EXAM 1 KEY

100 PTS.
Chem 454-2009
NAME _____

Part I. Multiple Choice: (12)

1. This energy source powers most ATP synthesis.
   (A) electrochemical potential of ion gradients
   B) electrochemical potential of stored glycogen
   C) high-energy intermediates
   D) All of the above.
   E) None of the above.

2. The reduced form of flavin adenine dinucleotide is
   A) FADH. B) FAD. C) $FADH^{++}$. (D) $FADH_2$. E) None of the above.

3. Which of the following is the electron donor used for reductive biosynthesis.
   A) NADH
   (B) NADPH
   C) $FADH_2$
   D) CoASH
   E) ATP

4. An example of an isomerization reaction would be
   A) the conversion of succinate to fumarate using FAD.
   B) the addition of carbon dioxide to pyruvate to form oxaloacetate.
   (C) the conversion of citrate to isocitrate.
   D) the hydrolysis of a peptide bond.
   E) none of the above.

5. Which of the following is an example of an oxidation reaction?

   (A) 

   B) 

   C) 

   D) None of the above.

6. Some of the mechanisms by which enzyme catalytic activity is controlled.
   A) allosteric control
   B) feedback inhibition
   C) covalent modification
   D) a and c
   (E) a, b, and c

Part II. Matching Questions(20)
Use the following to answer questions 1-10:
Choose the correct answer from the list below. Not all of the answers will be used.

a) obligate anaerobes
b) AMP
c) Embden Meyerhof pathway
d) NAD^+
e) gluconeogenesis
f) UDP-glucose
g) GLUT5
h) facultative anaerobes
i) ATP
j) magnesium
k) galactosemia
l) biotin

1. e This is the process by which noncarbohydrate precursor molecules are converted into glucose.
2. c This is another name for glycolysis.
3. a These organisms cannot survive in the presence of oxygen.
4. d This substance must be regenerated for glycolysis to proceed.
5. f This intermediate is necessary for the conversion of galactose to glucose.
6. i This molecule is an allosteric inhibitor of phosphofructokinase.
7. g This transporter is responsible for fructose uptake in the intestine.
8. k This condition is a result of a genetic deficiency of a single "transferase" enzyme.
9. j, l This essential nutrient is required for the carboxylation of pyruvate in humans.
10. b This is an allosteric activator of glycolysis.

PART III. Short Answer (12)

1. What astounding discovery was made by the Buchners (the funnel guys...)?

Fermentation could occur in a cell-free yeast extract.

2. What two ways can glycolysis be maintained under anaerobic conditions?

pyruvate → lactate

Pyruvate → → ethanol

3. How does citrate influence glycolysis?

inhibits it

4. Which metabolic steps differ from glycolysis in gluconeogenesis?

Pyr —X→ PEP
F,6-P —X→ FBP
G-6-P —X→ glucose

} Cannot be "reversed" ΔG° = too negative to overcome

Part IV. Problems. Be thorough and show all work. (56)

1. The mitochondrial membrane potential is 180 mV (inside -) and the pH inside the matrix is 8.0 and outside is 7.0. What is the minimum whole number of protons flowing in, energetically speaking, needed to spontaneously synthesize 1 molecule ATP (under standard conditions, hydrolysis ΔG° = -7.3 kcal/mol, T=25 C)?(12)

$1\ H^+_{out} = 2.3 \times 0.00199 \times 298k\,(1.0) + 23 \times 0.180V = 5.5\ kcal/mol$

So: $1\ H^+_{in} = -5.5\ kcal/mol$

so 2 H+ (-11 kcal/mol) is the minimum

a. The true stoichiometry of the ATP synthase is considered to be 4H+/ATP. Is this consistent with the calculation above? What is the % efficiency of the process?

Yes, because $-5.5 \times 4\ H^+ = 22\ kcal/mol$ is sufficient.

$\frac{-7.3\ ATP}{-22\ available} \times 100\% \approx 33\%$

b. If the membrane voltage were collapsed (with, *e.g.*, a K+ ionophore), but the pH gradient remained intact, would 4 H+ still be sufficient to synthesize ATP? Show why or why not.

$1\ H^+_{out} = 2.3 \times 0.00199 \times 298(1) + 0 = 1.4\ kcal/mol$

$1\ H^+_{in} = -1.4\ kcal/mol_{H^+} \times 4\ H^+ = -5.6\ kcal/mol$

It is not enough!!

Chem 454-EXAM 1 Add-on from problems 3

1. Excitable cells use a lot of ATP to generate transmembrane gradients and membrane potentials. Consider a neuron. It can be approximated as a cylinder with a volume of 6300 μm^3 and a surface area of 2700 μm^2. How many K+ ions would need to be actively pumped from inside to outside to charge the membrane to -70. mV. Considering the concentration of K+ inside a typical cell is 200. mM (and outside is 7 mM), would this pumping significantly change the internal [K+]? Show all work. (by significant I mean more than 5%). (10)

-0.070V = q/ 1.0 *10^{-6} *$(1/10000\ \mu m.cm)^2$ * 2700 μm^2
q=-2.7 *10^{-11} * 0.070=-1.9 * 10^{-12} C and # of charges = 1.9 * 10^{-12} C/1.6 * 10^{-19} C = 1.2 * 10^7 K+ ions pumped out

assume 1mL = 1 cm^3
ions inside if K+ = 200mM= 0.200 mol K+/L* **1L/1000mL (1cm/10000μm)^3** * 6300 μm^3 * 6.02 * 10^{23} K+/mole = 7.6 * 10^{11} K+ ions inside. So

7.6 * 10^{11}-1.2 * 10^7/ 7.6 * 10^{11} *100%= 99.9984% still left inside!! Not Significant

2. A newly discovered bacterium contains the following cytochromes. (10)

a. Predict the sequence of carriers in this ETS (from most reduced to most oxidized).
? →Flavoprot b→NAD+→cyt c→Flavoprot a→Ferroprot→?

b. How many molecules of ATP could be synthesized per pair of electrons under standard conditions using this pathway?

-nFΔE=-2(23)(0.85- -0.62)= -67 kcal/mol
-67/-7.3= 9.3 moles ATP or 9 molecules ATP whole number per electron pair

c. Why is it unlikely that oxygen is a terminal electron acceptor?

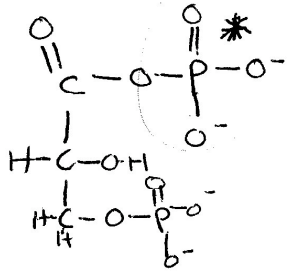
Because O_2 std reduction potential is 0.83 V and is more negative than the last carrier.

Reduction potentials for pathogenic gram-negative bacterium

Oxidant	Reductant	Electrons transferred	E'_0 (V)
NAD+	NADH	2	–0.32
Flavoprotein *b* (oxidized)	Flavoprotein *b* (reduced)	2	–0.62
Cytochrome *c* (+3)	Cytochrome *c* (+2)	1	+0.22
Ferroprotein (oxidized)	Ferroprotein (reduced)	2	+0.85
Flavoprotein *a* (oxidized)	Flavoprotein *a* (reduced)	2	+0.77

3. Inorganic phosphate labeled with radioactive ^{32}P is added with glucoses to a liver cell extract,and the mixture is then incubated in the absence of oxygen. After a short time 1,3-bisphosphoglycerate is isolated from the mixture. (6)

a. Show which carbon(s) of 1,3 BPG would you expect to find the radioactive phosphate?



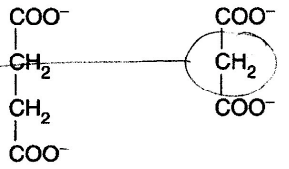
b. If you allow the incubation to continue for a longer period, will you find any change to this labeling pattern? Why or why not?

Yes. The ^{32}P phosphate will end up on ATP so anything phosphorylated with ATP will also be quickly labeled.

4. The malonate anion is a potent competitive inhibitor of succinate dehydrogenase.(10)

a. Why is malonate unreactive?

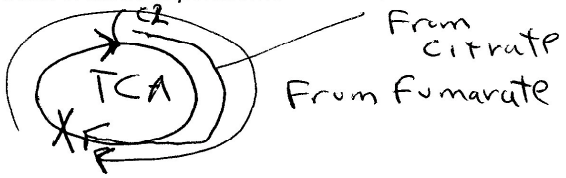
There is no position to unsaturate.



Succinate **Malonate**

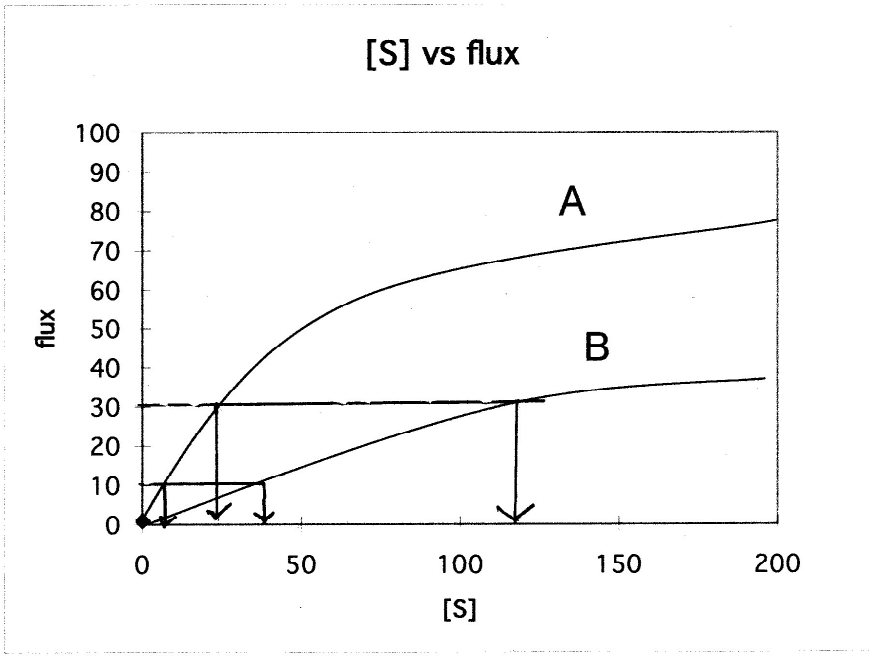
b. In the work that led to the elucidation of the citric acid (Krebs) cycle, Hans Krebs employed malonate as an inhibitor of succinate dehydrogenase. Earlier studies by Martius and Knoop had shown that in animal tissues there is a pathway from citrate to succinate. Krebs had also noticed that citrate catalytically enhances respiration in minced muscle tissue. Knowing that malonate reduces the rate of respiration in animal cells, he added citrate to malonate-poisoned muscle. In another experiment, Krebs added fumarate to malonate poisoned muscle. *What changes in succinate concentration do you think Krebs observed in each of these experiments with malonate treated muscle?*

Both Increased.



c. *Explain the significance of the findings to establishing the cyclic nature of the Citric Acid cycle.*

The experiment showed 2 routes from Fumarate → succinate and that suggested a cycle.



5. An assay for an enzyme step in a pathway in tissues from two different people is shown above. One is normal the other suffers from a genetic disease. (8)

a. What approximate [S] would support a flux of 10 for each? How much [S] for a sudden increase to a flux of 30?

for 10 : about 10 & 40
for 30 : about 20 & 120

b. What would problem would arise in person B?

The catastrophic rise in [S] could occur IF Flux increased. This could prove TOXIC.

c. Give one likely cause of the difference in the patients' activity.

1) Less [enzyme] synthesized in B
2) Same [enzyme], lower Kcat in B

d. Which would you guess belongs to a person with a genetic disease?

B.

BONUS

4/4

1. Predict the effects of the following mutations on glycolysis rate in the liver (increased, decreased, unchanged)

a. loss of allosteric site for ATP in phosphofructokinase-1 (increased, decreased, unchanged)
b. loss of binding site for citrate in phosphofructokinase-1 (increased, decreased, unchanged)
c. loss of phosphatase domain of the bifunctional phosphofructokinase-2 (increased, decreased, unchanged)
d. loss of binding site for fructose 1,6-bisphosphate in pyruvate kinase (increased, decreased, unchanged)

maybe