
  1. Hexokinase
  2. Phosphofructokinase-1
  3. Pyruvate kinase
- We will consider the control of glycolysis in two tissues: **muscle** and **liver**.

Regulation of Glycolysis in Muscle

Glycolysis in muscle is regulated to meet the **need of ATP**. Control of glycolysis in muscle depends on the ratio of **ATP** to **AMP (Fig. 10.9)**.

1. **Phosphofructokinase-1 (PFK-1)** is the most important primary regulatory enzyme in glycolysis.
   - High levels of ATP allosterically inhibit the enzyme by lowering the **phosphofructokinase-1** enzyme's affinity for its substrate, fructose 6-phosphate.
   - AMP reverses the inhibitory action of ATP and so the activity of the enzyme increases when the ATP/AMP ratio is lowered.
   - **A decrease in pH also inhibits phosphofructo**-kinase-1 activity. The pH might fall when muscles functioning anaerobically, producing excessive quantities of lactic acid. The inhibitory effect protects the muscle from damage that would result from the accumulation of too much of lactic acid.
2. **Hexokinase** is an allosteric enzyme catalyzing the first step of glycolysis in muscle is inhibited by its product **glucose-6-phosphate** in extrahepatic tissue.
   - High concentration of glucose-6-phosphate signal that the cell no longer requires glucose for energy or for the synthesis of glycogen, a storage form of glucose.

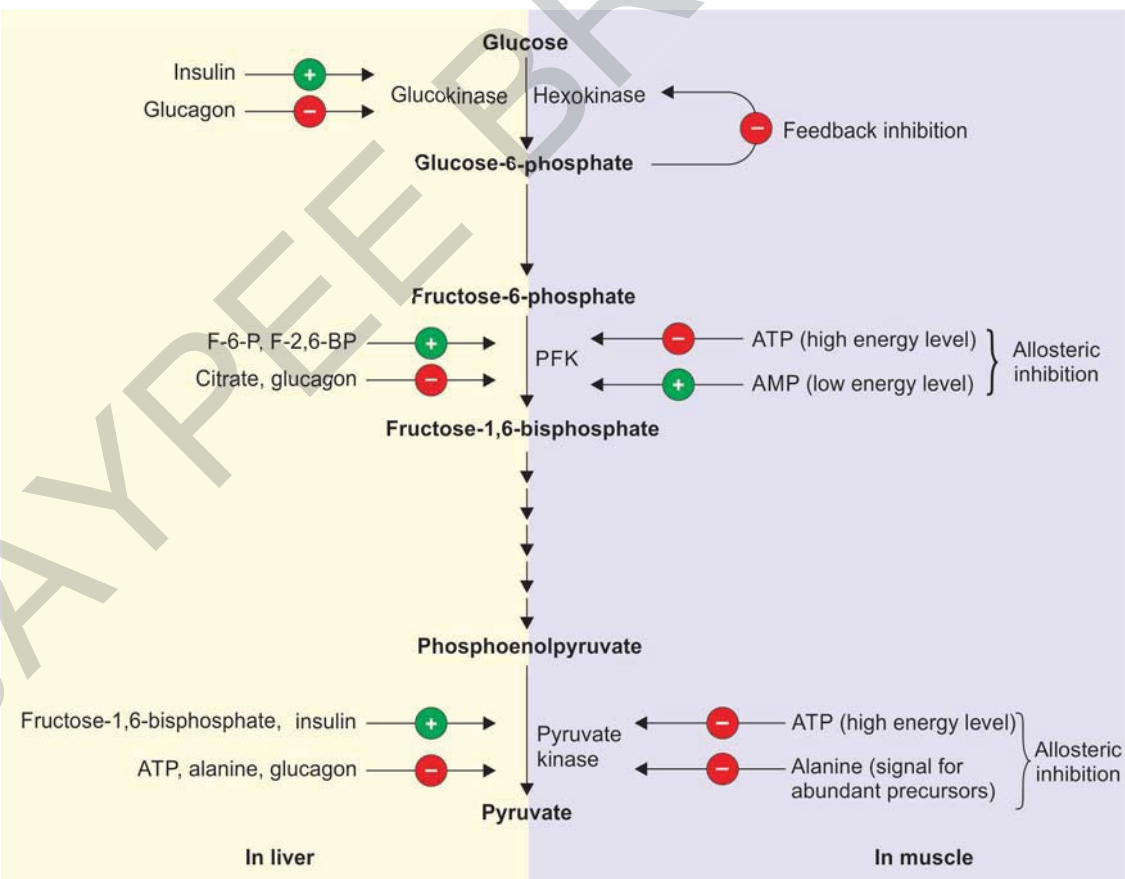

FIG. 10.9: Regulation of glycolysis in muscle and liver.
(PFK: Phosphofructokinase)

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- When phosphofructokinase-1 is inactive, the concentration of fructose-6-phosphate rises. In turn, the level of glucose-6-phosphate rises. Hence, the inhibition of phosphofructokinase-1 leads to the inhibition of hexokinase.

- Both **hexokinase and PFK-1** are important regulatory enzymes in glycolysis.
- But *PFK-1 is a more important primary committed regulatory enzyme of glycolysis than hexokinase because*:
  - PFK-1 reaction directs glucose to glycolysis, the only pathway for the metabolism of Fru-1,6-BP.
  - However in hexokinase reaction glucose-6-phosphate is not only a precursor for glycolysis, in muscle, glucose-6-phosphate can also be a precursor for the synthesis of glycogen or hexose monophosphate pathway.

3. **Pyruvate kinase** is the enzyme catalyzing the third irreversible step in glycolysis. This final step yields ATP and pyruvate that can be oxidized further or used as a precursor for synthetic reactions.
   - **ATP allosterically inhibits pyruvate kinase** to slow glycolysis when energy level is high.
   - **Alanine** also **allosterically inhibits pyruvate kinase** but in this case, it signals that precursors are abundant. Alanine is formed by pyruvate by accepting amino group. An increase in alanine concentration indicates an excess of pyruvate.

Regulation of Glycolysis in Liver

- The liver has more diverse biochemical functions than muscle:
  - The liver maintains blood glucose levels:
    - It stores glucose as glycogen when glucose is plentiful, and it releases glucose when it is low.
    - It provides precursors for synthesis of non-essential amino acids as well as glycerol, part of fat.
    - It also uses glucose to generate NADPH.
- Although the liver has many of the regulatory features of muscle glycolysis, the regulation of glycolysis in the liver is more complex.

Liver Phosphofructokinase-1

- Liver phosphofructokinase-1 can be regulated by ATP as in muscle, but such regulation is not as important since the liver does not experience the sudden ATP needs that a contracting muscle does.
- Likewise, low pH is not an important metabolic signal for the liver enzyme, because lactate is not normally produced in the liver.
- Glycolysis in the liver furnishes **precursors** for synthetic reactions and so a signal indicating whether precursors are abundant or scarce regulates phosphofructokinase.

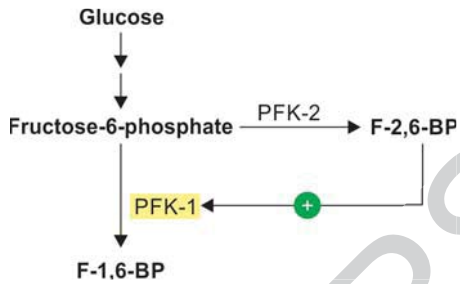

FIG. 10.10: Regulation of phosphofructokinase by fructose-2,6-bisphosphate.
(F-1,6-BP: fructose-1,6-bisphosphate; F-2,6-BP: fructose-2,6-bisphosphate; PFK: Phosphofructokinase)

  - In liver phosphofructokinase is inhibited by **citrate**, an intermediate in the citric acid cycle. A high level of citrate in the cytoplasm indicates that biosynthetic precursors are abundant, and so there is no need to degrade additional glucose for this purpose.
- One more means by which glycolysis in the liver is regulated is through **fructose 2,6-bisphosphate** (F-2,6-BP) a potent activator of **phosphofructokinase-1 (Fig. 10.10)**. In the liver, the concentration of fructose-6-phosphate rises when blood glucose concentration is high, and the abundance of fructose-6-phosphate accelerates the synthesis of F-2, 6-BP. The binding of F-2,6-BP increases the affinity of phosphofructokinase-1 for fructose-6-phosphate.
- Glycolysis is thus accelerated when glucose is abundant **(feed-forward stimulation)**.

Regulation of Glucokinase (Liver Hexokinase)

- Glucokinase reaction is the same in the liver as in muscle. However, the liver is involved in regulation of blood glucose levels, possesses another specialized isoenzyme of hexokinase, called **glucokinase**, which is not inhibited by **glucose-6-phosphate**.
- Glucokinase phosphorylates glucose only when glucose is abundant because the affinity of glucokinase for glucose is about 50-fold lower than that of hexokinase. The role of glucokinase is to provide glucose-6-phosphate for the synthesis of **glycogen** and for the formation of **fatty acids**.
- The activity of glucokinase is thus **influenced by carbo-hydrate intake**:
  - The activity increases with carbohydrate intake.
  - Decreases during **starvation** and **diabetes mellitus**.
- Liver glucokinase is an **inducible enzyme** that increases its synthesis in response to **insulin** and decreases in response to **glucagon**. Insulin signals the need to remove glucose from the blood for storage as glycogen or conversion into fat.

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Pyruvate Kinase

- Fructose 1,6-bisphosphate allosterically stimulates the enzyme, **pyruvate kinase** while **ATP** and **alanine** are allosteric inhibitors.
- Liver **pyruvate kinase** is an **inducible enzyme** that increases its synthesis in response to **insulin** and decreases in response to **glucagon**. Glucagon, secreted in response to low blood glucose and insulin secreted when blood glucose levels are adequate.

Energetics of Glycolysis

- The net reaction of aerobic glycolysis of glucose into two molecules pyruvate generates:
  - Two molecules of NADH
  - Four molecules of ATP at substrate level phosphorylation.
  - Two molecules of ATP per mole of glucose are consumed for reactions of **hexokinase** and **phosphofructokinase**.
- The net gain is **2 moles of ATP** and **2 moles of NADH**.
- The NADH produced transported into the mitochondria, where these undergo oxidative phosphorylation with the release of **5 moles of ATP** (2.5 moles of ATP per mole of NADH).
- The total net ATP gain in aerobic glycolysis is **7 moles** of ATP per mole of glucose oxidized to pyruvate **(Table 10.3)**.
- In anaerobic glycolysis, on the other hand, **only 2 moles of ATP** are produced per mole of glucose and no NADH is formed.

Anaerobic tissues metabolize glucose much more rapidly than aerobic tissues do to compensate for this meager (scanty) energy gain.

- When the anaerobic glycolysis occur using glycogen as a starting compound, the net production of ATP is increased from 2 to 3 by glycolytic pathway. This is due to bypass of hexokinase reaction (1 ATP consuming reaction), because glycogen through glycogenolysis provides glucose-6-phosphate directly to glycolysis **(*see* Fig. 10.26)**. Thus, in muscles anaerobic glycolysis from **glycogen generates 3 ATPs** but not two.
- In aerobic conditions, pyruvate is converted to **acetyl-CoA** which enters the TCA cycle for complete oxidation to CO_2 and H_2O. Complete oxidation of glucose through **glycolysis plus citric acid cycle** will yield net 32 moles of ATP.

Disorders of Glycolysis

Pyruvate Kinase Deficiency

- Pyruvate kinase deficiency (PKD) is the most common enzyme defect of glycolysis and an important cause of hereditary, non-spherocytic hemolytic anemia.
- Pyruvate kinase is a key enzyme in glycolysis, catalyzes the final step with formation of ATP. Genetic deficiency of pyruvate kinase in the erythrocyte leads to **hemolytic anemia, jaundice,** and **splenomegaly** due to excessive erythrocyte destruction. The presence of abnormal-shaped red blood cells (non-spherocytic, **bizarre erythrocytes)**, including **spiculated cells** on peripheral smear are the main features of anemia due to pyruvate kinase deficiency.
- The normal mature erythrocyte lacks mitochondria and is completely dependent on glycolysis for its energy in the form ATP. In erythrocytes deficiency of **pyruvate kinase** leads to reduced rate of glycolysis and the rate of ATP being inadequate to meet the energy needs of the cells and maintain the structural integrity of the erythrocyte membrane.
- Decreased ATP production affects the **cation pump** in the cell membrane. Ca^{2+} enters cells, while K^+ and H_2O leave the cell and cells become dehydrated and phagocytosed by cells in the spleen. The premature death and lysis of the red blood cells result in hemolytic anemia.

TABLE 10.3: Production of ATP in glycolysis.

Reaction	*Reaction catalyzed by*	*Mechanism of ATP production*	*No. of ATP formed*
Glyceraldehyde-3-phosphate to 1,3-bisphosphoglycerate	Glyceraldehyde-3-phosphate dehydrogenase	Respiratory chain oxidation of 2 NADH	+5*
1,3-bisphosphoglycerate to 3-phosphoglycerate	Phosphoglycerate kinase	Substrate level phosphorylation	+2
Phosphoenolpyruvate to pyruvate	Pyruvate kinase	Substrate level phosphorylation	+2
Consumption of ATP for reactions of hexokinase and phosphofructokinase			–2
			Net 7

*This assumes the NADH formed by glycolysis is transported into mitochondria by the **malate shuttle**.

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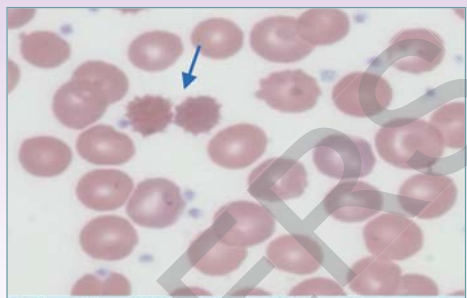
IMAGE-BASED QUESTION 10.1

What is the most likely diagnosis for the 2-year-old black girl presenting with severe anemia, splenomegaly, jaundice, normal hemoglobin electrophoresis, normocytic anemia, normal platelet and white blood cell counts, and the presence of bizarre erythrocytes including spiculated cells on peripheral smear?

a. Sickle cell disease
b. Thalassemia
c. Pyruvate kinase deficiency
d. G-6-PD deficiency



Two irregularly constricted with loss of central pallor and the presence of multiple spicules red cells (blue arrow) with pyruvate kinase deficiency

Discussion

a. **Sickle cell disease:** Sickle cell disease is characterized by the presence of hemoglobin S (HbS), which causes red blood cells to take on a sickle shape. This condition commonly leads to anemia, pain crises, and organ damage. However, in the scenario provided, the hemoglobin electrophoresis was normal, making sickle cell disease less likely.
b. **Thalassemia:** Thalassemias are genetic disorders that result in reduced production of hemoglobin and abnormal red blood cell formation. They can lead to anemia, but the hemoglobin electrophoresis typically shows abnormal hemoglobin patterns. Since the hemoglobin electrophoresis was normal in this case, thalassemia is less likely.
c. **Pyruvate kinase deficiency:** Pyruvate kinase deficiency is a rare inherited disorder that affects the red blood cells' ability to generate energy. It leads to hemolytic anemia, jaundice, and splenomegaly. The presence of bizarre erythrocytes, including spiculated cells on peripheral smear, is suggestive of this condition. The normal hemoglobin electrophoresis is consistent with this diagnosis, as it doesn't involve abnormal hemoglobin types.
d. **G-6-PD deficiency:** Glucose-6-phosphate dehydrogenase (G-6-PD) deficiency is an enzyme deficiency that can cause hemolytic anemia, especially in response to oxidative stress. While it can result in jaundice and anemia, the scenario doesn't mention abnormal erythrocyte morphology or spiculated cells on peripheral smear. G-6-PD deficiency is less likely in this case.

Answer: The most likely diagnosis that aligns with the patient's symptoms, peripheral smear findings, and normal hemoglobin electrophoresis is:
c. Pyruvate kinase deficiency.

Hexokinase Deficiency

- Genetic defects in the **hexokinase** of erythrocyte reduce the amount of oxygen that is available for tissues.
- Because hexokinase is the first enzyme in glycolysis, the red blood cells of these patients contain low concentration of **2,3-BPG** (glycolytic intermediate in erythrocytes) which normally allows hemoglobin to release oxygen in tissue.
- Consequently, due to low level of 2,3-BPG less oxygen is available for the tissue. This defect will result in anemia.

Phosphotriose Isomerase Enzyme Deficiency

- **Phosphotriose** isomerase deficiency is an autosomal recessive disorder characterized by congenital hemolytic anemia, and progressive neuromuscular dysfunction beginning in early childhood.
- Many patients die from respiratory failure in childhood.
- The neurologic syndrome is variable, but usually includes lower motor neuron dysfunction with hypotonia (decreased muscle tone), muscle weakness and atrophy, and hyporeflexia (muscles are less responsive to stimuli).
- Some patients may show additional signs such as dystonic posturing and/or spasticity.
- Laboratory studies show intracellular accumulation of dihydroxyacetone phosphate (DHAP), particularly in red blood cells.
- This deficiency is characterized by severe hemolytic anemia and neurodegeneration.

Lactic Acidosis

- Lactic acidosis is the accumulation of lactic acid in the blood to levels that significantly affect the blood pH. The high concentration of lactate results in lowered blood pH (7.2).
- Under normal conditions, lactate is metabolized in the liver and the blood lactate level is in between **1 and 2 mM**. With lactic acidosis, the blood lactate level may be **5 mM** or **more**.
- Lactate accumulation in the body fluids can be due to **increased formation** or **decreased utilization**.
- A common cause of lactic acidosis is tissue **hypoxia** caused by **shock**, **cardiopulmonary arrest**, and **hypo**

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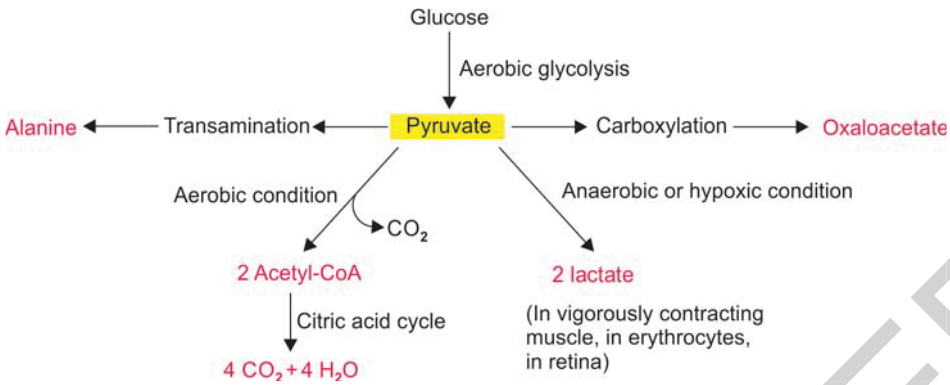

FIG. 10.11: Catabolic fates of pyruvate.

perfusion. Inadequate blood flow leads to deprivation of oxygen. Oxygen deprivation leads to decreased ATP production and accumulation of NADH, which promotes conversion of pyruvate to **lactate**. In order to survive, the cells use anaerobic glycolysis for generating ATP with increased formation of lactic acid.

- Laboratory assessment includes measurements of **blood lactate, pyruvate, beta-hydroxybutyrate** and **acetoacetate**.
- Blood **lactate to pyruvate** ratio below 25 suggests defects in a **gluconeogenic enzyme or pyruvate dehydrogenase**.
- The primary treatment of lactic acidosis involves correcting the basic cause such as reversal of circulatory failure.

Glycolysis in Cancer Cell

In fast growing cancer cells, glycolysis proceeds at a high rate. Cancer cells stimulate glucose uptake and glycolysis. As cancer cells grow more rapidly, blood vessels cannot supply oxygen efficiently to fulfill the required demand of oxygen by rapidly grown tumor cells. For the survival of tumor cells, some metabolic adaptations occur.

- They begin to grow in hypoxic (absence of oxygen) condition.
- Under these conditions glucose is oxidized anaerobically to **lactic acid** and this pathway becomes primary source of ATP for tumor cells.
- Thus fast growing cancer cells, leads to **lactic acidosis**.

METABOLIC FATES OF PYRUVATE

During aerobic glycolysis glucose is converted to pyruvate. Pyruvate is also formed in degradation of amino acids and has several fates depending on the tissue and its metabolic state. It can be converted to **acetyl-CoA**, **lactate**, **alanine**, or **oxaloacetate** depending on the needs of the cell. The fates of pyruvate and types of reactions in which it participates are given in **Figure 10.11**.

- **Conversion into acetyl-CoA:** Pyruvate is oxidized, with loss of its carboxyl group as CO_2 to yield **acetyl-CoA**.
- **Conversion into lactate in muscle and RBC (Anaerobic glycolysis (Fig. 10.7):** Pyruvate is reduced to **lactate**. Certain tissues and cell types (retina and erythrocytes for example) convert glucose to lactate even under aerobic conditions.
- **Conversion into alanine, or oxaloacetate:** The oxidation of pyruvate is an important catabolic process, but pyruvate has anabolic fates as well. It can provide the carbon skeleton for the synthesis of the amino acid **alanine** or **oxaloacetate**.

Conversion of Pyruvate into Acetyl-CoA

Pyruvate formed in **cytosol** by glycolysis, is transported into the **mitochondrion** by a specific carrier protein present in the mitochondrial membrane. Inside the mitochondria, it is **oxidatively decarboxylated** to **acetyl-CoA** by a multienzyme complex, **pyruvate dehydrogenase**. Pyruvate dehydrogenase is a large complex of **three distinct enzymes** and **five coenzymes (Table 10.4)**.

TABLE 10.4: Enzymes, coenzymes and vitamins required for pyruvate dehydrogenase complex.

Enzymes	• Pyruvate dehydrogenase • Dihydrolipoyl transacetylase • Dihydrolipoyl dehydrogenase
Coenzymes	• Thiamine pyrophosphate (TPP) • Flavin adenine dinucleotide (FAD) • Coenzyme A (Co-A or denoted as CoA-SH) • Nicotinamide adenine dinucleotide (NAD^+) • Lipoamide (Lipoic acid)
Vitamins	• Thiamine (in TPP) • Riboflavin (in FAD) • Niacin (in NAD^+) • Pantothenic acid (in CoA)

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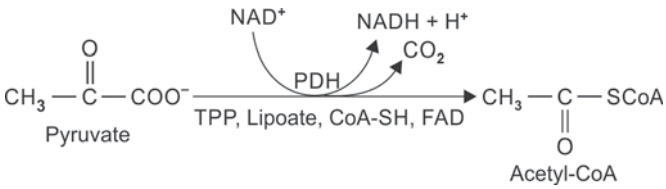

FIG. 10.12: Oxidative decarboxylation of pyruvate by the pyruvate dehydrogenase complex.

- In this reaction, the **carboxyl group** is removed from pyruvate as a molecule of **CO_2** and the two remaining carbons become the acetyl group of **acetyl-CoA** with the generation of a molecule of **NADH (Fig. 10.12)**.
- The transfer of electrons from NADH to oxygen ultimately generates **2.5 molecules of ATP** per molecule of pyruvate.
- Pyruvate dehydrogenase is regulated by **feedback inhibition**. Pyruvate dehydrogenase is inhibited by its product **acetyl-CoA** and **NADH**.
- It is also regulated by **phosphorylation** and **dephosphorylation** (covalent modification) of pyruvate dehydrogenase multienzyme complex. By phosphorylation active pyruvate dehydrogenase enzyme is converted to its inactive form.

INSIGHT 10.5

Lipoic Acid

- Lipoic acid is an **organosulfur compound** synthesized by humans and plants in mitochondria from octanoic acid and cysteine and is ***essential for aerobic metabolism***.
- α-Lipoic acid is found in small amounts in spinach, broccoli, peas, and potatoes.
- It acts as a cofactor for **pyruvate dehydrogenase** and **α-ketoglutarate dehydrogenase** activity.

Significance of Pyruvate to Acetyl-CoA Pathway

- The conversion of pyruvate to acetyl-CoA is a central step, linking the glycolytic pathway with citric acid cycle **(Fig. 10.13)** hence called **link reaction**.
- Acetyl-CoA is also an important intermediate in lipid metabolism, cholesterol biosynthesis and acetylation reactions.
- Different metabolic sources and fates of acetyl-CoA are shown in **Figure 10.14**.
- Fates of acetyl-CoA generated in the mitochondrial matrix include:
  - Complete oxidation of the acetyl group in the TCA cycle for energy generation.
  - In liver, conversion of excess acetyl-CoA into the ketone bodies, acetoacetate, and β-hydroxybutyrate.
  - Transfer of acetyl units as citrate to the cytosol for the synthesis of long chain fatty acids and cholesterol.

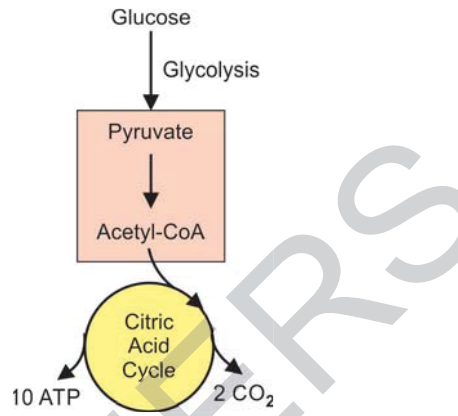

FIG. 10.13: The link between glycolysis and final common pathway citric acid.

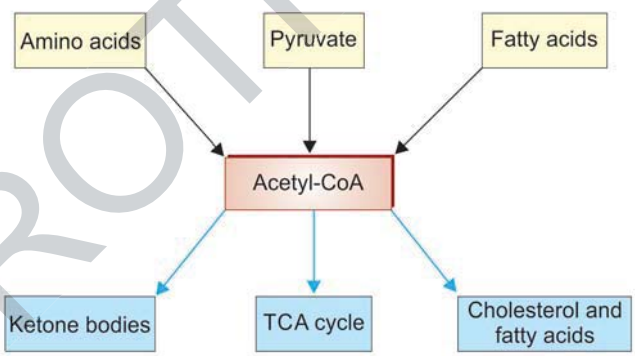

FIG. 10.14: Metabolic fates of acetyl-CoA.

Pyruvate Dehydrogenase Deficiency

- Pyruvate dehydrogenase deficiency is also known as **pyruvate dehydrogenase complex deficiency** or **PDCD** or **PDH deficiency**.
- Pyruvate dehydrogenase complex deficiency is a rare disorder of carbohydrate metabolism caused by a deficiency of **one of the three enzymes** (E1: pyruvate dehydrogenase, E2: dihydrolipoamide transacetylase and E3: dihydrolipoamide dehydrogenase) in the **pyruvate dehydrogenase complex**.
- Most individuals with PDCD have an abnormality in the PDHA1 gene located on the **X chromosome**. A smaller percentage follows autosomal recessive inheritance.
- A deficiency of any enzyme in these pathways results in inadequate removal of pyruvate and lactate from blood and tissues and causes **lactic acidosis**.

Symptoms

- Pyruvate dehydrogenase deficiency (PDHD) is typically characterized by:
  - **Neurological symptoms:** Poor coordination, seizures, weak muscles, developmental delay, abnormal eye movements, and ataxia.

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- **Metabolic symptoms:** Poor feeding, rapid breathing, lethargy, and lactic acidosis.
- **Brain abnormalities:** Abnormal brain structures, brain lesions, and atrophy of the cerebral cortex.
- **Facial dysmorphism:** Narrow head, frontal bossing, wide nasal bridge, long philtrum, and flared nostrils.
- **Other symptoms:** Intellectual disability, difficulty walking, and multiple fatty lumps.

Symptoms can vary from mild to severe and may be more noticeable during times of stress, illness, or after eating carbohydrates.



CASE STUDY 10.3: PYRUVATE DEHYDROGENASE COMPLEX DEFICIENCY

Case Scenario

A 3-year-old male patient presented to the emergency room due to respiratory failure, with generalized hypotonia, severe psychomotor development delay, and partial seizures. At the time of diagnosis, the child's laboratory result showed increase in lactic acid and alanine levels. Initial arterial blood gas analysis result was pH 6.80 (normal 7.35 to 7.45), PCO_2 131 mm Hg (normal 35 to 45 mm Hg), PO_2 75 mm Hg. The child was previously diagnosed with a mutation in the PDHA1 gene (which codes for pyruvate dehydrogenase complex) 2 months after birth. The patient was put on a ketogenic diet. Six months later, seizure frequency was reduced and psychomotor development had improved.

Questions

- What is the probable diagnosis?
- What is the cause of diagnosed disorder?
- What are the symptoms of the diagnosed disorder?
- What is the cause of lactic acidosis and neurological problems?
- What is the dietary therapy?

Discussion

- This patient is presenting with respiratory failure, hypotonia, and partial seizures. Laboratory testing demonstrates elevated alanine and lactate levels along with a PDHA1 gene mutation. In combination, these findings are concerning for **pyruvate dehydrogenase complex (PDC) deficiency**.
- Pyruvate dehydrogenase complex deficiency (PDCD) is an X-linked recessive mitochondrial disorder caused by **mutations in the PDHA1 gene** which encodes the **E1-alpha subunit** of the PDH complex. The pyruvate dehydrogenase complex is made up of multiple copies of three enzymes:
  - E1: Pyruvate dehydrogenase,
  - E2: Dihydrolipoamide transacetylase and
  - E3: Dihydrolipoamide dehydrogenase.
- Symptoms of PDC deficiency typically begin during infancy. Pyruvate dehydrogenase deficiency is characterized by the increase in **lactic acid** and a variety of **neurological problems**. The most common feature is **lactic acidosis (metabolic acidosis)**, which can cause nausea, vomiting, severe breathing problems, and an abnormal heartbeat. The neurological problems include, delayed development of mental abilities and motor skills such as sitting and walking, intellectual disability, seizures, weak muscle tone (hypotonia), poor coordination, and difficulty walking.
- A decrease in functional E1 alpha leads to reduced activity of the pyruvate dehydrogenase complex. PDC catalyzes the irreversible decarboxylation of pyruvate into acetyl-CoA, which enters the Krebs cycle to produce ATP. In PDC deficiency, acetyl-CoA production is impaired, and there is reduced ATP synthesis. Hence, the condition primarily affects cells with high energy requirements, such as **neurons** and **myocytes** resulting in the **neurological** and **neuromuscular degeneration**. At the same time, pyruvate accumulates and is converted into **lactate and alanine**. The excess lactic acid causes **lactic acidosis**. Thus pyruvate dehydrogenase complex deficiency (PDCD) disorder commonly associated with **lactic acidosis, progressive neurological** and **neuromuscular degeneration** and, usually, **death** during childhood.
- Lactic acidosis can be aggravated by carbohydrate consumption. Therefore, treatment consists of adopting a ketogenic diet, which is a diet low in carbohydrates and high in fat and ketogenic amino acids. This diet results in the generation of ketone bodies, which can be used as an alternative energy source by body tissues. This dietary approach can help to reduce lactate levels. Thiamine, a co-factor of pyruvate dehydrogenase, is also used routinely but only a minority show response to thiamine. Patients with pyruvate dehydrogenase E1-alpha deficiency exhibit the most favorable response to thiamine.

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CITRIC ACID CYCLE

The citric acid cycle is the final common pathway for the oxidation of fuel molecules, **carbohydrates**, **fatty acids**, and **amino acids**. Most fuel molecules enter the cycle as **acetyl coenzyme A**. In eukaryotes, the reactions of citric acid cycle take place in the matrix of the mitochondria in contrast with those of glycolysis, which take place in cytoplasm.

- The citric acid cycle is a series of reactions in mitochondria that bring about the catabolism of **acetyl-CoA** to **CO_2** and **H_2O**, liberating reducing equivalents, which, upon oxidation through respiratory chain of mitochondria, generate **ATP**.
- Citric acid cycle is also called **Krebs cycle** or **Tricarboxylic Acid Cycle (TCA):**
  - It is called **citric acid cycle** because citrate was one of the first compounds known to participate.
  - It is called **Krebs cycle**, because its reaction was formulated into a cycle by **Sir Hans Krebs**.
  - The most common name for this pathway is the **Tricarboxylic acid cycle** or **TCA cycle**, due to involvement of the tricarboxylate **citrate** and **isocitrate**.

Location of Citric Acid Cycle

The enzymes of citric acid cycle are located in the **mitochondrial matrix**, which facilitates the transfer of reducing equivalents to the respiratory chain, situated in the inner mitochondrial membrane.

Reactions of Citric Acid Cycle

The citric acid cycle has **eight** steps **(Fig. 10.15)**.

1. **Formation of citrate:** The first reaction of cycle is the condensation of acetyl-CoA with oxaloacetate to yield citrate, catalyzed by **citrate synthase**.
2. **Formation of isocitrate via cis-aconitate:** Citrate is converted to isocitrate through the intermediary formation of the tricarboxylic acid **cis-aconitate** by an enzyme **aconitase** (aconitate hydratase), which contains iron in the Fe^{2+} state. This conversion takes place in two steps, dehydration to cis-aconitate, and rehydration to isocitrate. The reaction is inhibited by **fluoroacetate**.
3. **Oxidation of isocitrate to α-ketoglutarate and CO_2:** Isocitrate undergoes oxidative decarboxylation catalyzed by **isocitrate dehydrogenase** to form α-ketoglutarate. The formation of **NADH** and liberation of **CO_2** occurs at this stage.

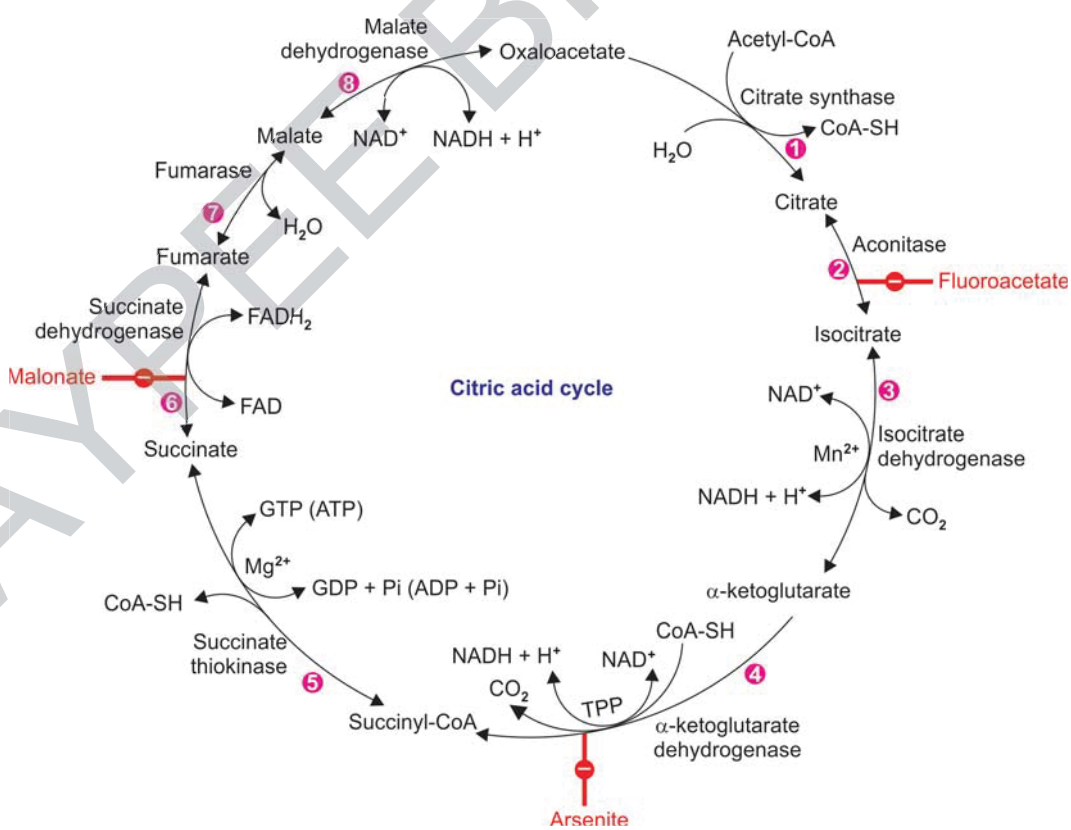

FIG. 10.15: Reactions of citric acid cycle.

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BIOCHEMISTRY

The seventh edition of ***Biochemistry*** has been thoroughly updated in accordance with the revised guidelines of the Competency-Based Medical Education (CBME) curriculum 2024, issued by the Undergraduate Medical Education Board (UGMEB).

Salient Features

- This edition comprises 34 chapters, up from 30 in the previous (sixth) edition. The four newly added chapters are: *Metabolism of Alcohol, Instrumentation and Techniques, Artificial Intelligence in Healthcare Industry,* and *AETCOM Module 1.1*.
- Each chapter begins with clearly defined competencies and learning objectives aligned with the CBME framework.
- Applied aspects of biochemistry are emphasized through: Clinical case boxes, clinical approach discussions, image-based questions, rich clinical illustrations related to key disorders, and "insight" boxes highlighting important and practical facts.
- This comprehensive approach eliminates the need for a separate book on clinical case studies.
- To reinforce learning, each chapter concludes with a variety of assessment questions, including: Long-answer type questions, short notes, scenario-based case studies, reasoning questions, multiple choice questions (MCQs).
- This CBME-aligned textbook has been recommended by several universities and medical colleges across India as part of the curriculum for the Indian Medical Graduates (IMG).

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In addition, she has actively participated in various academic committees under MUHS, contributing to the enhancement of biochemistry education. Dr Naik is also an accomplished author with three notable publications to her credit, published nationally and internationally, including as eBooks: *Essentials of Biochemistry*—recommended textbook for BDS, BTh, and BOT courses by MUHS and various other universities; *Textbook of Biochemistry for BSc Nursing*—as per the revised Indian Nursing Council (INC) syllabus; *Biochemistry Laboratory Practical Manual*—designed specifically for Phase-I MBBS students under the CBME framework.

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