INTRODUCTION TO METABOLISM

Course: Biochemistry (BIOC 230)

Instructor: Dr. Mahmoud A. Srour

Textbook:
Lehninger Principles of Biochemistry, 5th Ed. Chapters 14, 16 & 19

Introduction to metabolism

Chapter 14, p.527

- **Key topics:**
- Harnessing energy from glucose via glycolysis
- Fermentation under anaerobic conditions
- Gluconeogenesis
- Cellular respiration
- Conversion of pyruvate to activated acetate
- Reactions of the citric acid cycle
- Electron transport chain in mitochondria
- Building up the proton-motive force
- Synthesis of ATP in mitochondria

Central Importance of Glucose

- Glucose is an excellent fuel
  - Yields good amount of energy upon oxidation
  - Can be efficiently stored in the polymeric form
  - Many organisms and tissues can meet their energy needs on glucose only
- Glucose is a versatile biochemical precursor
- Bacteria can use glucose to build the carbon skeletons of:
  - All the amino acids
  - Membrane lipids
  - Nucleotides in DNA and RNA
  - Cofactors needed for the metabolism

Four Major Pathways of Glucose Utilization

1. When there's plenty of excess energy, glucose **can be stored** in the polymeric form (starch, glycogen)
2. Short-term **energy needs** are met by oxidation of glucose via glycolysis
3. Pentose phosphate pathway generates **NADPH** that is used for detoxification, and for the biosynthesis of lipids and nucleotides
4. **Structural polysaccharides** (e.g. in cell walls of bacteria, fungi, and plants) are derived from glucose

- Organisms that do not have access to Glucose, how they can overcome this obstacle like in case of plants?

Major pathways of glucose utilization

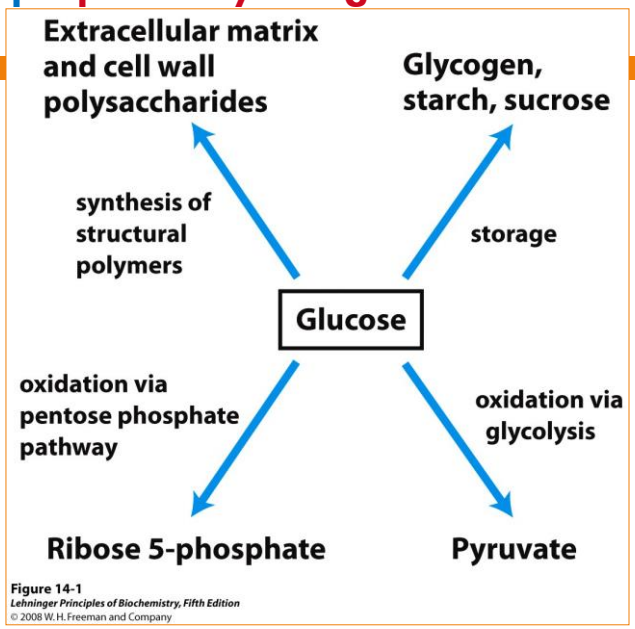

Figure 14-1
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Glycolysis: Importance

- Glycolysis is a sequence of enzyme-catalyzed reactions by which **glucose** is converted into **pyruvate**
  - Pyruvate can be further aerobically or anaerobically oxidized
  - Pyruvate can be used as a precursor in biosynthesis
- In the process, some of the oxidation free energy is captured by the synthesis of ATP and NADH

Glycolysis

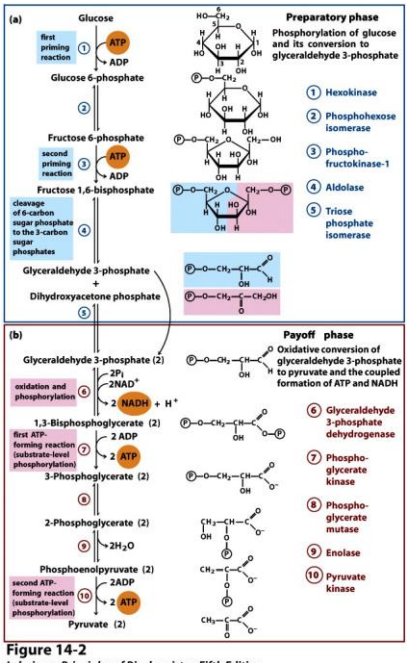
- Glycolysis is almost universal central pathway of glucose catabolism
- Glycolysis is the sole source of metabolic energy in some mammalian tissues and cell types (erythrocytes, renal medulla, brain and sperms for example)
- Glycolysis is probably the most ancient biochemical mechanism for obtaining energy from organic fuel molecules
- Glycolysis differs among species only in the details of its regulation and subsequent metabolic fate of pyruvate formed

Fermentation

- Fermentation is a general term for the anaerobic degradation of glucose or other organic nutrients to obtain energy, conserved as ATP

The two phases of Glycolysis: For each molecule of glucose that passes through the **preparatory phase (a)**, two molecules of glyceraldehyde 3-phosphate are formed; both pass through the **payoff phase (b).** Pyruvate is the end product of the second phase of glycolysis.

For each glucose molecule, 2 ATP are consumed in the preparatory phase and 4 ATP are produced in the payoff phase.



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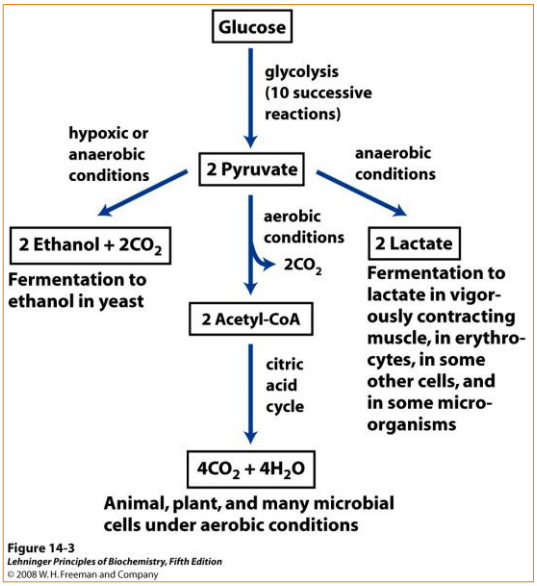
Chemical transformations in Glycolysis

- In the sequential reaction of glycolysis, three types of chemical transformations are particularly noteworthy:
  1. Degradation of carbon skeleton of glucose to yield pyruvate
  2. Phosphorylation of ADP to ATP by high-energy phosphate compounds formed during glycolysis
  3. Transfer of hydride ion to NAD+ forming NADH

Three possible catabolic fates of the pyruvate formed in glycolysis



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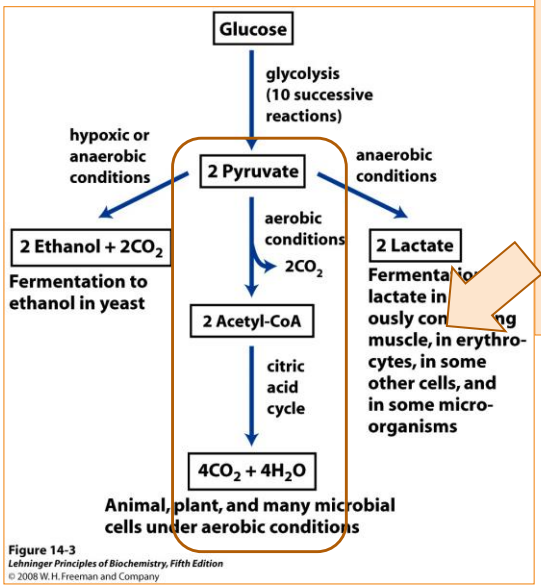
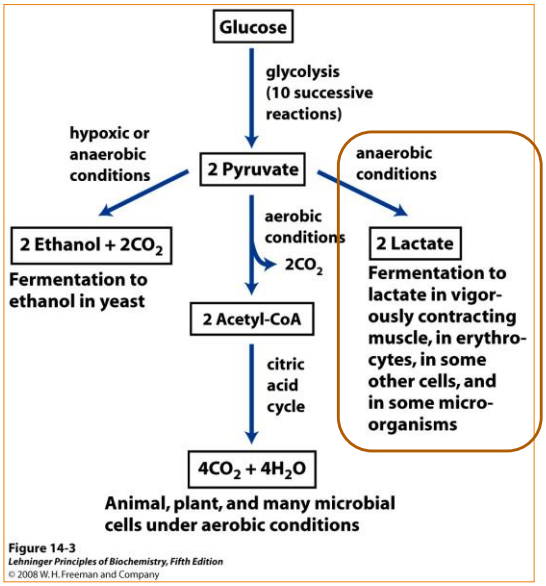


In aerobic organisms & under aerobic conditions, glycolysis is the only first stage in complete degradation of Glucose

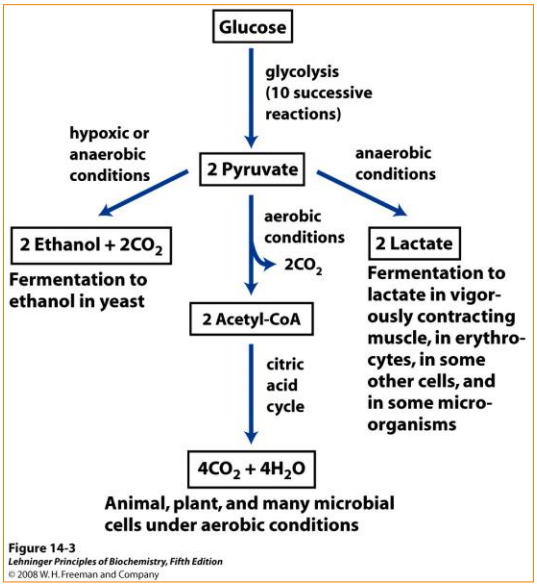
Three possible catabolic fates of the pyruvate formed in glycolysis



Lactic acid fermentation: Regeneration of NAD+ necessary for glycolysis to continue

Ethanol (alcohol) fermentation: in some plant tissues and brewers yeast, pyruvate is converted under hypoxic or anaerobic conditions into Ethanol and CO2

Three possible catabolic fates of the pyruvate formed in glycolysis



Anabolic fates for pyruvate

- Pyruvate provides the carbon skeleton for the synthesis of the amino acid alanine or for the synthesis of fatty acids.

ATP formation coupled to glycolysis

- During glycolysis some of the energy of the GLUCOSE molecule is conserved in ATP, which much remains in the product, pyruvate: Glucose + 2NAD+ + 2ADP +2Pi → 2 pyruvate + 2NADH+ $2H^+$+ 2ATP+$2H_2O$
- Energy remaining in pyruvate: glycolysis releases only a small fraction of the total available energy of the glucose molecule. The two molecules of pyruvate formed by glycolysis still contain most of the energy of glucose, which is extracted by oxidative using oxidative phosphorylation

Importance of phosphorylated intermediates

- Plasma membrane generally lacks transporters for phosphorylated sugars, which cannot leave the cell
- Phosphoryl groups are essential components in enzymatic conversation of metabolic energy
- Binding energy resulting from the binding of phosphate group to active sites of enzymes lowers the activation energy

Pyruvate Kinase is Subject to Regulation

- Pyruvate kinase requires divalent metals (Mg^{2+} or Mn^{2+}) for activity
- Under physiological conditions, the activity of pyruvate kinase is limited by the level of Mg^{2+}
- When there is plenty of ATP, the Mg ions are sequestered by ATP; this slows down pyruvate kinase
- Increased concentration of metabolites in the glycolytic pathway slows down glucose utilization

Pyruvate Kinase is Subject to Regulation (cont'd)

- The flux of glucose through the glycolytic pathway is regulated to maintain nearly constant ATP levels; as well as adequate supplies of glycolytic intermediates that serve biosynthetic roles
- How?
- By a complex interplay of ATP consumption, NADH regeneration and allosteric regulation of several glycolytic enzymes including hexokinase, PFK-1 and pyruvate kinase and concentration of key metabolites

Pyruvate Kinase is Subject to Regulation (cont'd)

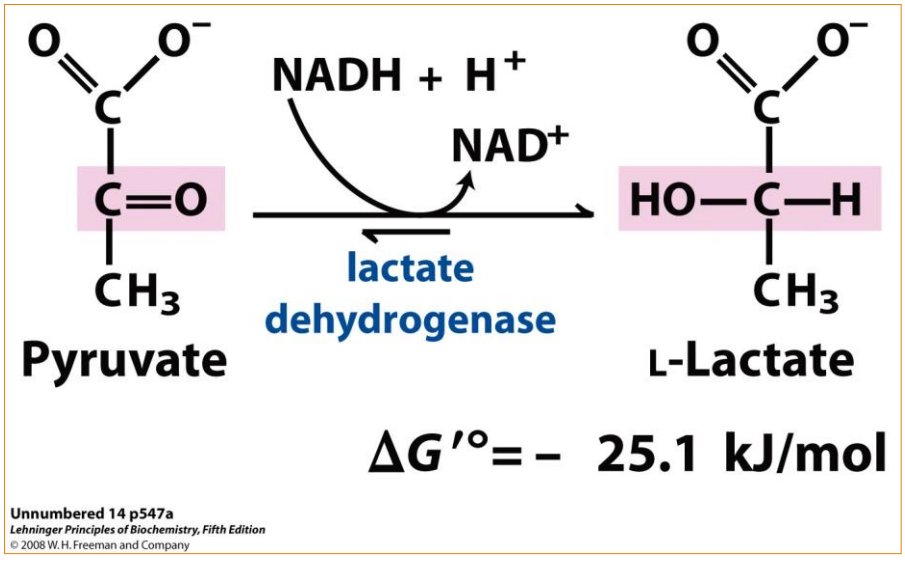
- On the long term; Glycolysis is regulated by hormones glucagon, epinephrine and insulin and by changes in expression of genes for several glycolytic enzymes

Cancerous tissues has deranged glucose catabolism

- Glucose uptake and glycolysis proceed about 10X faster in most solid tumors than in non-cancerous tissues
- Cancer tissues commonly experience hypoxia due to lack of an extensive capillary network.
- Cancer cells more than 100-200 um from nearest capillaries depend on anaerobic glycolysis for much of their ATP production
- Cancer cells have smaller number of mitochondria
- Many cancer cell overproduce several glycolytic enzymes including an isoenzyme of hexokinase

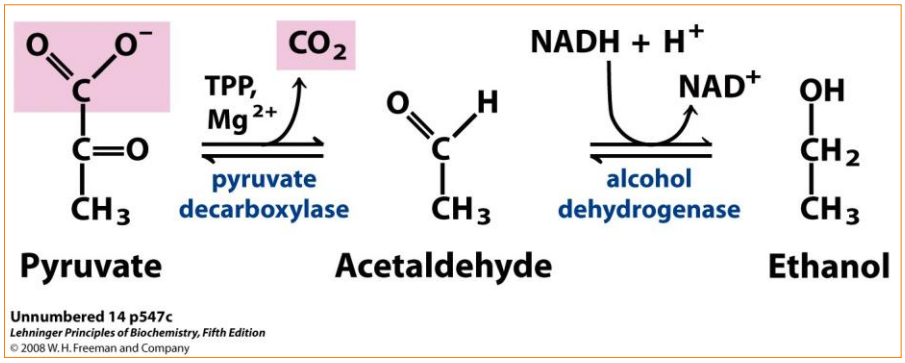
Under Anaerobic Conditions, Animals Reduce Pyruvate to Lactate

- During strenuous exercise, lactate builds up in the muscle
- The acidification of muscle prevents its continuous strenuous work
- The lactate can be transported to liver and converted to glucose there



Under Anaerobic Conditions, Yeast Ferments Glucose to Ethanol

- Both steps require cofactors
  - Mg2+ and thiamine pyrophosphate (TPP) in pyruvate decarboxylase
  - Zn2+ and NAD+ in alcohol dehydrogenase
- Pasteur effect: the rate and total amount of glucose consumed under anaerobic conditions is greater than under aerobic conditions?



Fermentation yields a variety of common foods and industrial chemicals

- Certain microorganisms present in raw food products ferment carbohydrates and yield metabolic products that give the food their characteristic forms
- Examples: yogurt is produced when *Lactobacillus bulgaricus* ferments carbo in milk producing lactic acid
- Propionibacterium freudenreichii ferments milk to produce propionic acid and CO2; propionic acid precipitates milk protein and CO2 bubbles cause the holes characteristic of Swiss cheese
- Other examples: pickles, brewing beer
- Fermentation causes a drop in pH that helps preserve foods

Gluconeogenesis: Precursors for Carbohydrates

- Notice that mammals cannot convert fatty acids to sugars
- The pathway from phosphoenolpyruvate to glucose 6-phosphate is common to the biosynthetic conversion
- The path from pyruvate to phosphoenolpyruvate leads through oxaloacetate
- Any compound that can be converted to either pyruvate or oxaloacetate can serve as starting material for gluconeogenesis

Carbohydrate synthesis from simple precursors

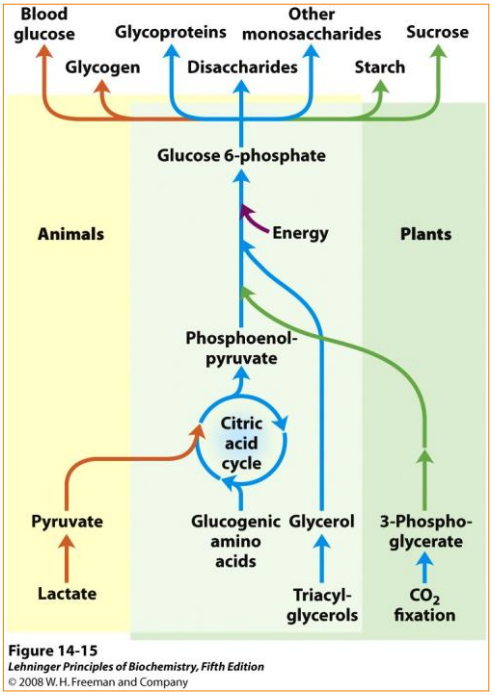


Figure 14-15
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Glycolysis vs. Gluconeogenesis

- Glycolysis occurs mainly in the muscle and brain
- Gluconeogenesis occurs mainly in the liver
- Both processes are NOT identical pathways running in opposite directions; but they share several steps. SEVEN of the ten enzymatic rxns of gluconeogenesis are the reverse of glycolysis rxns.
- Steps 1, 3 and 10 in glycolysis are irreversible in vivo and cannot be used in gluconeogenesis.

Opposing pathways of glycolysis and gluconeogenesis in rat liver

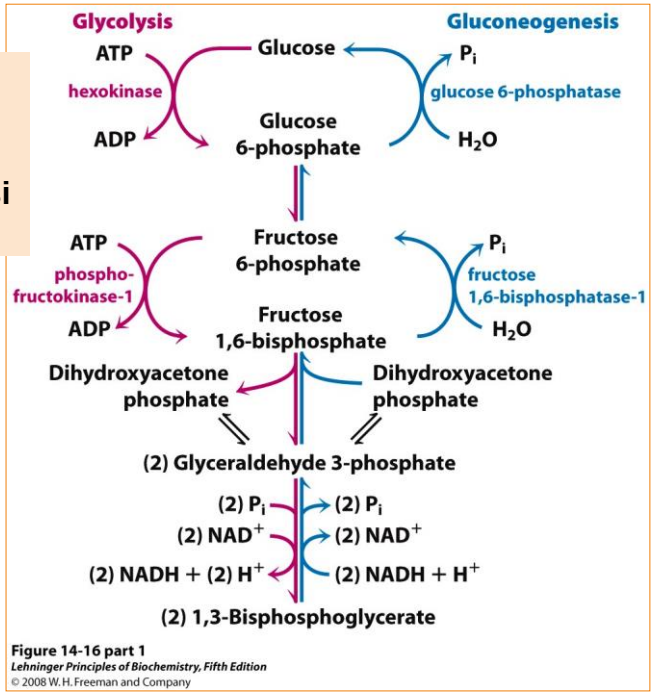

Figure 14-16 part 1
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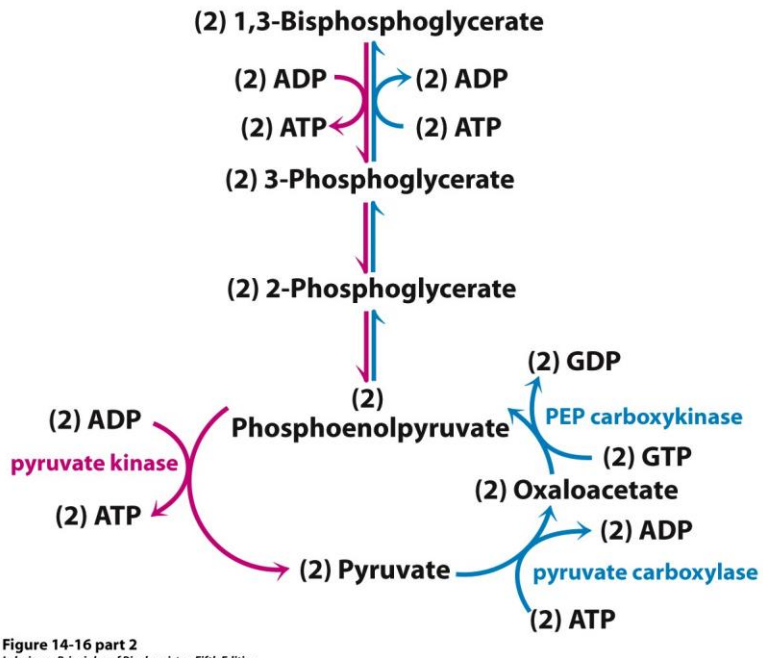

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Alternative paths from pyruvate to phosphoenolpyruvate

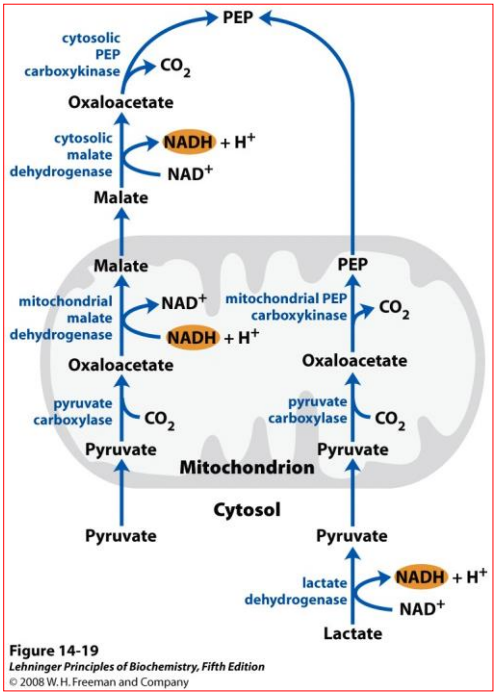

Figure 14-19
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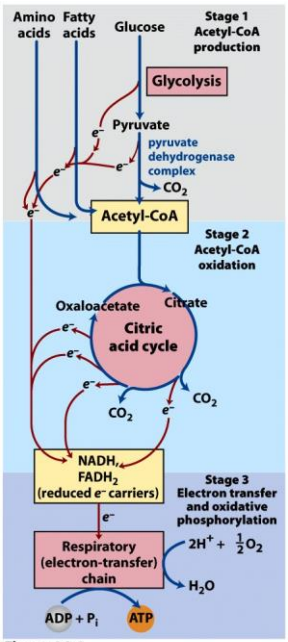
Chapter 16/ p.615

Cellular Respiration

- Respiration: the aerobic phase of catabolism
- Process in which cells consume O2 and produce CO2
- Provides more energy (ATP) from glucose than glycolysis
- Also captures energy stored in lipids and amino acids
- Evolutionary origin: developed about 2.5 billion years ago
- Used by animals, plants, and many microorganisms
- Occurs in three major stages:
  - acetyl CoA production
  - acetyl CoA oxidation
  - electron transfer and oxidative phosphorylation

Catabolism of proteins, fats, and carbohydrates in the three stages of cellular respiration



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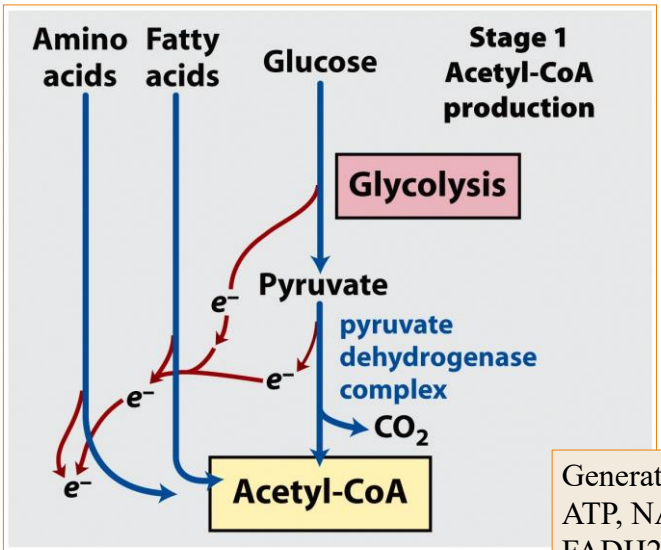
Respiration: Stage 1



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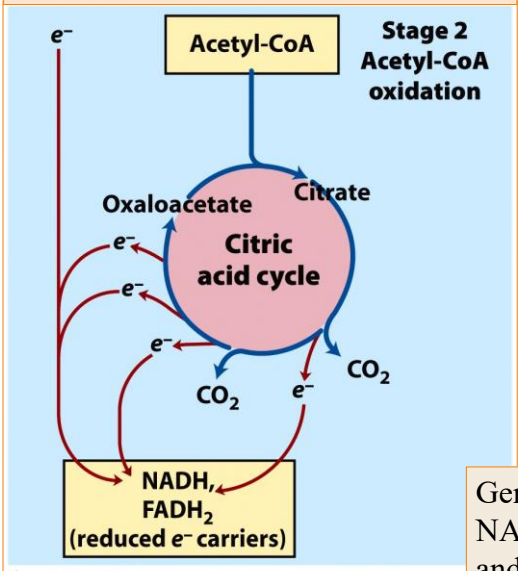
Generates some: ATP, NADH, FADH2

Respiration: Stage 2



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Generates more: NADH, FADH2 and one GTP

Respiration: Stage 3

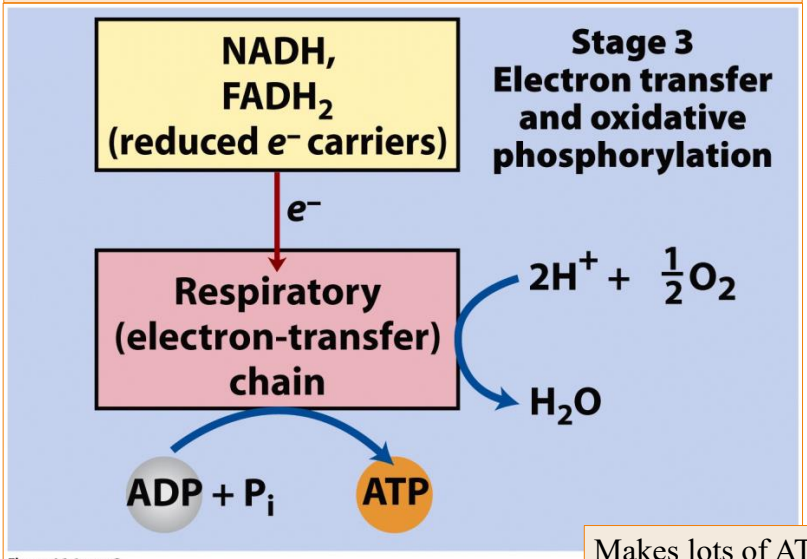

Figure 16-1 part 3
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Makes lots of ATP

In Eukaryotes, Citric Acid Cycle Occurs in Mitochondria

- Glycolysis occurs in the ***cytoplasm***
- Citric acid cycle occurs in the ***mitochondrial matrix*** (Except succinate dehydrogenase, which is an integral inner membrane protein)
- Oxidative phosphorylation occurs in the ***inner membrane***

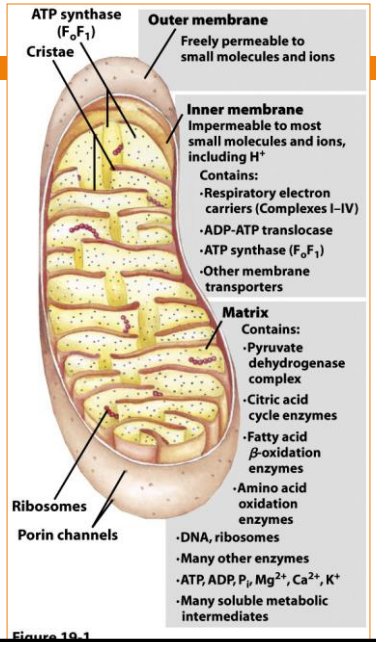

Figure 19-1

Chemistry of Oxidative Decarboxylation of Pyruvate

- NAD+ and CoA-SH are co-substrates
- TPP, lipoyllysine and FAD are prosthetic groups
- *Coenzymes or co-substrates are not a permanent part of the enzymes' structure; they associate, fulfill a function, and dissociate*
- The function of CoA is to accept and carry acetyl groups
- *Prosthetic groups are strongly bound to the protein*
- Lipoic acid is covalently linked to the enzyme via a lysine residue

NAD: nicotinamide adenine dinucleotide; FAD: Flavin adenine dinucleotide; TPP: thiamine pyrophosphate; Coenzyme A: CoA o CoA-SH

Citric acid cycle

- In aerobic organisms, glucose, other sugars, fatty acids and most amino acids are ultimately oxidized to CO2 and H2O
- The **carbon skeleton of sugars and fatty acids** are **degraded to the acetyl group of Acetyl-CoA**, the form in which the cycle accepts most of its fuel input
- Many **amino acid carbons** also enter the cycle this way, although **several amino acids are degraded to other cycle intermediates**

The Citric Acid Cycle

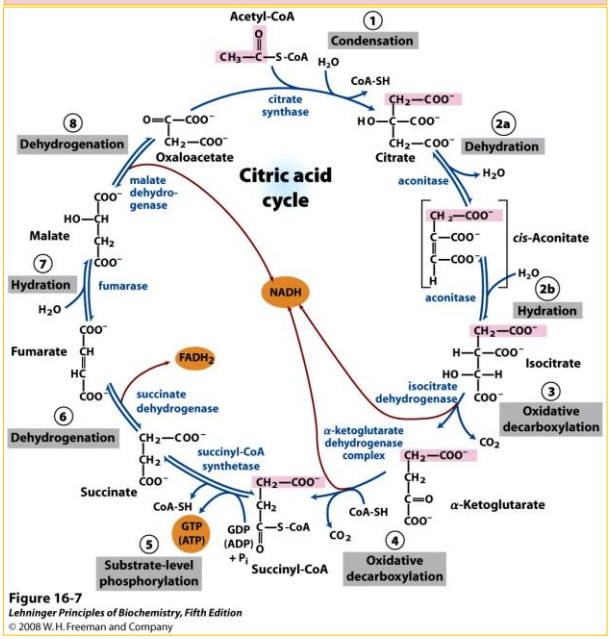

Figure 16-7
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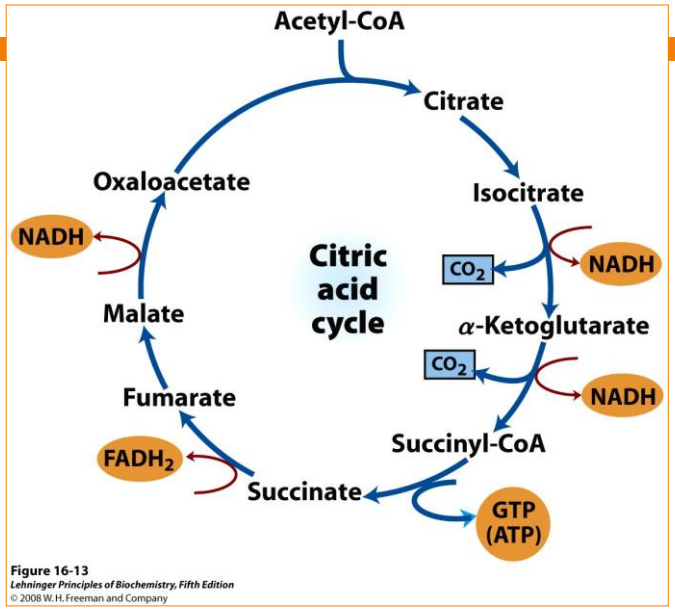
Sequence of Events in the Citric Acid Cycle

- **Step 1:** C-C bond formation to make citrate
- **Step 2:** Isomerization via dehydration, followed by hydration
- **Steps 3-4:** Oxidative decarboxylations to give 2 NADH
- **Step 5:** Substrate-level phosphorylation to give GTP
- **Step 6:** Dehydrogenation to give reduced FADH2
- **Step 7:** Hydration
- **Step 8:** Dehydrogenation to give NADH

Products from One Turn of the Cycle



Net Effect of the Citric Acid Cycle

- **Acetyl-CoA + 3NAD+ + FAD + GDP + Pi + 2 H2O → 2CO2 +3NADH + FADH2 + GTP + CoA + 3H+**
- carbons of acetyl groups in acetyl-CoA are oxidized to CO2
- electrons from this process reduce NAD+ and FAD
- one GTP is formed per cycle, this can be converted to ATP
- intermediates in the cycle are not depleted

Why is the oxidation of acetate is so complicated?

- The 8 steps of cyclic oxidation of simple 2-carbon acetyl group to CO2 may seem unnecessarily cumbersome and not in keeping with the biological principle of maximum economy
- The role of CAC is not confined to the oxidation of acetate
- The CAC is the hub of intermediary metabolism. 4 and 5-carbon end products of many catabolic processes feed into the cycle to serve as fuels
- Examples: Oxaloacetate and α–ketoglutarate are produced from Asp and Glu, when proteins are degraded

Why is the oxidation of acetate is so complicated? (cont'd)

- Under some metabolic situations, intermediates are drawn out of the cycle to be used as precursors in a variety of biosynthetic pathways
- In aerobic organisms, CAC is an amphipathic pathway that serves in both catabolic and anabolic pathways

Oxidative Phosphorylation

- Electrons from the reduced cofactors NADH and FADH2 are passed to proteins in the respiratory chain
- In eukaryotes, oxygen is the ultimate electron acceptor for these electrons
- Energy of oxidation is used to phosphorylate ADP

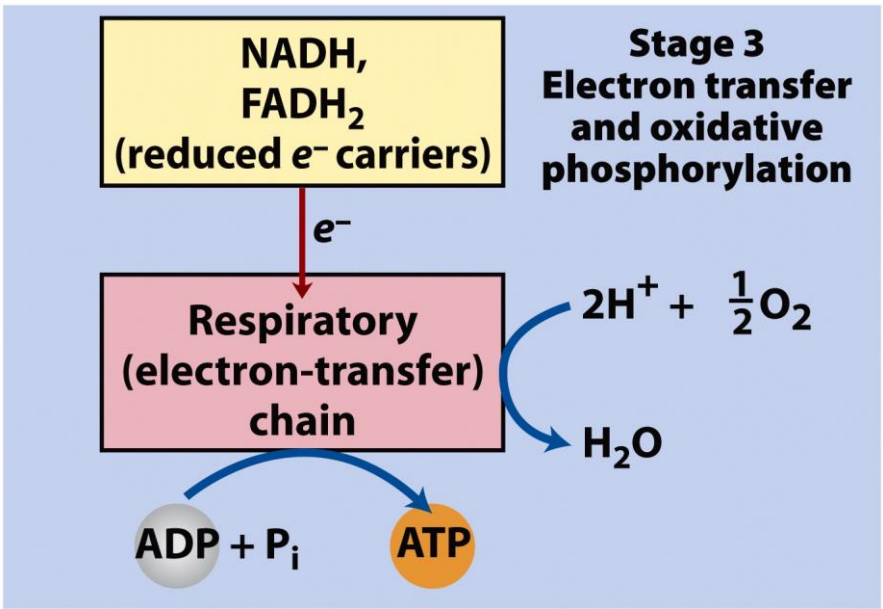


Figure 16-1 part 3
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Chemiosmotic Theory

- How to make an unfavorable ADP + Pi → ATP possible?
- Phosphorylation of ADP is not a result of a direct reaction between ADP and some high energy phosphate carrier
- Energy needed to phosphorylate ADP is provided by the flow of protons down the electrochemical gradient
- The electrochemical gradient is established by transporting protons against the electrochemical gradient during the electron transport

Chemiosmotic Energy Coupling Requires Membranes

- The proton gradient needed for ATP synthesis can be stably established across a topologically closed membrane
  - Plasma membrane in bacteria
  - Cristae membrane in mitochondria
  - Thylakoid membrane in chloroplasts
- Membrane must contain proteins that couple the "downhill" flow of electrons in the electron transfer chain with the "uphill" flow of protons across the membrane
- Membrane must contain a protein that couples the "downhill" flow of proton to the phosphorylation of ADP

Electrons are collected to universal electron acceptors

TABLE 19–1 Some Important Reactions Catalyzed by NAD(P)H-Linked Dehydrogenases

Reaction*	Location†
NAD-linked	
α-Ketoglutarate + CoA + NAD^+ ⇌ succinyl-CoA + CO_2 + NADH + H	M
L-Malate + NAD^+ ⇌ oxaloacetate + NADH + H^+	M and C
Pyruvate + CoA + NAD^+ ⇌ acetyl-CoA + CO_2 + NADH + H^+	M
Glyceraldehyde 3-phosphate + P_i + NAD^+ ⇌ 1,3-bisphosphoglycerate + NADH + H^+	C
Lactate + NAD^+ ⇌ pyruvate + NADH + H^+	C
β-Hydroxyacyl-CoA + NAD^+ ⇌ β-ketoacyl-CoA + NADH + H^+	M
NADP-linked	
Glucose 6-phosphate + $NADP^+$ ⇌ 6-phosphogluconate + NADPH + H^+	C
NAD- or NADP-linked	
L-Glutamate + H_2O + $NAD(P)^+$ ⇌ α-ketoglutarate + NH_4^+ + NAD(P)H	M
Isocitrate + $NAD(P)^+$ ⇌ α-ketoglutarate + CO_2 + NAD(P)H + H^+	M and C

*These reactions and their enzymes are discussed in Chapters 14 through 18.
†M designates mitochondria; C, cytosol.
Table 19-1
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Flavoproteins: contains FMN or FAD

Chemiosmotic coupling allows non-integral stoichiometries of O_2 consumption and ATP synthesis

1. Most experiments have yield P/O ratio (ATP to $1/2O_2$) of between 2 to 3 when NADH was the electron donor, and between 1 to 2 when succinate was the eletron donor.
2. Given the assumption that P/O should have an intergral value, most experimenters agreed that the P/O ratios must be 3 for NADH and 2 for succninate
3. However, there was no theoretical requirement for P/O to be intergral.
4. The consensus values for number of protons pumped out per pair of electrons are 10 for NADH and 6 for succinate.
5. The most widely accepted experimental value for number of protein required to drive the synthesis of an ATP molecule is 4.
6. Thus P/O for NADH is 2.5 and for succinate is 1.5.
7. The value 3.0 and 2.0 are still common in the biochemical literature.

TABLE 19–5 ATP Yield from Complete Oxidation of Glucose

Process	Direct product	Final ATP
Glycolysis	2 NADH (cytosolic)	3 or 5*
	2 ATP	2
Pyruvate oxidation (two per glucose)	2 NADH (mitochondrial matrix)	5
Acetyl-CoA oxidation in citric acid cycle (two per glucose)	6 NADH (mitochondrial matrix)	15
	2 $FADH_2$	3
	2 ATP or 2 GTP	2
Total yield per glucose		30 or 32

*The number depends on which shuttle system transfers reducing equivalents into the mitochondrion.

Table 19-5
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