BIOL 230: Cell & Molecular Biology

Fall 2019 17-205 MW, Sept. 30-Oct. 2

http://accounts.smccd.edu/staplesn/biol230/

1. Pre-Lab writeups due each Mon. (for both M&W!!) at the start of lab. (*briefly*, **What**? **Why**? **How**? for each expt.). Question & **Hypothesis**?!
2. **LAB this week: nPAGE DATA, Nitrate Oxidation!!!, & Photosynthesis!**
3. **Ch. 10, PHOTOSYNTESIS lecture will be posted online by next week!!**
4. **Native PAGE data** to be posted under **"Add'l Materials."** (Enzyme Rpt. Due 10/9 Online)
5. ***Research Topic paragraph!! ☺ ***Due THIS WED., Oct. 2 with a Professional, Primary Reference!!***
   - ***What is your topic? Why does it interest you?***
   - ***How does it directly apply to BIOL 230?***
6. **Midterm #1 was returned last week!! M/C Answer key will be under "Additional Materials."** ***REVIEW your exam WITHOUT the key first. SEE ME THIS week, if you scored ≤70!! (Mandatory!)***
7. **Extra Credit: STEM SPEAKER SERIES,** Weds. @ 5pm-6pm, Sept. 11- Nov. 6. (NOT Oct. 9) in 6-102. *Write 1 page summary by the following week, and upload to CANVAS*. Extra-Extra credit: Ask the speaker a scientific question, and write about the answer.
8. **QUIZ #3 Due WEDNESDAY!!! (Do NOT forget!!)**

1

REVIEW

1. Define **energetic coupling** and provide an example. What types of molecules can couple chemical reactions?
2. Explain how the **change in free energy** affects the equilibrium of a reaction.
3. Diagram and describe **three ways that an enzyme can speed up a chemical reaction**. How does the enzyme affect the energy and equilibrium of a catalyzed reaction?

TODAY's Objectives: Students should be able to....

1. List and describe the effects of 5 factors that can **regulate** enzyme activity.
2. Diagram and describe the **forms in which energy** may be transferred between molecules and reactions in cells.
3. Define **Glycolysis &** describe how **redox** reactions & **phosphorylations** drive the process.
4. Diagram and describe the **forms in which energy** may be transferred between molecules and reactions in cells.
5. Outline or diagram the **energy** inputs and outputs of Glycolysis and Cellular Respiration. What types of **cofactors** and biomolecules are involved in these processes?
6. Diagram the inputs & outputs of **carbons** during Glycolysis and Cellular Respiration.
7. Explain how ATP is synthesized in mitochondria, including the electron transport process. *Define **substrate-level phosphorylation, chemiosmosis, & oxidative phosphorylation.***

- ***Objectives and Study Guide Questions are your HOMEWORK between classes!!! DUE WED. at the end of Lecture!!***

2

C. Molecular Structure Determines Enzyme Function *(Imagine that!! ☺)*

- The ***active site*** where substrate binds
  - determines the **specificity** of an enzyme.
  - Upon binding, some enzymes **change shape**, facilitating catalysis.

Lock & Key

vs.

Induced Fit:

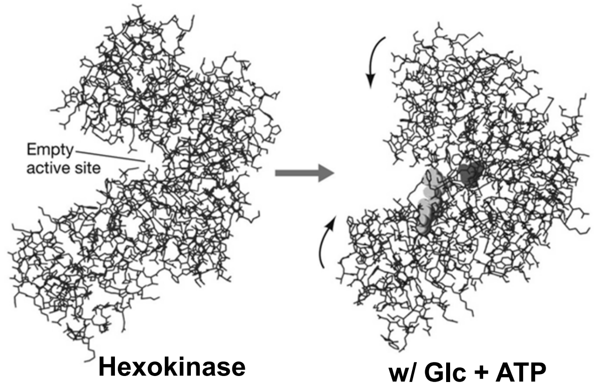


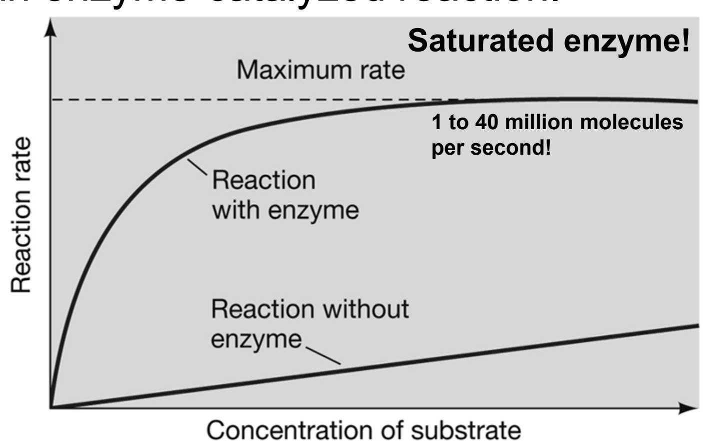
Fig. 6.12

LIFE 9e, Figure 8.12

3

8.3) Enzymes Regulation: A. Environmental Factors

1. **Substrate concentration** affects the rate of an enzyme-catalyzed reaction.

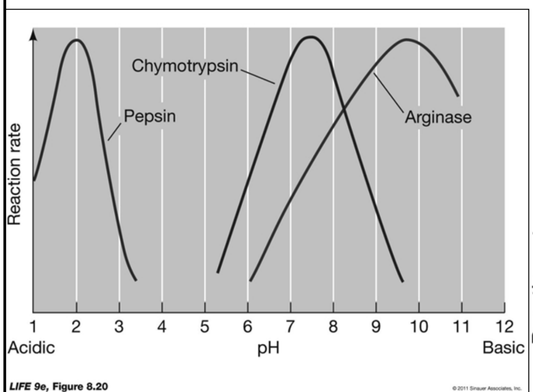


LIFE 9e, Figure 8.13

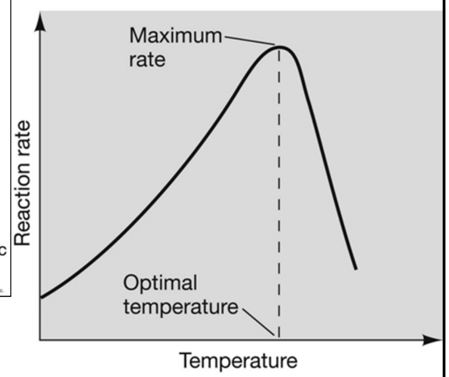
4

Metabolism and the Regulation of Enzymes

- Enzymes are sensitive to their environment.
  - Both (2.) **<u>pH</u>** and
  - (3.) **<u>temperature</u>** affect enzyme activity.



LIFE 9e, Figure 8.20



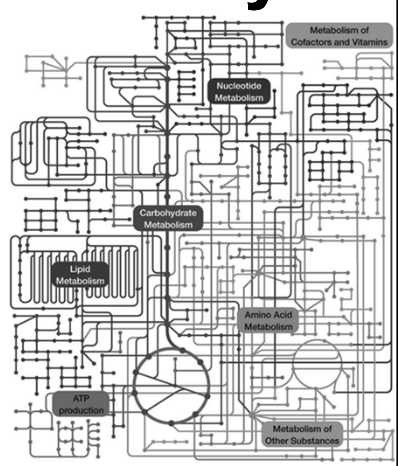
LIFE 9e, Figure 8.21

5

B. Metabolic Pathways

❖ Metabolism is organized into <u>pathways</u>:

- the product of one reaction is a reactant for the next.
- *Each reaction is catalyzed by a separate and specific enzyme.*



A → B → C → <u>D</u> → E → F

↘ G → H → I

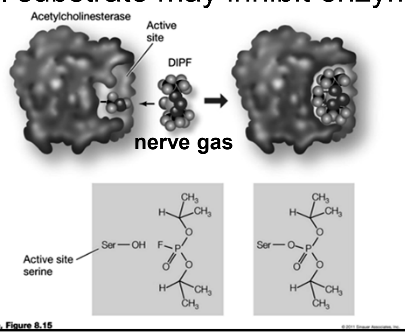
http://highered.mcgraw-hill.com/olcweb/cgi/pluginpop.cgi?it=swf::535::535::/sites/dl/free/0072437316/120070/bio09.swf

6

C. Enzyme Inhibitors: Chemical Regulators

- **Irreversible Inhibitors**: permanently reduce their catalytic activity. Covalent binding.
- **Reversible Inhibitors**: inhibit enzyme action temporarily. (***Competitive*** or ***Noncompetitive***)
  - A compound structurally similar to an enzyme's normal substrate may inhibit enzyme action.

Figure 8.15

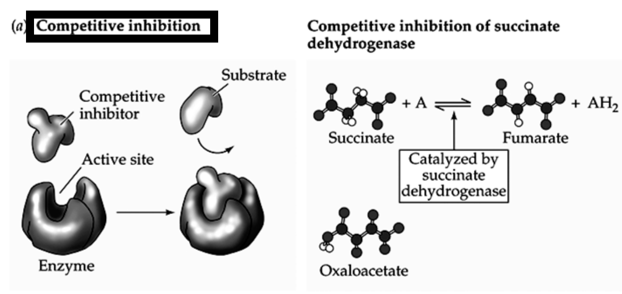


7

Reversible Inhibitors

1. **Competitive:**

   http://highered.mheducation.com/olcweb/cgi/pluginpop.cgi?it=swf::535::535::/sites/dl/free/0072437316/120070/bio10.swf



2. **Non-Competitive:**
   - ***Allosteric*** = "different" + "shape"

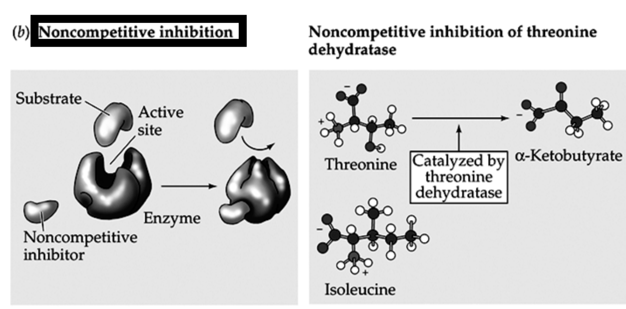


Figure *8e:* 6.17, *9e:* 8.16

8

Allosteric enzymes

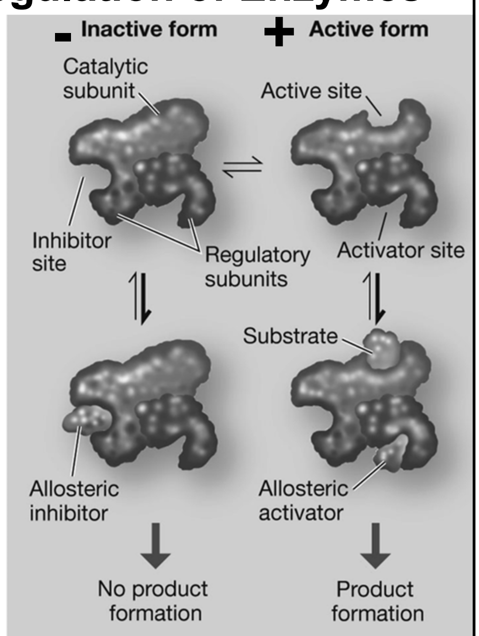
1. ***Allosteric*** inhibitors bind to a site different from the active site (***Noncompetitive***)
   - *stabilize the inactive form of the enzyme.*
   - Most allosteric enzymes have quaternary structure.

2. The multiple catalytic subunits of many allosteric enzymes interact ***cooperatively***.
   - *Binding to one subunit facilitates binding to others*

9

Fig 8.17 Allosteric Regulation of Enzymes

- **Binding at allosteric site changes shape of separate, active site!!**
  - **Conformational Change**
- **Allosteric Regulation: Activate or Inhibit**
- **Binding to one subunit facilitates binding to others**
  - **Activation site**
  - **Cooperative Binding**

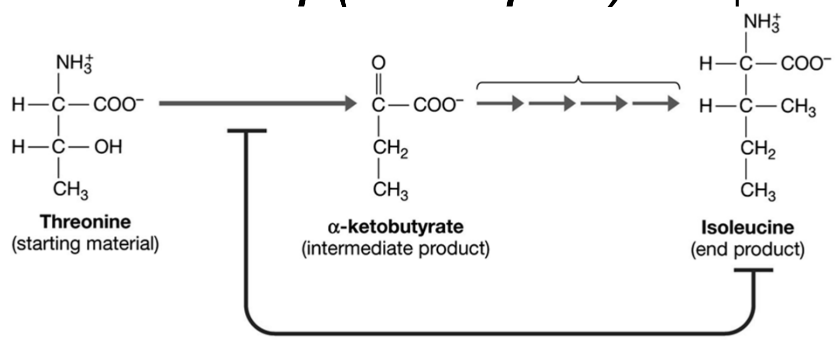


8.17

10

G. Allosteric regulation of metabolism

- The ***end product*** of a metabolic pathway may inhibit the allosteric enzyme that catalyzes the ***commitment step (branch-point)*** of the pathway.



Feedback Inhibition: accumulation of an end product signals to end its own synthesis. Usually ALLOSTERIC inhibition.

LIFE 9e, Figure 8.19 http://highered.mcgraw-hill.com/olcweb/cgi/pluginpop.cgi?it=swf::535::535::/sites/dl/free/0072437316/120070/bio10.swf

11

H. Some Enzymes Require Non-protein "accessories" to work

Some enzymes require "partners":

- **Cofactors:** inorganic ions (metals).
- **Coenzymes:** not bound permanently to enzymes.
- **Prosthetic groups:** non-amino acid groups bound to enzymes.

TABLE 8.1

Some Examples of Nonprotein "Partners" of Enzymes

TYPE OF MOLECULE	ROLE IN CATALYZED REACTIONS
COFACTORS	
Iron (Fe^{2+} or Fe^{3+})	Oxidation/reduction
Copper (Cu^{+} or Cu^{2+})	Oxidation/reduction
Zinc (Zn^{2+})	Helps bind NAD
COENZYMES	
Biotin	Carries $-COO^-$
Coenzyme A	Carries $-CO-CH_3$
NAD	Carries electrons
FAD	Carries electrons
ATP	Provides/extracts energy
PROSTHETIC GROUPS	
Heme	Binds ions, O_2, and electrons; contains iron cofactor
Flavin	Binds electrons
Retinal	Converts light energy

12

Part I – Major Themes So Far!!

1. **Electronegativity, charge** and **polarity** govern the major chemical and functional properties of water and biomolecules.
2. Molecular <u>shape</u>/**<u>structure</u>** → Molec./Biol. **<u>Function</u>**
   – Lipids, Polysacch., Proteins! (strx. Levels)…., RNA, DNA
3. Biological reactions in eukaryotes are **<u>compartmentalized</u>**.
   – Mitoch., chloroplasts, nucleus, nucleolus, lysosome, RER, SER, vacuole, Golgi…….
4. Membranes are more than just barriers:
   a) Dynamic – cycling contents!
   b) regulate transport – passive, active (1°, 2°) and bulk (endocyt., exocyt.)
   c) transduce signals and energy; enzyme alignment
5. Biochemical energy can be harvested to do cellular work. *COUPLING*!!
   - Endergonic reactions can be powered by exergonic reactions by energetic coupling
     - ***Uses ATP and enzymes/cofactors to transfer the energy between reactions***
6. **<u>Enzyme Regulation:</u>** *Physical factors, Inhibition, Allostery, Cooperativity*

13

Chapter 9: Cellular Pathways That Harvest Chemical Energy

1. <u>An Overview: Releasing Energy from Glucose</u>
2. <u>Glycolysis: From Glucose to Pyruvate</u>
3. <u>Pyruvate Oxidation</u>
4. <u>The Citric Acid Cycle: Obtaining Energy and Electrons from Glucose</u>
5. <u>The Respiratory Chain: Electrons, Proton Pumping, & ATP</u>
6. <u>Fermentation: ATP from Glucose, without O_2</u>
7. <u>Contrasting Energy Yields</u>
8. <u>Metabolic Pathways</u> & <u>Regulation</u>

14

Cellular Pathways

- **Metabolic pathways:**
  1. occur in small steps,
  2. each catalyzed by a specific enzyme,
  3. often compartmentalized, and are
  4. highly regulated (allowed by #s 1-3).

15

9.1) Obtaining Energy & Electrons from Glucose

- When glucose burns (combustion), energy is released as heat and light:

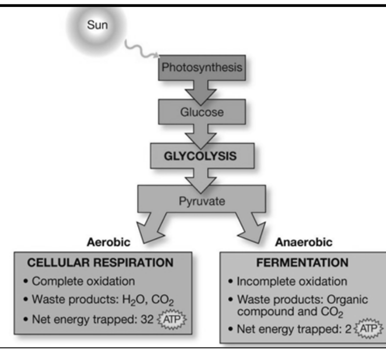
$$C_6H_{12}O_6 + 6\ O_2 \rightarrow 6\ CO_2 + 6\ H_2O + \text{energy}$$

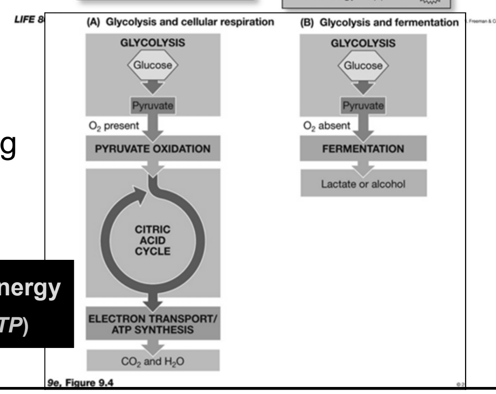
(-686 kcal/ mol, *if complete! = ~57ATP*)

- The same equation applies to the metabolism of glucose by cells,
  - but in ***many separate steps***
  - so energy can be captured in **ATP** and **electron-carriers**.
  - *****Incremental harvesting of released Energy!!***

16

Energy for life: Contrasting Yields!





9.4 Energy-Producing Metabolic Pathways

$C_6H_{12}O_6 + 6\ O_2 \rightarrow 6\ CO_2 + 6\ H_2O$ + energy

(-686 kcal/ mol, *if complete! = ~57 ATP*)

17

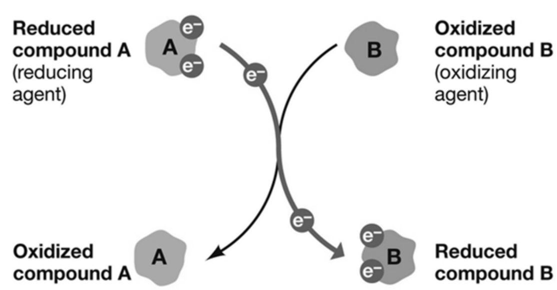
A. Redox Reactions: Transferring Electrons

- As a material is <u>oxidized</u>, the electrons it loses transfer to another material, which is thereby <u>reduced</u>.

  $AH_2 + B \rightarrow BH_2 + A$

  (A is oxidized, B is reduced)

  For GLC: glc = Reducing Agent, O_2 = Oxidizing agent



- Such ***<u>redox reactions</u>*** transfer a lot of energy.
  - Much of the energy liberated by the oxidation of the reducing agent
  - is captured in the reduction of the oxidizing agent.

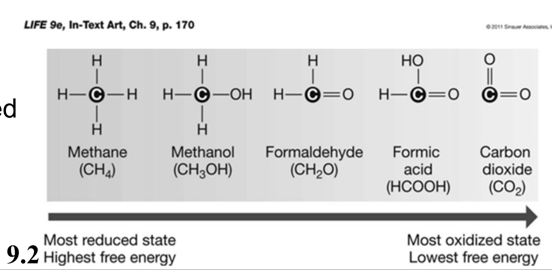


Fig. 9.2

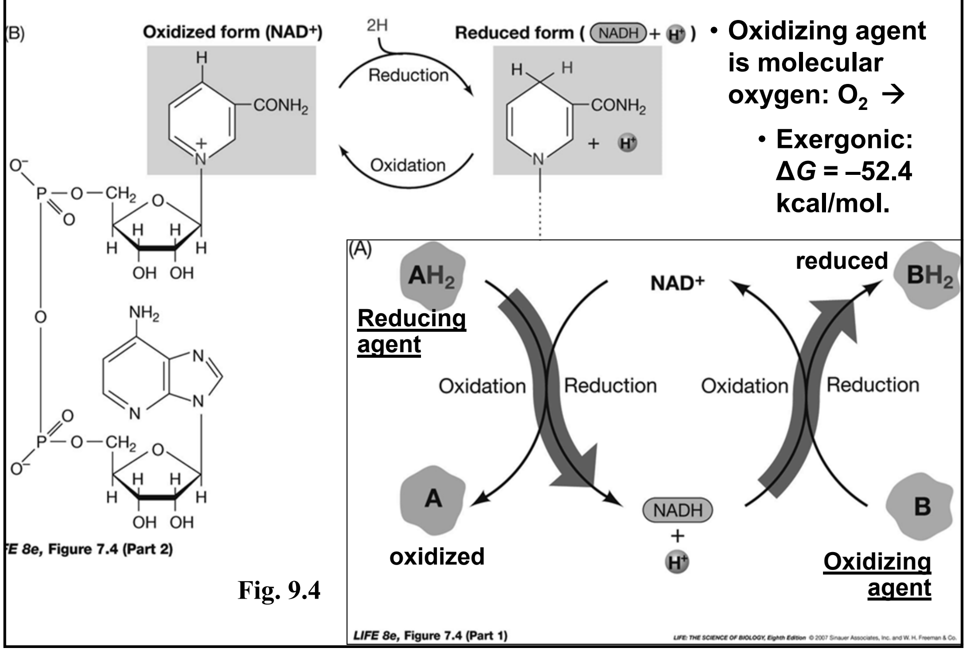
18

Redox Reactions with Glucose

- The ***coenzyme NAD*** is a key electron carrier in biological redox reactions.
  - Two forms:
    - oxidized (NAD^+)
    - ***reduced ($NADH + H^+$)*** = includes electrons with their associated Hydrogen nuclei.
      - ***Transfer H atom (hydride ion & H+) = equivalent of electrons being passed/ redox rxn.***

19

NAD Is an Energy Carrier in Redox Reaction Cycles



- **Oxidizing agent is molecular oxygen: O_2 →**
  - **Exergonic: Δ*G* = –52.4 kcal/mol.**

Fig. 9.4

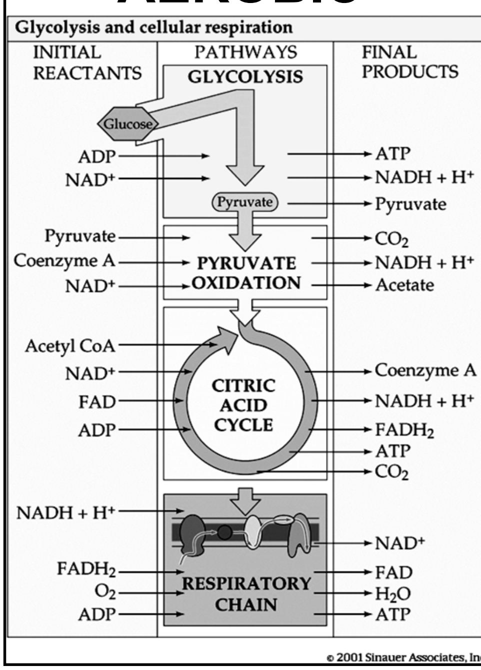
20

B. An Overview: Releasing Energy from Glucose

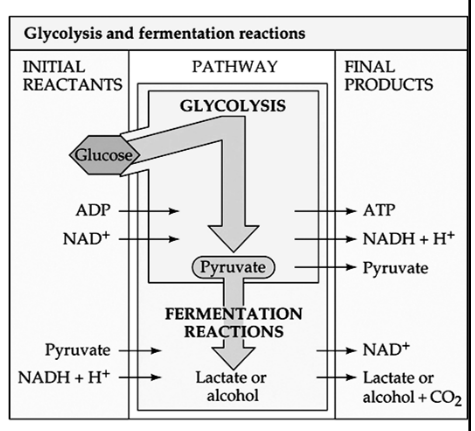
- ***Glycolysis operates in the presence or absence of O_2.***
- Under aerobic conditions (w/ O_2), ***cellular respiration*** continues the breakdown process.

21

AEROBIC



"ANAEROBIC"



(CAN happen in the presence of O_2!!)

Figure 7.5 (2004)

22

Releasing Energy from Glucose

1. ***Pyruvate oxidation*** and the ***citric acid cycle (TCA / Krebs)***
   – produce $\underline{CO_2}$ and <u>hydrogen</u> atoms
   – carried by <u>NADH</u> and $\underline{FADH_2}$.
2. The ***respiratory chain***
   – combines the hydrogens with O_2,
   – releasing enough energy for ATP synthesis.
3. In some cells under anaerobic conditions, pyruvate can be reduced by NADH **(*fermentation*)**
   – to form <u>lactate</u> or <u>ethanol</u>
   – to ***regenerate the NAD*** needed to sustain glycolysis

23

9.2) Overview: Releasing Energy from Glucose

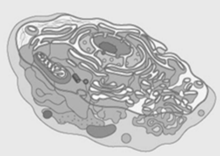
- <u>In **eukaryotes**,</u>
  – glycolysis & fermentation occur in the <u>*cytoplasm*</u> (outside of the mitochondria)
  – pyruvate oxidation, the citric acid cycle (TCA), & the respiratory chain (ETC) operate in <u>*mitochondria*</u>
- <u>In **prokaryotes**,</u>
  – glycolysis, fermentation, & TCA take place in the <u>*cytoplasm*</u>
  – pyruvate oxidation & ETC operate in association with the <u>*plasma membrane*</u>

24

Cellular Locations of Energy Pathways

TABLE 9.1

Cellular Locations for Energy Pathways in Eukaryotes and Prokaryotes



EUKARYOTES	PROKARYOTES
External to mitochondrion	**In cytoplasm**
Glycolysis	Glycolysis
Fermentation	Fermentation
	Citric acid cycle (TCA)
Inside mitochondrion	**On plasma membrane**
Inner membrane	Pyruvate oxidation
Respiratory chain	Respiratory chain
Matrix	
Citric acid cycle	
Pyruvate oxidation	

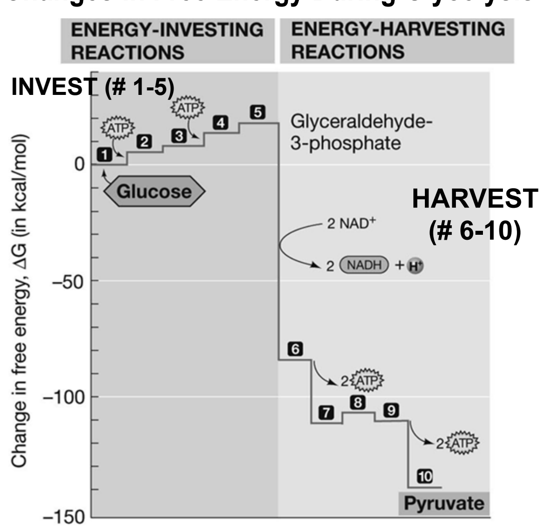
Inside a CELL: http://learn.genetics.utah.edu/content/begin/cells/insideacell/

25

A. Glycolysis: From Glucose to Pyruvate

- **Glycolysis** is a pathway of **ten enzyme-catalyzed reactions**
  - located in the cytoplasm (-sol).
  - provides ***starting materials for both cellular respiration & fermentation***.
  - +2ATP, no $-CO_2$

Changes in Free Energy During Glycolysis



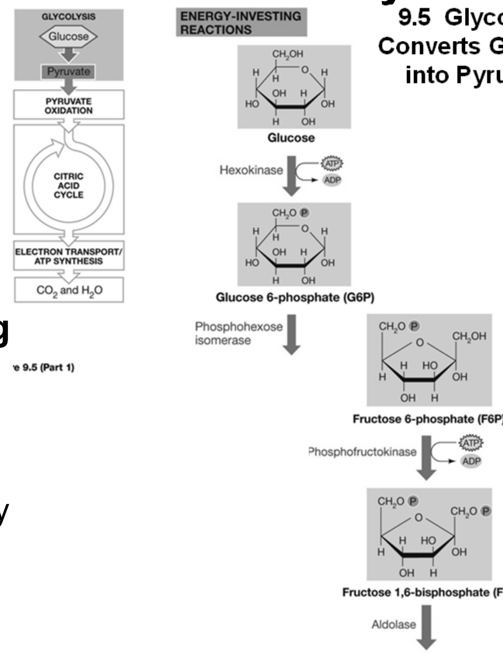
Each glucose yields: 2 Pyruvate, 2 ATP, 2 NADH + 2 H^+

7.6 LIFE: THE SCIENCE OF BIOLOGY, Eighth Edition © 2007 Sinauer Assoc

26

Glycolysis: From Glucose to Pyruvate

- **Energy-investing** reactions of glycolysis (per glc):
  - use 2 ATPs
  - yield 2 glyceraldehyde-3-phosphates (g3p)
- **Energy-harvesting** reactions produce (per glc):
  - 2 NADH molecules (-52 kcal/mol each)
  - 4 ATP molecules by *substrate-level phosphorylation*
  - two pyruvates



9.5 Glycolysis Converts Glucose into Pyruvate

e 9.5 (Part 1)

27

Glycolysis: From Glucose to Pyruvate

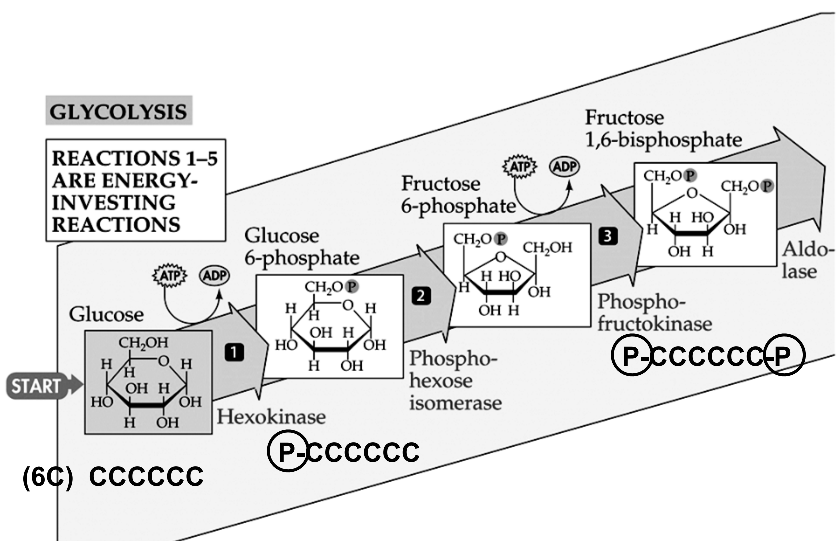


figure 07-06 (2001)

http://www.iubmb-nicholson.org/swf/glycolysis.swf -- molecular detail!!!

28

Glycolysis: From Glucose to Pyruvate

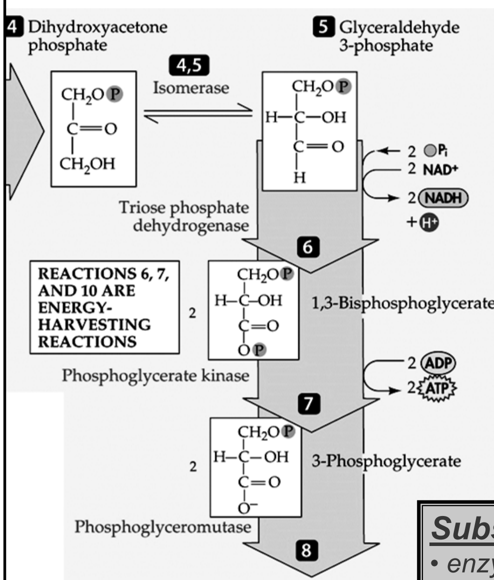


Figure 7.6 – Part 2

Split P-CCCCCC-P (f1,6bp)

↓ 4,5

2x CCC-P (g3p)

↓ 6

2x P-CCC-P (1,3bpg)

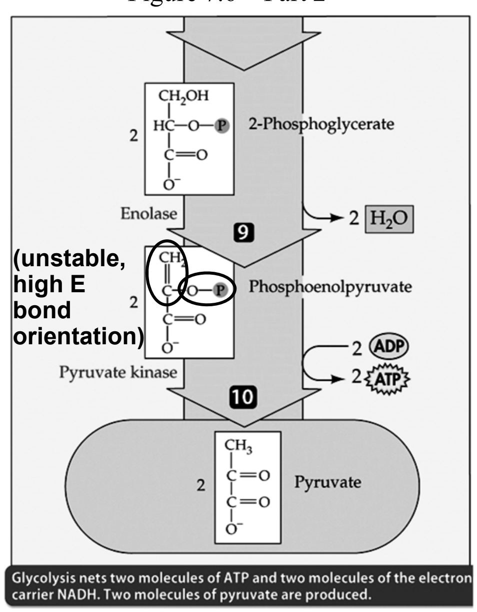
↓ 7

2x CCC-P (3pg) + 2 ATP!!

Substrate-Level Phosphorylation:
• enzyme-catalyzed transfer of phosphates from donor molecules to ADP→ATP

29

Figure 7.6 – Part 2



↓ 7

[2x CCC-P (3pg) + 2 ATP!!]

↓ 8

2x CCC (2pg) (P on middle C)

↓ 9

2x CCC (PEP) + 2 H_2O (P on middle C)

↓ 10

2x CCC (Pyruvate) + 2 ATP!!

2nd Substrate-Level Phosphorylation

Invest 2 ATP, Harvest 4 ATP = ***net 2ATP/glc***

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

30

B. Pyruvate Oxidation (2 per Glc)

Pyruvate dehydrogenase complex (= Huge! 72 subunits, 4.6 MDa) catalyzes three reactions:

1. **Pyruvate = ox'd to acetyl group, releasing 1 CO_2 & energy**
2. **Some energy is captured when NAD^+ is reduced to $NADH + H^+$ (stores *52 kcal/mol*)**
3. **Remaining energy is captured in acetyl CoA (stores 7.5 kcal/mol)**
   - ***S-bond = high energy!***

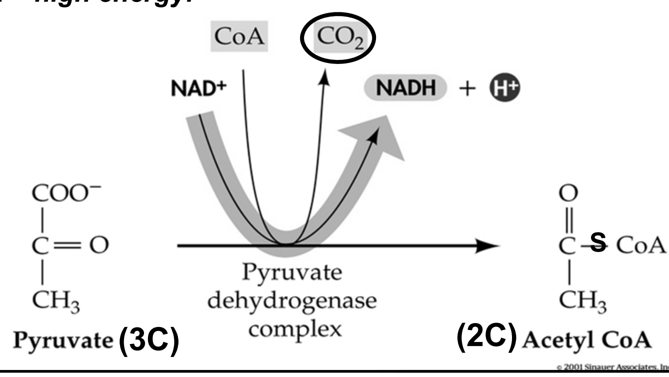


Figure 7.8 (2004)

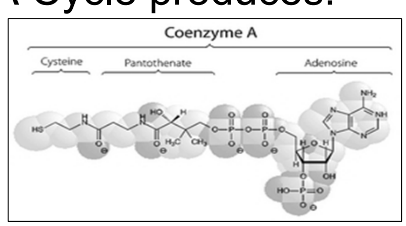
31

C. The Citric Acid Cycle (TCA)

1. Energy in acetyl CoA drives rxn:
   - **Acetate (2C) + oxaloacetate (4C) → citrate (6C).**

2. TCA Cycle = series of rxns that *oxidize CITR and regenerate OAA (OXAL)*

3. For each acetyl CoA, TCA Cycle produces:
   - **2 CO_2**
   - **3 NADH** (52 kcal/mol)
   - **1 $FADH_2$** (43 kcal/mol)
   - **1 ATP** (12 kcal/mol)



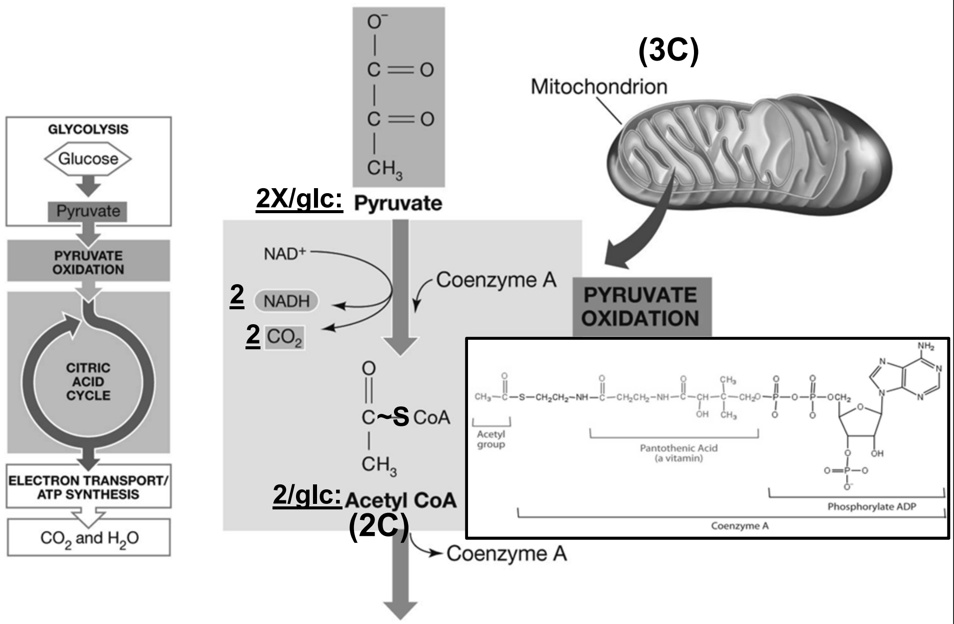
http://www.science.smith.edu/departments/Biology/Bio231/krebs.html

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

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

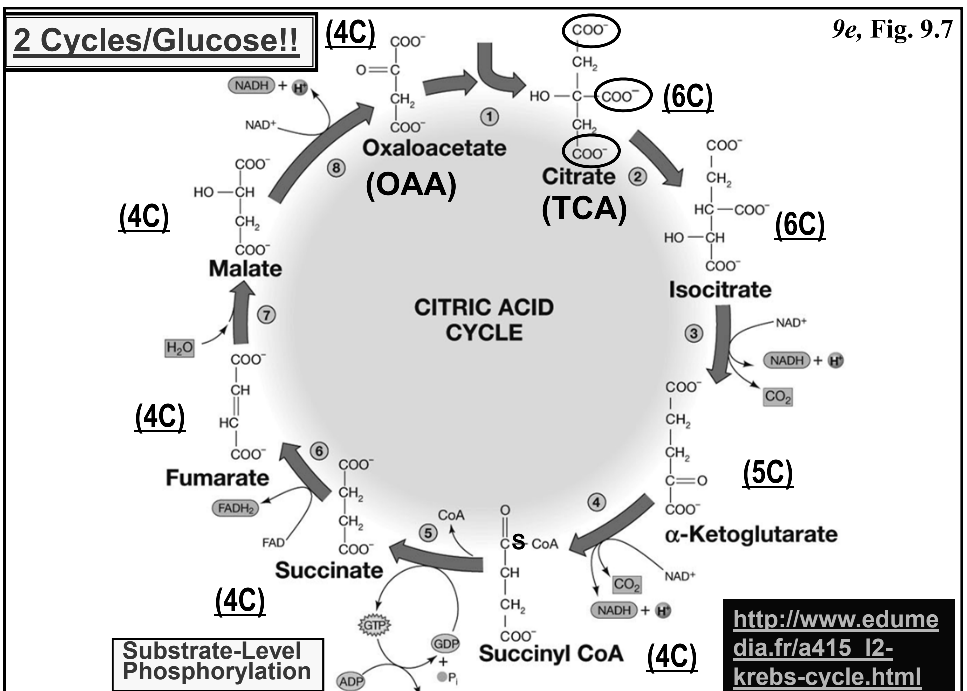
32

Pyruvate Oxidation & the Citric Acid Cycle



LIFE 9e, Figure 9.7 (Part 1)

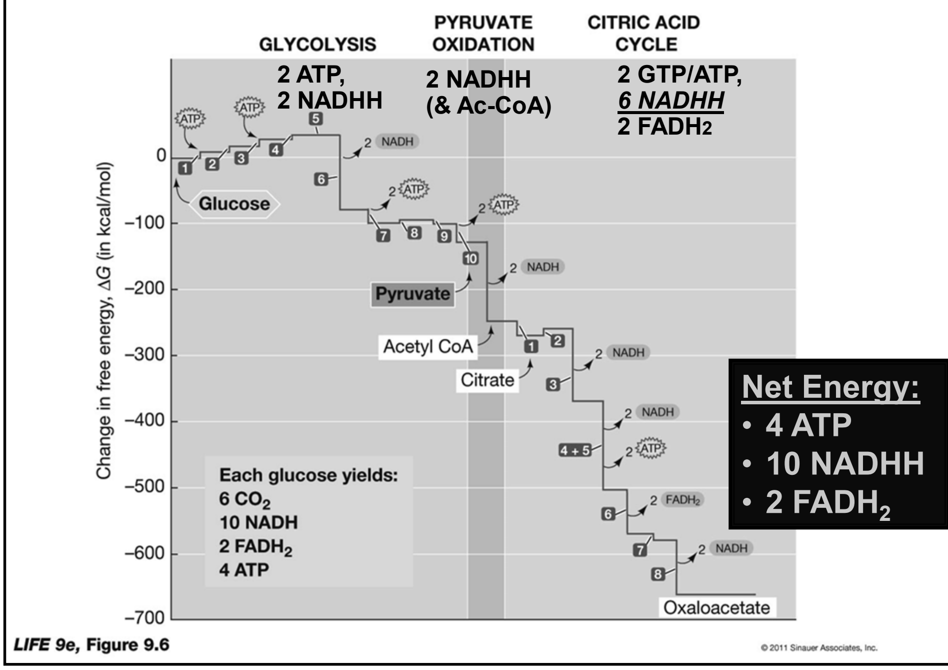
33



LIFE 8e, Figure 7.8 (Part 2)

34

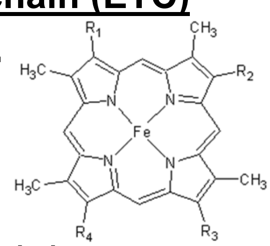
TCA Releases Much More Free Energy (G) than Glycolysis



35

D. The Respiratory Chain (ETC): Electrons, Proton Pumping, and ATP

1. ***NADH + H^+*** and ***$FADH_2$*** (Glyc, Pyr Ox, TCA)
   - **oxidized by the respiratory chain (ETC)**
   - **regenerating NAD^+ and FAD.**

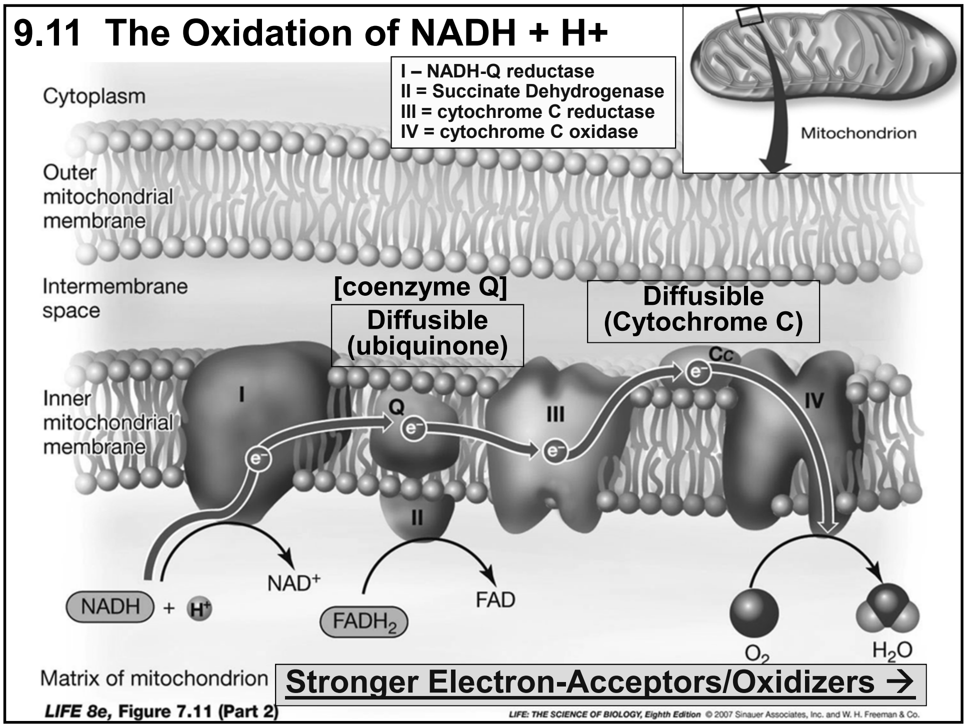


Porphyrin Ring

2. Most of the enzymes and other electron carriers of ETC are part of the *inner mitochondrial membrane.*
3. ***O_2 is the final acceptor*** of electrons and protons, forming H_2O.

36

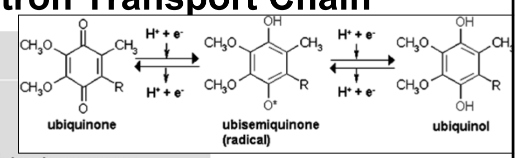
9.11 The Oxidation of NADH + H+

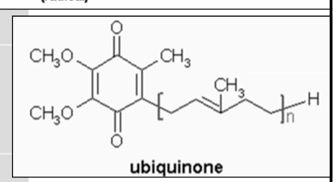


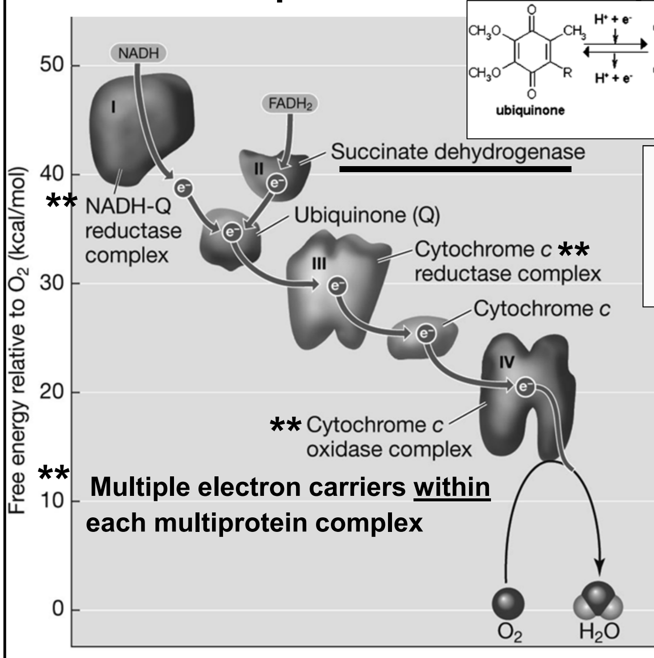
LIFE 8e, Figure 7.11 (Part 2)

37

9.12 The Complete Electron Transport Chain







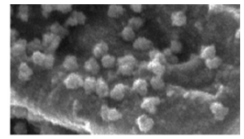
– ΔG!!

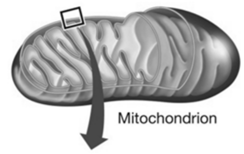
http://vcell.ndsu.nodak.edu/animations/etc/movie-flash.htm

e 9.8

38

1. CHEMIOSMOSIS



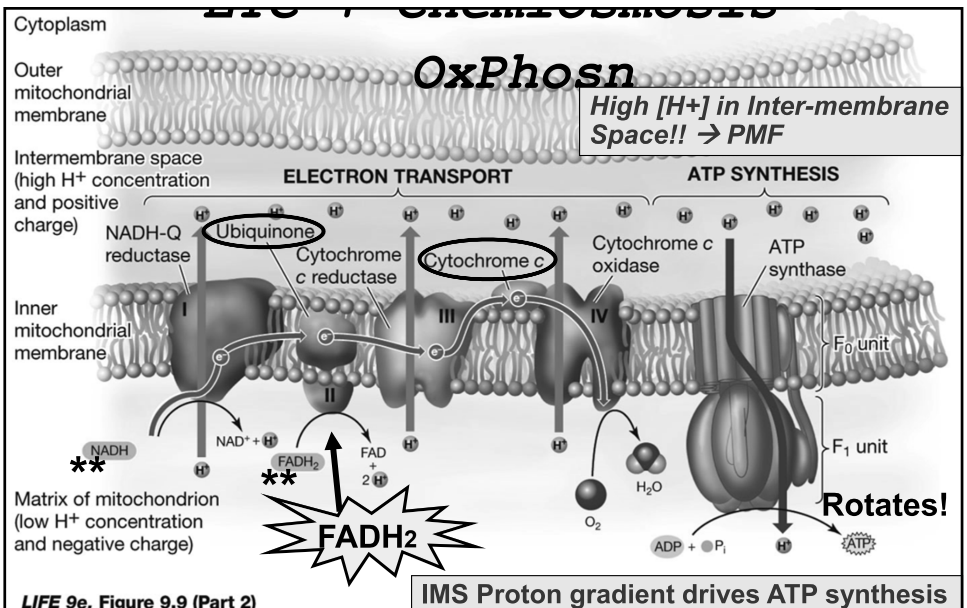


- The **chemiosmotic mechanism** *couples* proton transport to **oxidative phosphorylation**.
  - (PMF → ATP synthesis)

- Electrons moving down the ETC release energy:
  - captured by proton (H^+) pumps:
    - *actively transport* H^+ out of the mitochondrial matrix (Ch. 5)

❖ *create gradient of [H+] and electric charge — the **Proton-Motive Force**.*

39



***LIFE 9e*, Figure 9.9 (Part 2)**

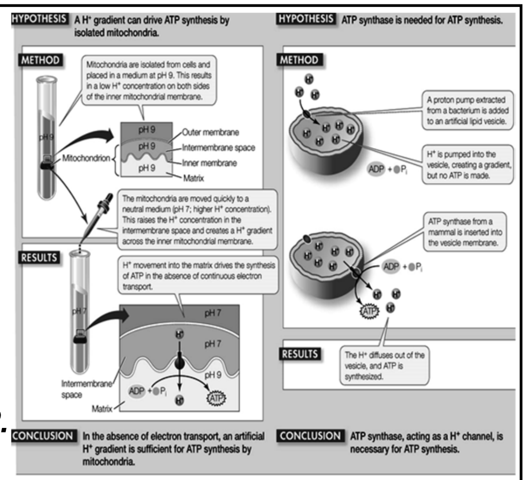
❖ **Each NADH → ~3 ATP**
❖ **Each $FADH_2$ → ~2 ATP**

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

40

2. PMF

- The **Proton-Motive Force** causes **H+ to diffuse** back into the mitochondrial interior (MATRIX) **thru ATP synthase**, which
  - ***couples H+ diffusion to the production of ATP.***



e, Figure 9.10

- ***Uncoupling → Heat!!***
  (***thermogenin*** in newborns)
  - Proton pore, without ATP synthase;
  - also in ***brown fat***
- *Voodoo Lily* – (***calorigen***) early pollination, aromatic amines & indoles

41

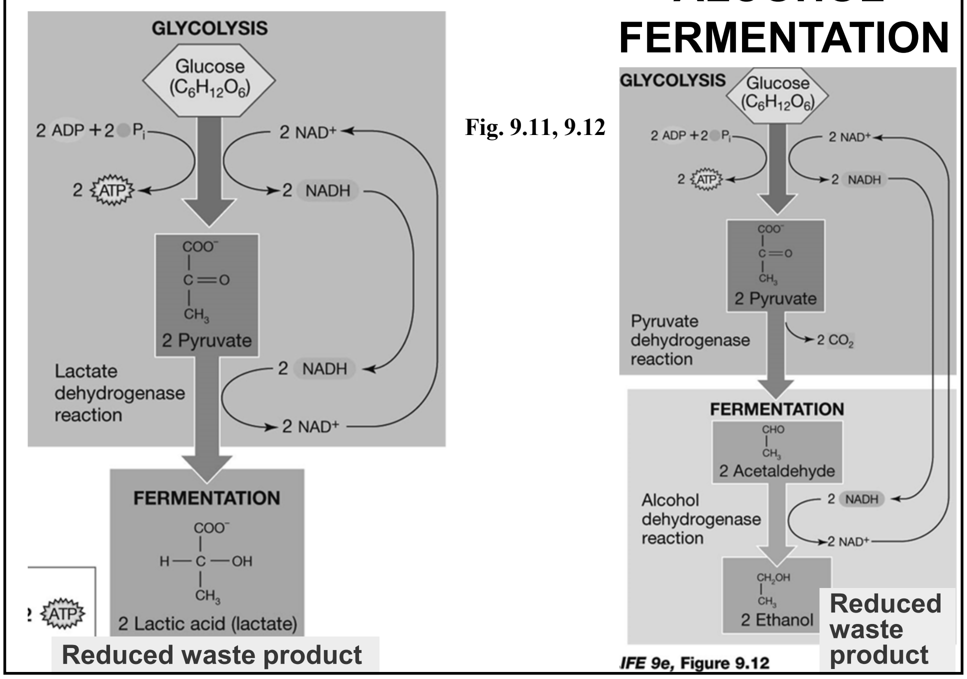
E. Fermentation: ATP from Glucose, without O_2

- Anaerobic organisms
  - energy from ***glycolysis & fermentation.***
  - partly oxidize glucose
    - generate energy-containing products
      - (Lactate, EtOH)
  - anaerobically oxidize the NADH + H^+ produced in glycolysis → ***recycle NAD+.***

42

LACTATE FERMENTATION

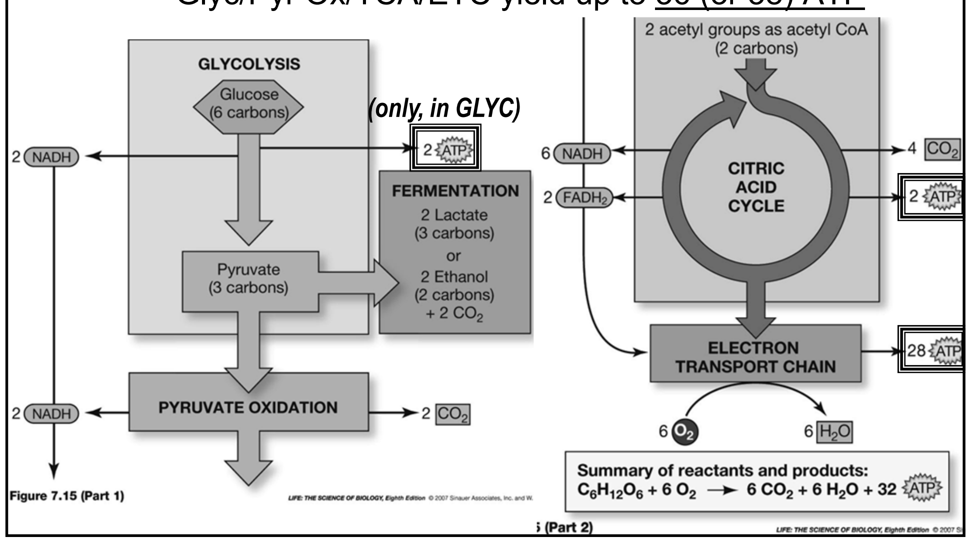
ALCOHOL FERMENTATION



43

9.3) Contrasting Energy Yields

- **For each GLUCOSE:**
  - fermentation yields 2 ATP
  - Glyc/Pyr Ox/TCA/ETC yield up to 36 (or 38) ATP



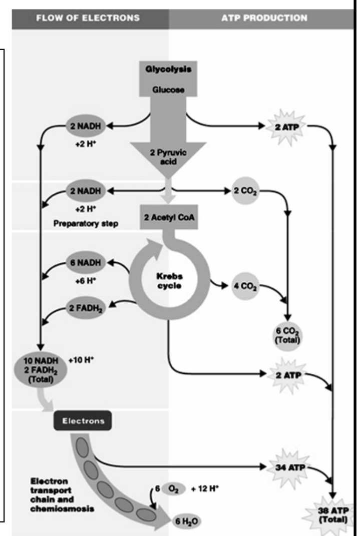
44

Oxidative Metabolic Yield, Summary:

NET:

1. **Glyc** = 2 ATP
   (4 made, 2 invested)
   2 NADHH → 4 ATP
   (use 2 ATP** on transport into **mito. I.M.!!)
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



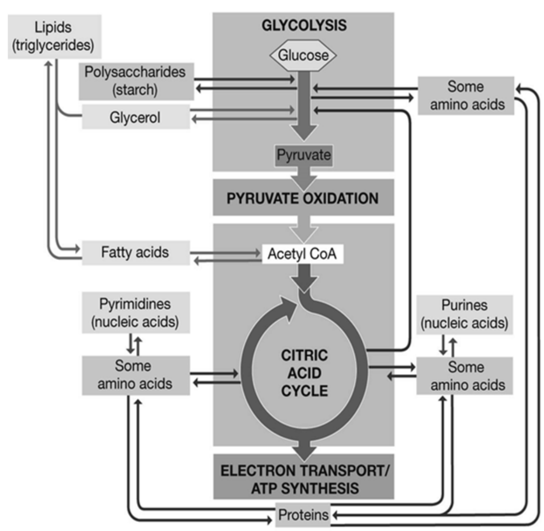
http://vcell.ndsu.nodak.edu/animations/atpgradient/movie.htm

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

45

9.4) Metabolic Pathways: Catabolic pathways feed into the Respiratory pathways

1. **Polysaccharides → glucose**
   - enters *glycolysis*
2. **Fats → Glycerol**
   - enters glycolysis (DHAP)
3. **Fatty acid degradation →Acetyl CoA**
   - enters *TCA cycle*
4. **Proteins → amino acids**
   - enter glycolysis & TCA

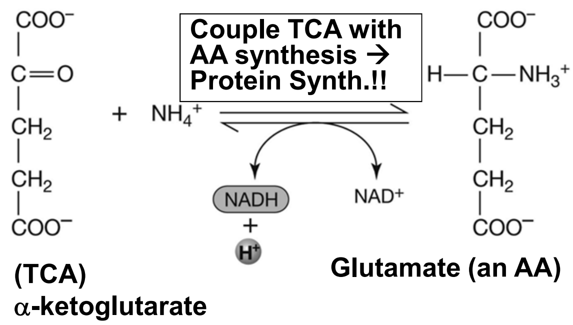


igure 9.14

46

9.5) Anabolic pathways: ***coupled*** to catabolism by enzymes

- use respiratory intermediate compounds of metabolism.
  - fats, amino acids, and others
- synthesize essential building blocks for cellular structure & function.

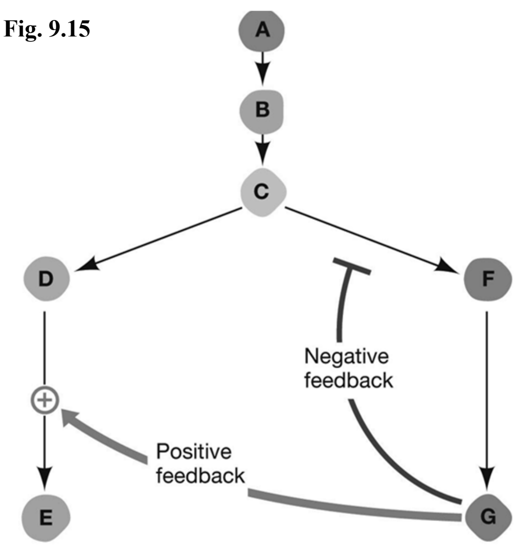


LIFE 8e, Figure 7.17

47

9.6) Regulating Energy Pathways

Fig. 9.15



7.18

- Rates of GLYC and TCA are increased or decreased by:
  - ATP, ↓
  - ADP, ↑
  - NAD^+, or ↑
  - $NADH + H^+$ ↓
- (all act on ***allosteric*** enzymes)

Abundant product "G":
- Inhibits own production
  - (***neg. feedback***)
- Activates alternate branch
  - (***pos. feedback***)

http://www.science-groove.org/Now/Glucose.html https://youtu.be/jJvAL-iiLnQ

48