BIOL 240: General Microbiology

Spring 2020 Rm. 23-203 MW, Mar. 2-4

http://accounts.smccd.edu/staplesn/biol240/

1. **Pre-Lab Writeup #4 due!: ALL of Expts. 8 & 9.** Prepare before each Monday's labs (for BOTH Mon. & Wed.)!! ***(What? Why? How? are we doing in the lab? Question? HYPOTHESIS?)***
2. ***0.5-1 hour of OPEN LAB *expected* each week. Goal: 8-16h by May! ☺***
3. **LAB: Oral bacteria!! Lactic acid bacteria.**
4. **Study Guides & Lesson Objectives (see slides) due THIS WED. in Lab (Ch. 4b, 5a)!**
5. **MIDTERM EXAM #1 was returned! Review and reexamine** the test & **meet with me TODAY**, if scored <70%!!
6. ***Extra Credit Opportunity: Starting This Wed: Wed. evenings, 5-6 pm, (2/4-3/25/2020) in Bldg. 6, Room 102 – STEM SPEAKER Series. 1 page summary & reflection due (on CANVAS) the following week.***
   - **https://www.canadacollege.edu/stemcenter/speaker-series.php**
   - **NOTE: YOU may upload MULTIPLE Speaker Summaries, but only I can see all of them. You can only see the last upload! It's OK!! ☺**
7. **Read Chs. 6 & 7!! This week! See Ch. 7 lecture ONLINE!! Objs. due next week!**

1

REVIEW:

1. Recognize and describe the properties and functions of the various **Eukaryotic Organelles**. *(REVIEW!!)*
2. Illustrate and explain the evidence supporting the predominant **Theory** on the **origin of energetic organelles** in Eukaryotes.

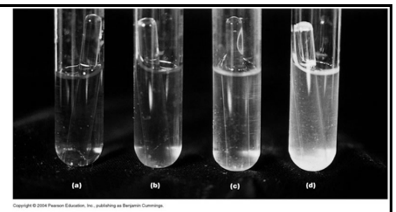
OBJECTIVES: Students should be able to: …..

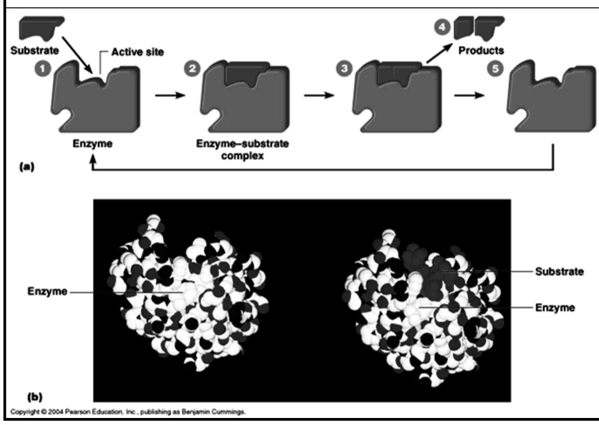
1. Describe **how enzymes speed up chemical reactions**, and how they affect the energy and equilibrium of a reaction.
2. Describe 6 different physical and chemical factors that can **regulate enzyme activity**.
3. Explain how Enzymes and ATP participate in **Energetic Coupling**.
4. Diagram the **investment and release of Energy** and **Carbon** atoms from Glycolysis (through the Citric Acid Cycle).
5. Diagram or outline the process by which **high energy electron carriers** are produced by glycolysis, pyruvate oxidation, and the Krebs/TCA cycle.
6. Diagram how high energy **electrons are used to produce ATP** in the mitochondrial inner membrane (or bact. plasma mem).
7. Compare and contrast the **energy inputs and outputs of Fermentation & Aerobic Respiration/Oxidative** phosphorylation.
8. Explain how lipids and proteins are catabolized and energy harvested thru pathways shared with glucose metabolism.

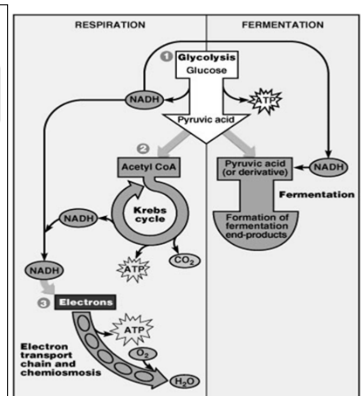
- ***These questions are your HOMEWORK between classes!!!***
  - ***DUE (and/or Study Guide questions) WED. at the start of Lab!!***

2

Chapter 5
Microbial Metabolism







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Microbial Metabolism

1. ***Metabolism*** is the sum of the chemical reactions in an organism.
2. ***Catabolism*** is the breaking-down complex molecules; energy-releasing processes.
3. ***Anabolism*** is building up complex molecules from simpler subunits; energy-using processes.

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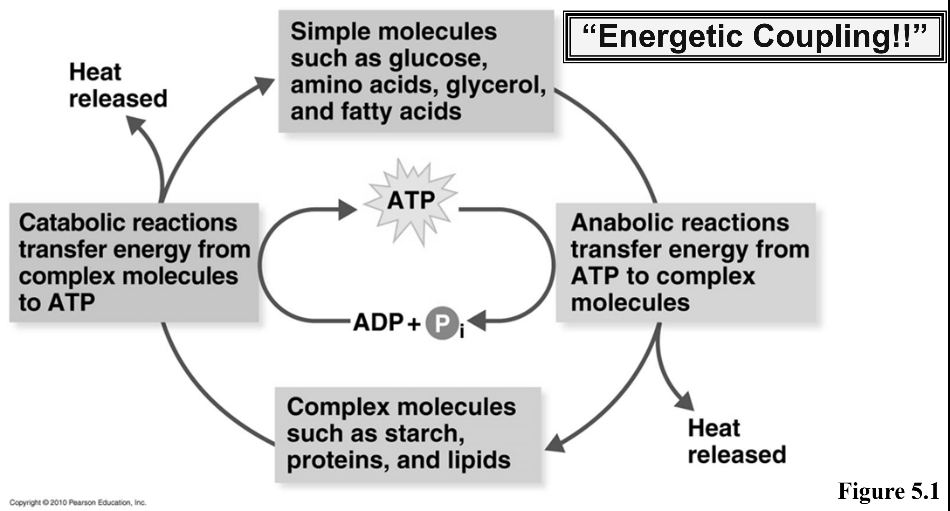
Microbial Metabolism

- Catabolism provides the building blocks and energy for anabolism.



5

Metabolism

- A ***metabolic pathway*** is a ***sequence of enzymatically catalyzed chemical reactions*** in a cell.

A → B → C → D → E → F
D → G → H → I

1. Metabolic pathways are determined by enzymes.
2. Enzymes are encoded by genes……. Therefore:

➢ ***Genes drive metabolism!!!***

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- The ***Collision Theory:*** chemical reactions can occur when atoms, ions, and molecules collide – allowing exchange of electrons.

- ****___Activation energy___ is needed to disrupt electronic configurations.**
- ***Reaction rate*** is the frequency of collisions with enough energy to bring about a reaction.
  - Reaction rate can be increased by ***enzymes*** or by increasing temperature or pressure.

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5.1) Enzymes

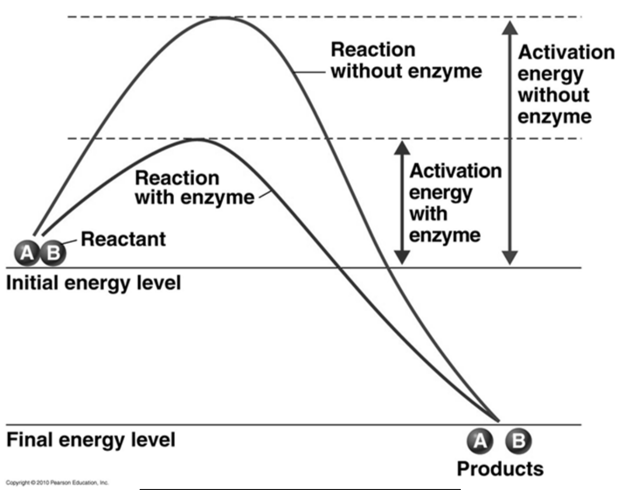

Figure 5.2

FURTHER READING:
http://blog.dearbornschools.org/renkomapbio/2010/10/28/enzymes/

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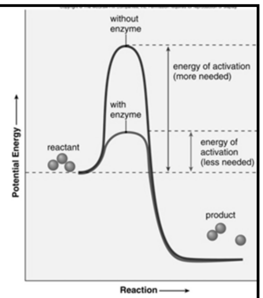
Enzymes



❖ **Biological catalysts**

1) **Specific** for a chemical reaction;
2) not used up in that reaction; usually protein.
3) **Apply:**
   a) **tension,**
   b) **temporary charges, or**
   c) **proper orientation/alignment of reactants/bonds to speed up a reaction.**
4) ***Do **NOT** change the **equilibrium** or difference in **energy** between reactants and products, but only speed up **HOW FAST** equilibrium state is reached.***
5) **Often catalyze reactions in BOTH directions.**

****

- ***Holoenzyme***: Apoenzyme + cofactor
  - ***Apoenzyme**:* protein
  - ***Cofactor**:* Nonprotein component
- ***Coenzyme***: Organic cofactor

****

- ***RIBOZYMES***: = Catalytic RNA's!!
  - RNA that cuts and splices RNA;
  - in peptide synthesis (ribosome).

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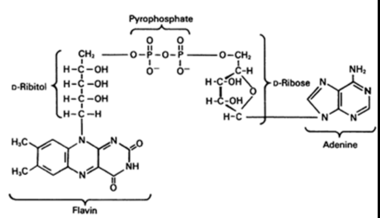
Enzymes & Important Coenzymes

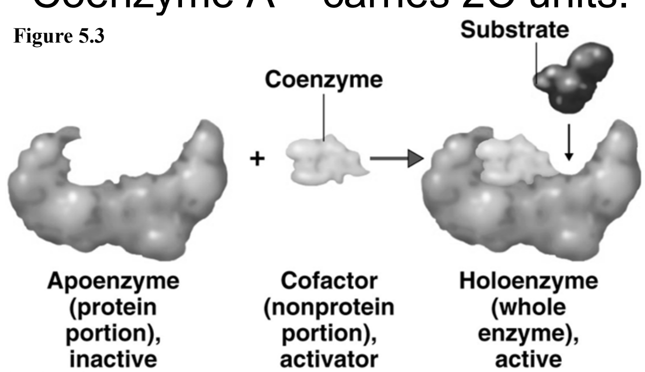
- NAD+
- NADP+
- FAD

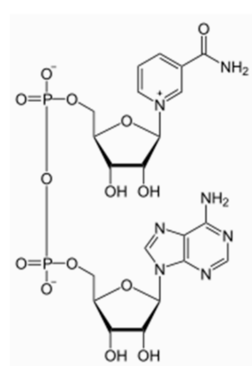
} ***electron carriers***



- Coenzyme A – carries 2C units.

Figure 5.3





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Enzymes

- The turnover number is generally 1-10,000 molecules per second.

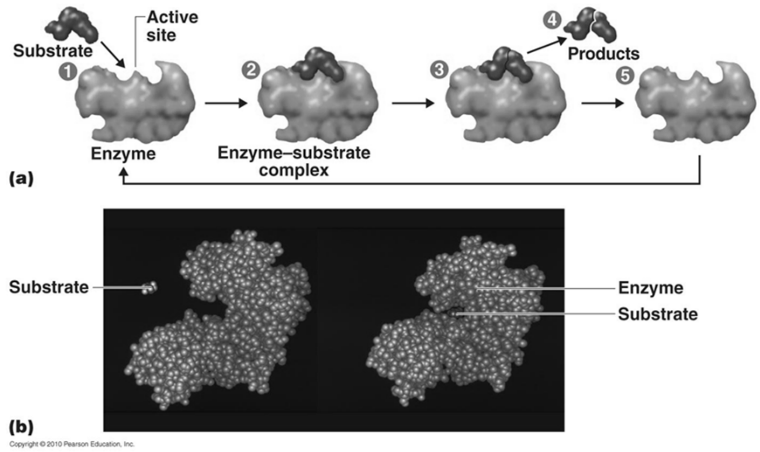

Figure 5.4

http://highered.mcgraw-hill.com/sites/0072495855/student_view0/chapter2/animation__how_enzymes_work.html

http://www.stolaf.edu/people/giannini/flashanimat/enzymes/prox-orien.swf

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5.2) Factors Influencing Enzyme Activity

- Enzymes can be ***denatured*** by temperature and pH. (& high salt, nonpolar solvents….)

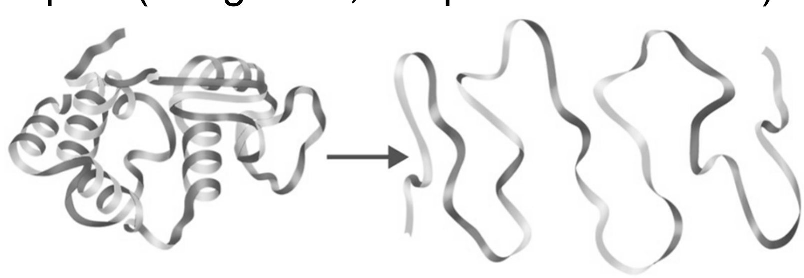

Figure 5.6

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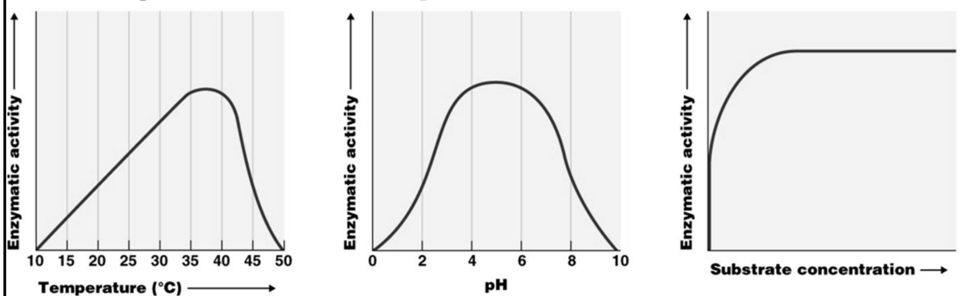
A. Factors Influencing Enzyme Activity: <u>Physical Conditions</u>

- **Temperature**
- **pH**
- **Substrate concentration**



(a) Temperature. The enzymatic activity (rate of reaction catalyzed by the enzyme) increases with increasing temperature until the enzyme, a protein, is denatured by heat and inactivated. At this point, the reaction rate falls steeply.

(b) pH. The enzyme illustrated is most active at about pH 5.0.

(c) Substrate concentration. With increasing concentration of substrate molecules, the rate of reaction increases until the active sites on all the enzyme molecules are filled, at which point the maximum rate of reaction is reached.

Figure 5.5

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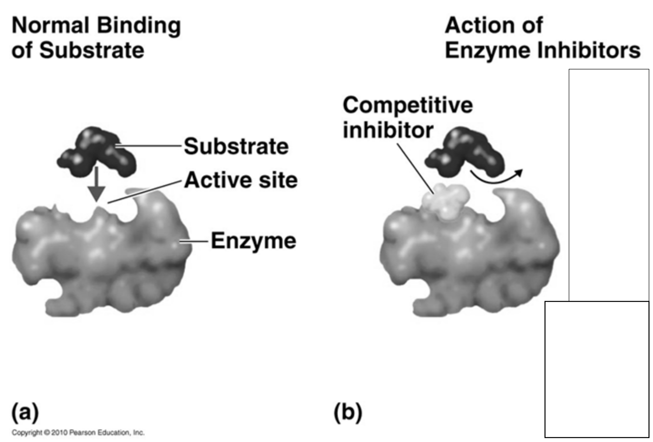
B. Factors Influencing Enzyme Activity: <u>Competitive Inhibition</u>

❖ ***<u>Competitive Inhibition</u>***

Figure 5.7a,b



http://bcs.whfreeman.com/thelifewire/content/chp06/0602001.html

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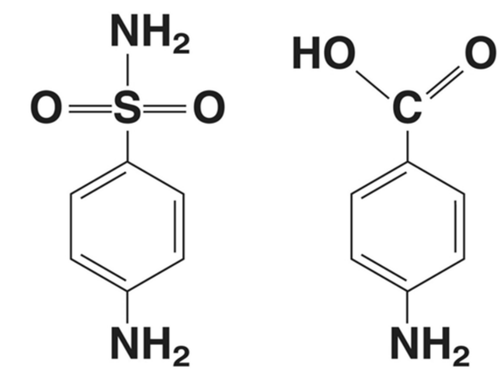
Competitive Inhibition

- Eg: **Sulfanilamide**
- Competes with **PABA**
  - ***= Sulfa Drug***
  - Prevents conversion of PABA to **folic acid**
  - Cells don't grow



Sulfanilamide **PABA**

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C. Factors Influencing Enzyme Activity: Noncompetitive Inhibition

❖ ***Noncompetitive Inhibition***

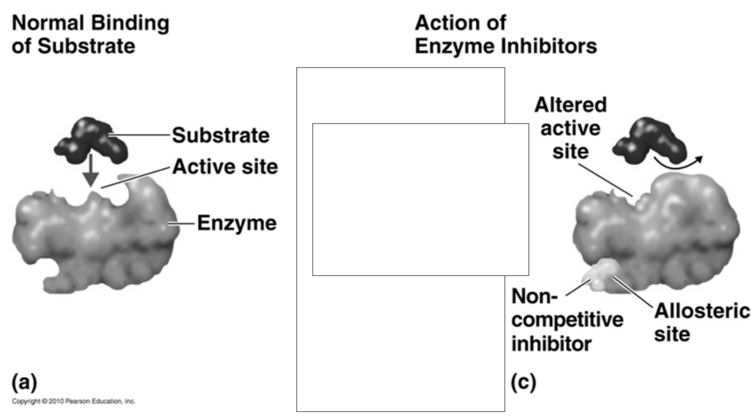

Figure 5.7a, c

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➢ ***Allosteric site (regulatory)*** – **"different shape" induced**

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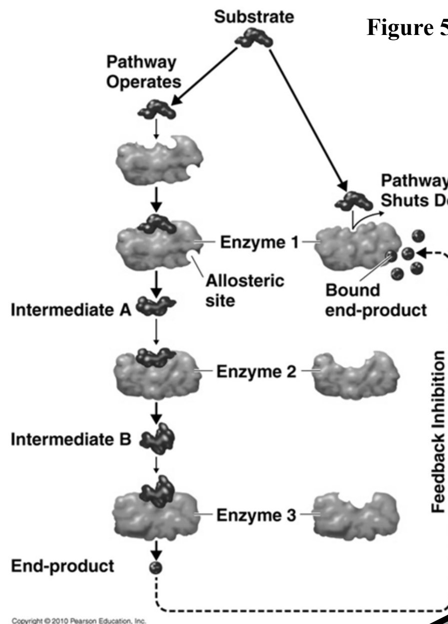
D. Factors Influencing Enzyme Activity

Figure 5.8



- ***Feedback inhibition***
  - ***Often noncompetitive***
    - ***Allosteric*** site involved
  - Can be competitive.

http://highered.mcgraw-hill.com/sites/0072943696/student_view0/chapter2/animation__feedback_inhibition_of_biochemical_pathways.html

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5.3) Oxidation-Reduction

- ***Oxidation*** is the removal of electrons.
- ***Reduction*** is the gain of electrons.
- **Redox reaction** is an oxidation reaction paired with a reduction reaction (always!).

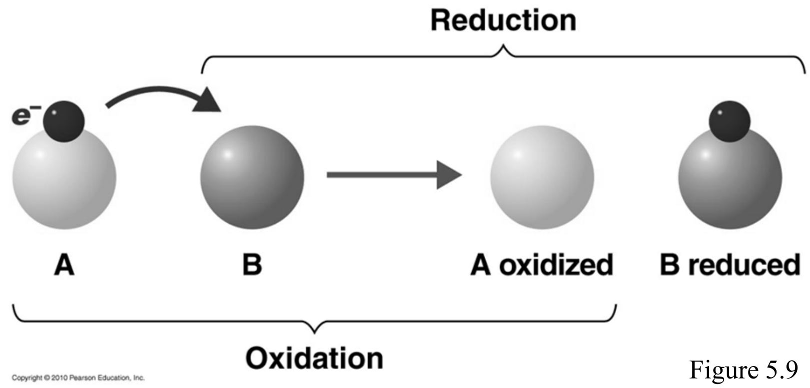

Figure 5.9

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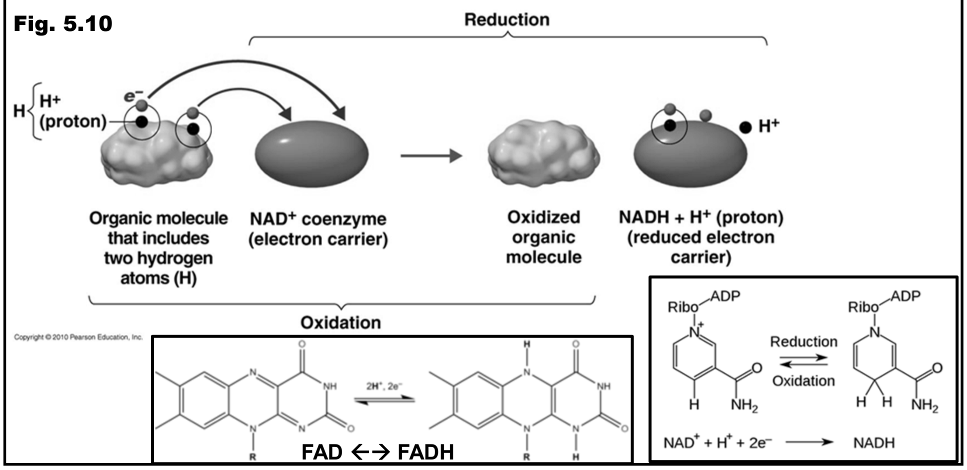
Oxidation-Reduction

- In biological systems, the **electrons** are often associated with **hydrogen atoms**.
- Biological oxidations are often ***dehydrogenations***.

Fig. 5.10



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5.4) The Generation of ATP

- ATP is generated by the phosphorylation of ADP.

$$\underbrace{\text{Adenosine}-P\sim P}_{\text{ADP}} + \text{Energy} + P \rightarrow \underbrace{\text{Adenosine}-P\sim P\sim P}_{\text{ATP}}$$

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The Generation of ATP

❖ ***Substrate-Level Phosphorylation*** is the transfer of a high-energy organic PO_4^- to ADP.

– The **phosphate** comes from an ***organic molecule (substrate)***.

C—C—C ~ Ⓟ + ADP → C—C—C + ATP

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The Generation of ATP

- ***Energy released from the transfer of electrons (oxidation)***
  - ***of one compound to another (reduction)***
  - ***is used to generate ATP by chemiosmosis.***
- **Light** causes **chlorophyll** to give up electrons.
  - Energy released from the transfer of electrons (oxidation)
    - from chlorophyll through a system of carrier molecules is used to generate ATP.

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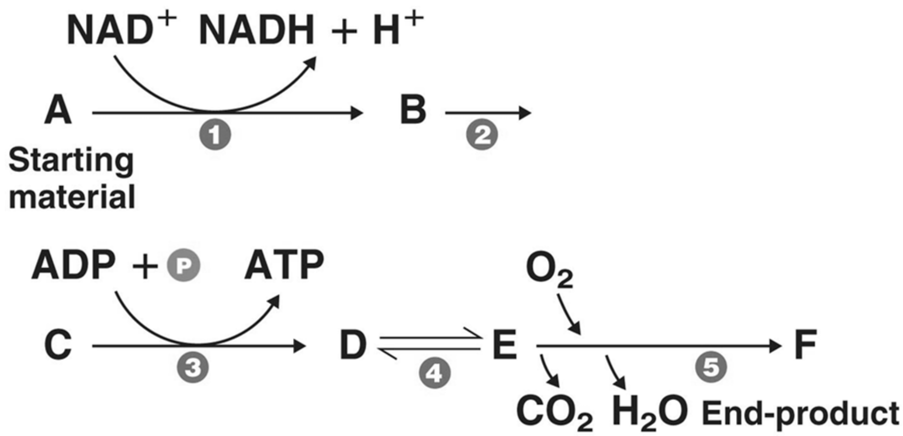
5.5) Metabolic Pathways



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Carbohydrate Catabolism

- The breakdown of carbohydrates to release energy:
  1) **Glycolysis**
  2) **Pyruvate Oxidation**
  3) **Krebs Cycle**
  4) **Electron Transport Chain**

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A. Glycolysis

- The oxidation of glucose to pyruvic acid, produces ATP and NADH.

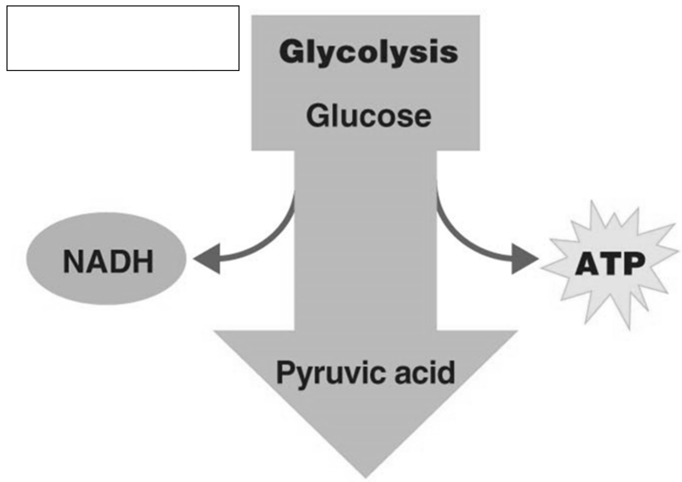

Figure 5.11

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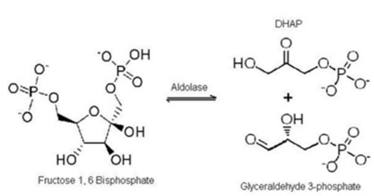
Energy-Consuming Stage

- **2 ATP**s are used
- Glucose is split to form 2 *Glyceraldehyde-3-phosphates* (**G3P**)



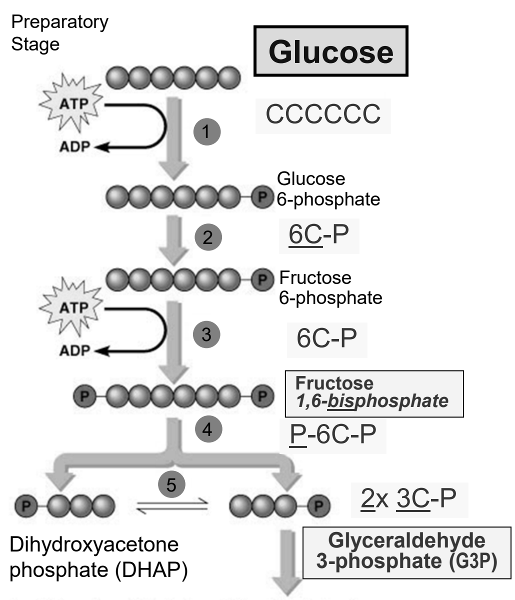


Figure 5.12.1

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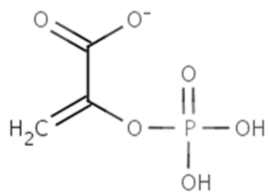
Energy-Releasing Stage

- 2 Glyceraldehyde-3-phosphates oxidized to 2 Pyruvic acid.
- **4 ATP** produced.
- **2 NADH** produced.



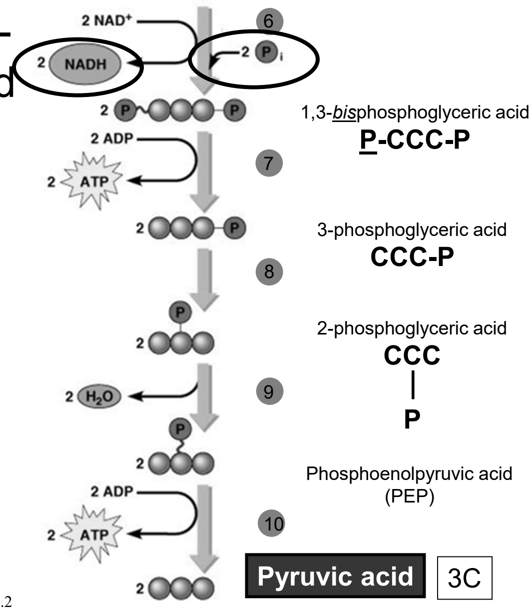


Figure 5.12.2

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Glycolysis

- ***Glucose*** **+ <u>2 ATP</u> + 2 ADP + 2 PO_4^- + 2 NAD^+**

→ *2 pyruvic acid* + <u>4 ATP</u> + <u>2 NADH</u> + $2H^+$

❖ So far, ONLY = ***<u>Substrate-Level Phosphorylation!!</u>***

http://www.science.smith.edu/departments/Biology/Bio231/glycolysis.html

http://highered.mcgraw-hill.com/sites/0072507470/student_view0/chapter25/animation__how_glycolysis_works.html

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COMPLETE Oxidation of Glucose: Cellular Respiration

- ***Oxidation of molecules liberates electrons for an electron transport chain.***

- **ATP** generated by ***Oxidative Phosphorylation.***

http://www.qcc.cuny.edu/biologicalSciences/Faculty/UGolebiewska/respiration.html

http://www.sumanasinc.com/webcontent/animations/content/cellularrespiration.html

http://www.brookscole.com/chemistry_d/templates/student_resources/shared_resources/animations/oxidative/oxidativephosphorylation.html

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B. Intermediate Step: Pyruvate Oxidation

- Pyruvic acid (from glycolysis) is oxidized and decarboyxlated.
- 2X/glc!!………

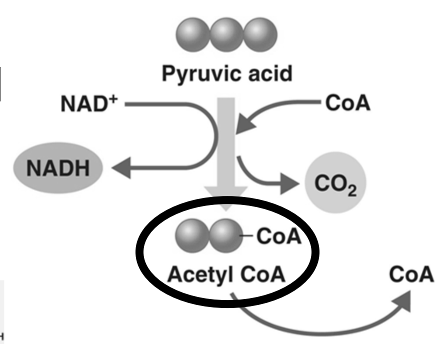
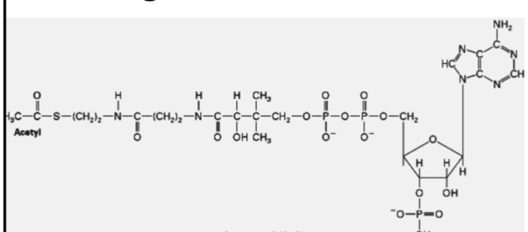


Figure 5.13.1



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C. Krebs Cycle
(TCA Cycle)

- Oxidation of acetyl CoA produces:
  - (6) **NADH** and
  - (2) **$FADH_2$** per GLC
  - And (2) **ATP**

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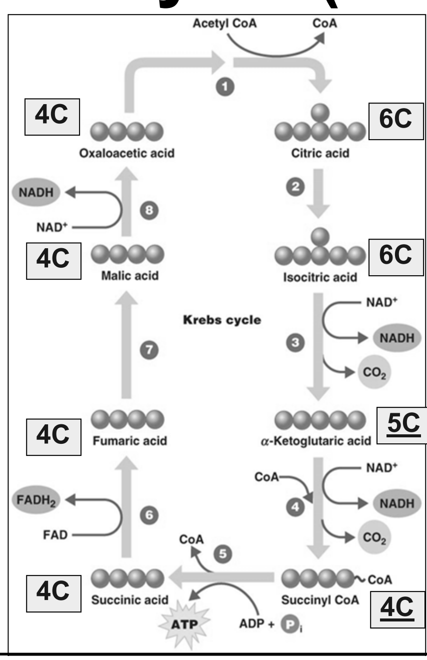
Krebs Cycle (2x/glc)



http://www.science.smith.edu/departments/Biology/Bio231/krebs.html

http://highered.mcgraw-hill.com/sites/0072507470/student_view0/chapter25/animation__how_the_krebs_cycle_works__quiz_1_.html

Figure 5.13.2

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D. The Electron Transport Chain

- A series of carrier molecules that are, in turn, oxidized and reduced as electrons are passed down the chain.
- Energy released can be used to produce ATP by ***ChemiOsmosis***.

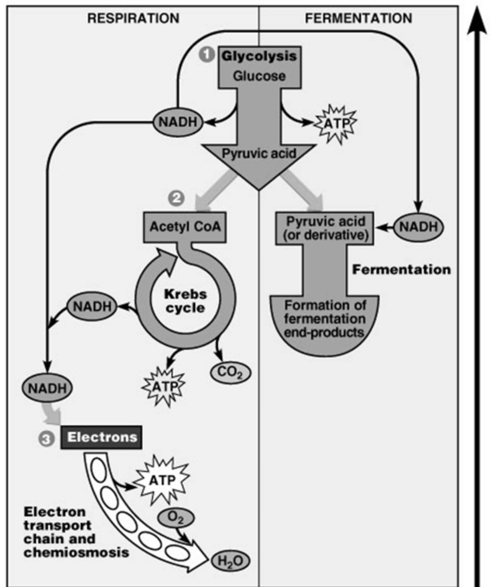

Figure 5.14

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E. *Chemi-osmosis*

- Each NADH → ~3 ATP
- Each $FADH_2$ → ~2 ATP

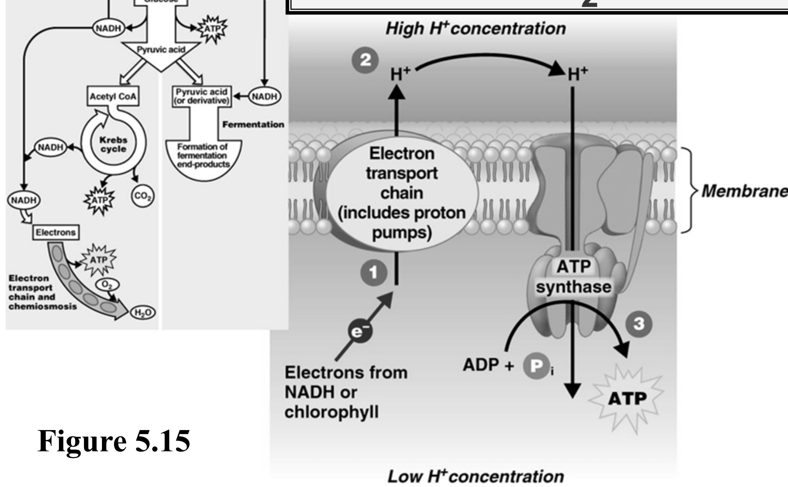

Figure 5.15

➢ ***Create <u>gradient of [H+]</u> and <u>electric charge</u> — the <u>Proton-Motive Force</u>.***

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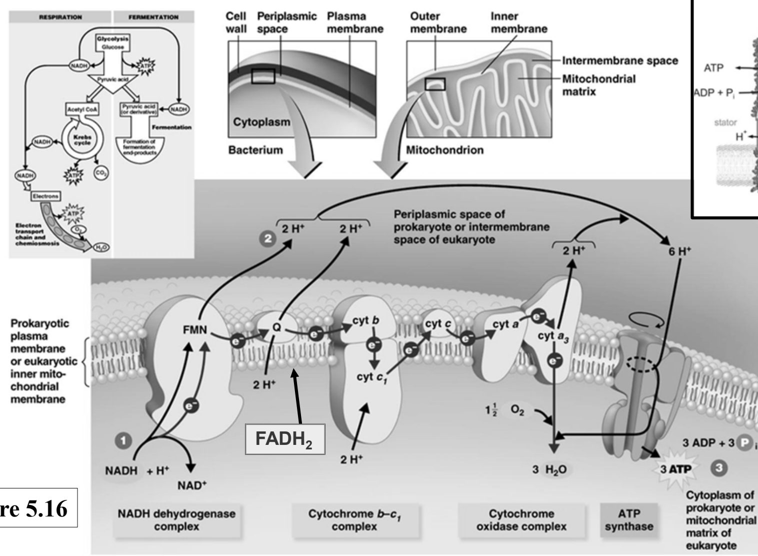
Chemiosmosis



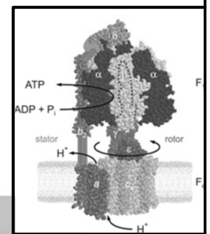


Figure 5.16

- Stronger Electron-Acceptors/Oxidizers →→

http://vcell.ndsu.nodak.edu/animations/etc/movie.htm

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5.6) Respiration ★

- ***Aerobic respiration:*** The final electron acceptor in the electron transport chain is molecular oxygen (O_2).
- ******ETC + Chemiosmosis = Oxidative Phosphorylation!!******
- ***<u>Anaerobic</u> respiration:*** The final electron acceptor in the electron transport chain is not O_2.
  - Yields less energy than aerobic respiration
    - only part of the Krebs cycle operates w/out O_2.

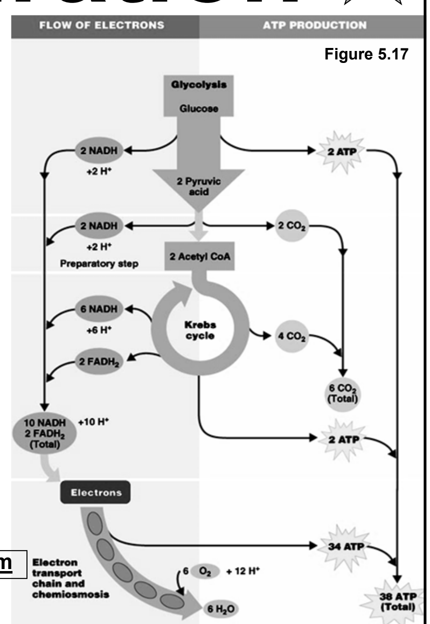

Figure 5.17

http://vcell.ndsu.nodak.edu/animations/etc/movie.htm

http://www.science.smith.edu/departments/Biology/Bio231/krebs.html

http://www.science.smith.edu/departments/Biology/Bio231/etc.html

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A. Aerobic Respiration

Pathway	Eukaryote	Prokaryote
Glycolysis	Cytoplasm	Cytoplasm
Intermediate step (Pyruvate Ox'n)	Mito. Inner Memb	Cytoplasm
Krebs cycle	Mitochondrial matrix	Cytoplasm
ETC	Mitochondrial inner membrane	**Plasma membrane**

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- **Energy produced (types)** – **from complete oxidation of 1 glucose using aerobic respiration**

Pathway	ATP produced	NADH produced	$FADH_2$ produced
Glycolysis	2	2	0
Pyruvate Oxidation	0	2	0
Krebs cycle	2	6	2
Total	**4**	**10**	**2**

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❖ **ATP produced (chemical processes)** from complete oxidation of **1 glucose** using aerobic respiration.

Pathway	By Substrate-Level Phosphorylation	By Oxidative Phosphorylation	
		From NADH	From $FADH_2$
Glycolysis	2	6	0
Pyruvate Oxidation	0	6	0
Krebs cycle	2	18	4
Total	**4**	**30**	**4**

- **38** ATPs are produced in ***prokaryotes***, **36** in ***Euk***.

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Aerobic Metabolic Yield, Summary (Eukaryotes):

NET:

1. **Glyc = 2 ATP** (4 made, 2 invested)
   2 NADHH → 4 ATP ***(use 2 ATP** on transport into **mito IM!!)***
2. **Pyr.Ox. = 2 NADHH → 6 ATP**
3. **TCA = 6 NADHH → 18 ATP**
   2 GTP → 2 ATP
   2 FADH2 → 4 ATP

36 ATP total NET from Glycolysis & Oxidative Respiration

http://vcell.ndsu.nodak.edu/animations/atpgradient/movie.htm

http://www.wiley.com/legacy/college/boyer/0470003790/animations/electron_transport/electron_transport.swf

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B. Anaerobic Respiration

= truly *RESPIRATION*, and NOT glycolysis/fermentation!

Electron acceptor	**Products**
NO_3^-	NO_2^-; N_2 + H_2O
SO_4^{2-}	H_2S + H_2O
CO_3^{2-}	CH_4 + H_2O

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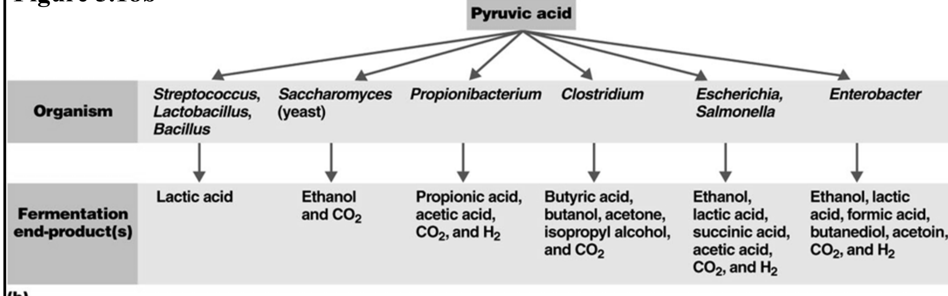
5.7) Fermentation

1. Releases energy from oxidation of organic molecules
2. Does not require oxygen
3. Does not use the Krebs cycle or ETC
4. Uses an organic molecule as the final electron acceptor

Figure 5.18b



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Types of Fermentation

A. ***<u>Alcohol fermentation</u>*** - Produces ethyl alcohol + CO_2

B. ***<u>Lactic acid fermentation</u>*** - Produces lactic acid.

– ***Homolactic fermentation*** - Produces lactic acid only.

– ***Heterolactic fermentation*** - Produces lactic acid and other compounds (eg: acetoin).

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Fermentation

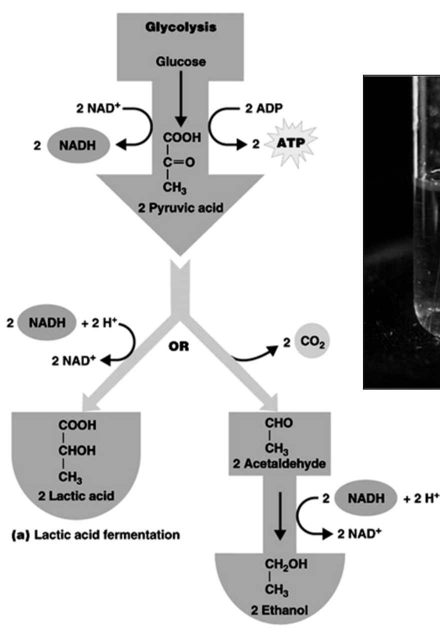
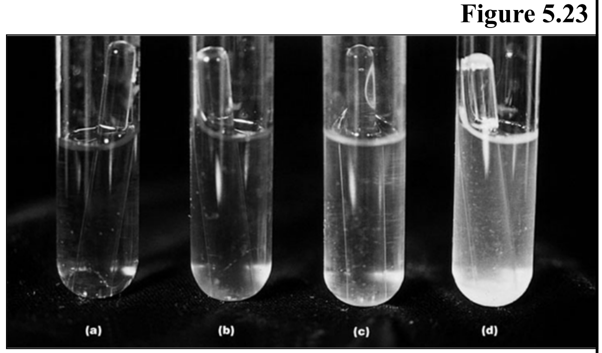


Figure 5.19

Figure 5.23



The Glucose song!!! …
https://youtu.be/jJvAL-iiLnQ

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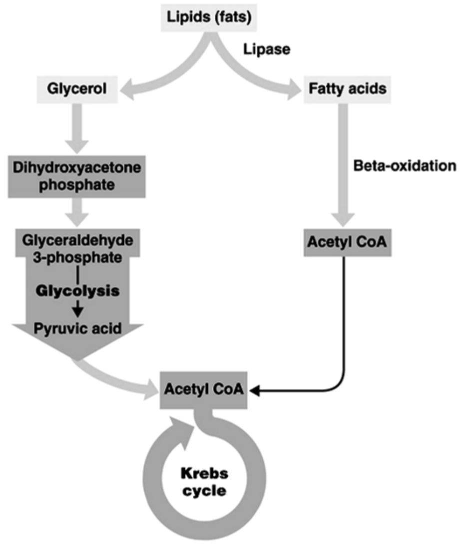
5.8) Other Catabolism: A. Lipid Catabolism



http://www.wiley.com/college/pratt/0471393878/student/animations/citric_acid_cycle/index.html

Figure 5.20

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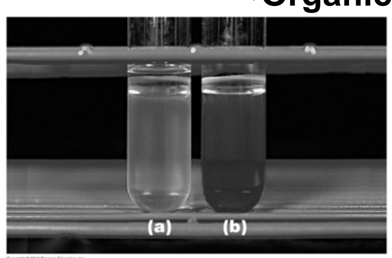
B. Protein Catabolism

Protein —*Extracellular proteases*→ **Amino acids**

—*Deamination (→ acid), decarboxylation (→ amine), dehydrogenation*→ **Organic acid** → Krebs cycle



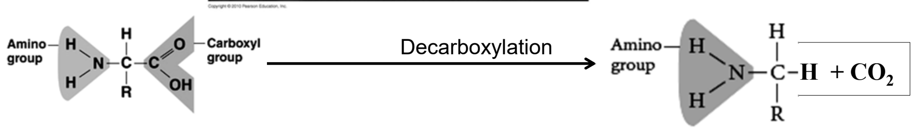


Figure 5.22

http://www.wiley.com/legacy/college/boyer/0470003790/animations/tca/tca.htm

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** Biochemical tests **

- **Used to identify bacteria.**

<u>Dichotomous Key:</u>

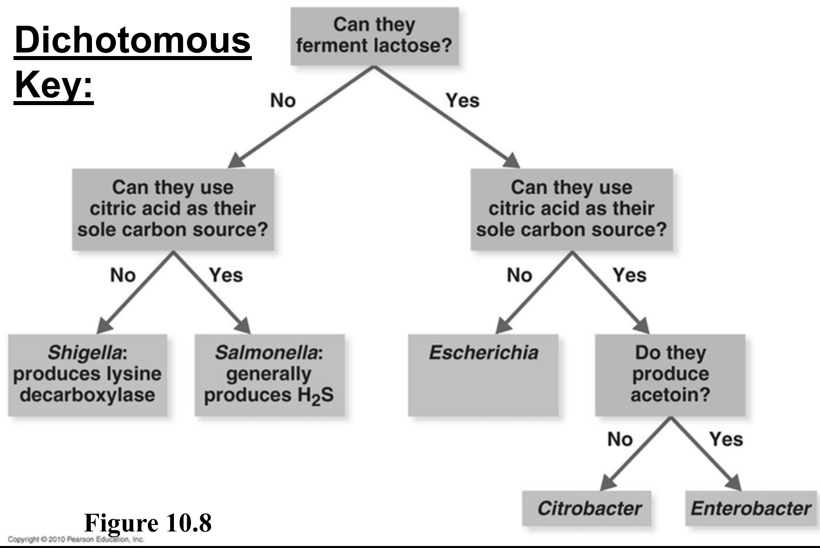

Figure 10.8

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5.9) Photosynthesis

- ***<u>Photo:</u>*** Conversion of light energy into chemical energy (ATP).
  - Light-dependent (light) reactions

- ***<u>Synthesis:</u>*** Fixing carbon into organic molecules.
  - Light-independent (dark) reaction, Calvin-Benson cycle
  - ➢ ***<u>Oxygenic:</u>***

    $6\ CO_2 + 12\ H_2O$ + Light energy $\rightarrow C_6H_{12}O_6 + 6\ O_2 + 6\ H_2O$
  - ➢ ***<u>Anoxygenic</u>***:

    $CO_2 + 2\ H_2S$ + Light energy $\rightarrow [CH_2O] + 2\ S + H_2O$

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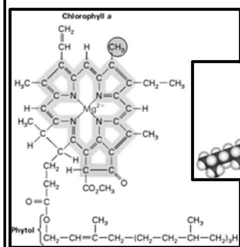
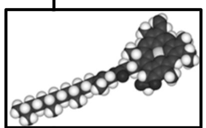
A. Cyclic Photophosphorylation

ATP ONLY!!!! (No NADPH)





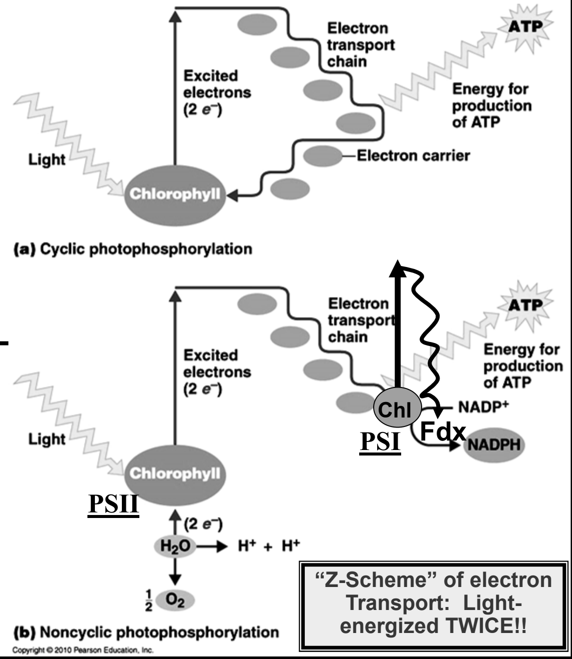


Figure 5.25

B. Noncyclic Photophosphorylation

ATP AND NADPH made!!!!

"Z-Scheme" of electron Transport: Light-energized TWICE!!

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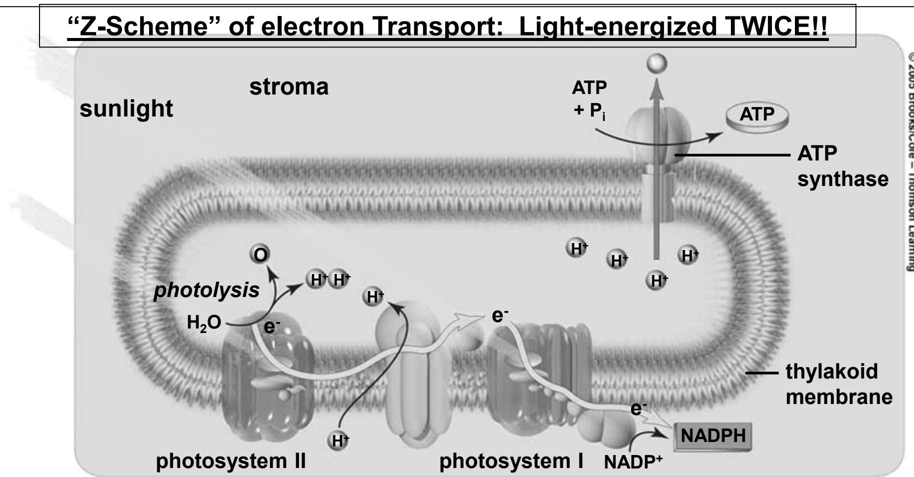
Noncyclic Pathway

"Z-Scheme" of electron Transport: Light-energized TWICE!!



http://vcell.ndsu.nodak.edu/animations/photosynthesis/movie.htm

http://www.science.smith.edu/departments/Biology/Bio231/ltrxn.html

http://www.fw.vt.edu/dendro/forestbiology/photosynthesis.swf

http://www.stolaf.edu/people/giannini/flashanimat/metabolism/photosynthesis.swf

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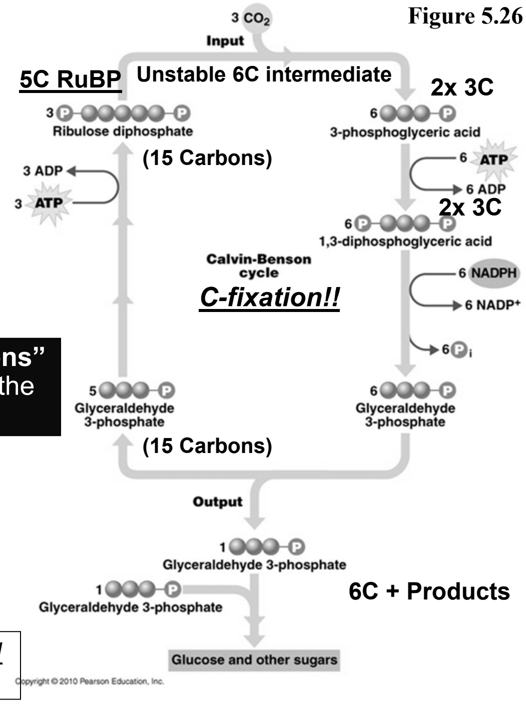
C. Calvin-Benson Cycle: *Carbon-Fixation!*



- **SYNTHESIS!!! = "Dark Reactions"**
  - Using ATP & NADPH made in the light reactions!!

Step-By-Step Narration: http://www.cells.de/cellseng/1medienarchiv/Zellfunktionen/Memb_Vorg/Photosynthese/Dunkel_u_Staerke/Calvin-Benson-Zyklus/index.jsp

**http://www.science.smith.edu/departments/Biology/Bio111/calvin.html

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Table 5.6 Photosynthesis Compared in Selected Eukaryotes and Prokaryotes

Characteristic	*Eukaryotes*	*Prokaryotes*		
	Algae, Plants	**Cyanobacteria**	**Green Bacteria**	**Purple Bacteria**
Substance That Reduces CO_2	H atoms of H_2O	H atoms of H_2O	Sulfur, sulfur compounds, H_2 gas	Sulfur, sulfur compounds, H_2 gas
Oxygen Production	Oxygenic	Oxygenic (and anoxygenic)	Anoxygenic	Anoxygenic
Type of Chlorophyll	Chlorophyll *a*	Chlorophyll *a*	Bacteriochlorophyll *a*	Bacteriochlorophyll *a* or *b*
Site of Photosynthesis	Chloroplasts with thylakoids	Thylakoids	Chlorosomes	Chromatophores
Environment	Aerobic	Aerobic (and anaerobic)	Anaerobic	Anaerobic

****Study figure 5. 28!! ** -- nutritional flow chart of organisms****

- ***Halobacterium*** uses **bacteriorhodopsin**,
  - not chlorophyll,
  - to generate electrons for a *chemiosmotic proton pump*.
    - Different pools drying, with different salinities.
      - Different pigmented species of *Halobacteria* grow in diff't [salt]s.

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