

1. Cytochrome  $a$  and  $a_3$  are both part of cytochrome  $c$  oxidase. The  $a$ -type hemes have different standard reduction potentials because of the different protein environments around them. SHOW ALL CALCULATIONS!!!! - redox potential values= in table on back (10)

a. Could the transfer of two electrons between these two cytochromes ( $a$  and  $a_3$ ) have sufficient energy ( $\Delta G^\circ$ ) to lead to ATP synthesis from ADP and  $P_i$  under standard conditions? Show why or why not.

$$\Delta G = -n F \Delta E^\circ$$

$$\Delta G = -2 (23,000) (0.35 - 0.29)$$

$$= -2.8 \text{ kcal/mol}$$

$$(-11.7 \text{ KJ/mol})$$

Not enough. You need -7.3 kcal/mol

3

b. Would transfer of two electrons from  $FADH_2$  to CoQ be sufficient for ATP synthesis (theoretically)? Show why or why not. (ATP hydrolysis = -7.3 kcal/mol)

$$\Delta G = -2 (23,000) (0.96 - 0.22)$$

$$= -46,000 (0.28)$$

$$= 12.9 \text{ kcal/mol}$$

$$(1.54 \text{ kcal/mol})$$

should be 0.06 not 0.031  
Yes. enough.

3

c. Experimentally, the protonmotive force generated by the 2 electron transfer process of  $FADH_2$  to CoQ in mitochondria is 0.160 V. If the transmembrane voltage is 0.090 V (inside negative), what  $\Delta pH$  would be necessary to achieve this pmf? Would the pH be acidic or basic inside the matrix of the mitochondria? (show work)

$$PMF = 0.160 \text{ V} = \Delta \psi + \frac{RT}{F} \ln 10 \Delta pH$$

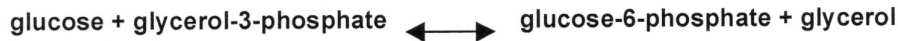
$$= 0.090 \text{ V} + \frac{2.303 RT}{F} \Delta pH$$

$$+ 0.070 = 0.0592 \Delta pH$$

$$\Delta pH = 1.18$$

4

2. A recent article in *Science* claims researchers have discovered a new biochemical reaction that is the first step in the glycolytic pathway in certain bugs. It is shown below (10)



a. Is this reasonable (spontaneous) under standard conditions? Show why or why not.

$$\begin{array}{r} +13.8 \text{ kJ/mol} \\ -9.2 \\ \hline +4.6 \text{ kJ/mol} \end{array} = \Delta G^\circ \quad \text{Not spontaneous} \quad 3$$

b. The normal reaction is :



What is the  $\Delta G^\circ$  for this reaction?

$$\begin{array}{r} -30.5 \\ +13.8 \\ \hline -16.7 \text{ kJ/mol} \end{array} \quad 3$$

c. When you read farther in the article you find that under cellular conditions in the bug [glycerol] = 1.0 mM; [glycerol-3-phosphate] = 20.0 mM; [glucose] = 5 mM; [glucose 6-phosphate] = 0.0050 mM. Temp = 37°C. pH = 7.0. What is the  $\Delta G'$  for the reaction under these conditions? Does this new information make the original claim more or less reasonable? Show and Explain

$$\Delta G' = \Delta G^\circ + (8.31)(310) \ln \frac{(0.005)(1)}{(5)(20)}$$

$$+4.2 + -25.5 \quad 4$$

$$= -21.3 \text{ kJ/mol}$$

Under real conditions, this reaction would be spontaneous.

1. Why does succinate stimulate oxygen use (respiration)? 4
2. Why is mitochondrial respiration dependent upon ADP? Explain the results of adding KCN (cytochrome oxidase blocker). 4
3. Under normal circumstances, what would be the effect of adding oligomycin on oxygen use (an ATP synthase proton channel blocker)? 4
4. What do the data tell you about the mechanism of DNOC? 4
5. Why were the respiratory rate and body temperature elevated and fat depleted? 4

1) Succinate feeds into the TCA producing both NADH and FADH<sub>2</sub>. Both will consume O<sub>2</sub> via the ETS.

2) ADP + P<sub>i</sub> → ATP is the ultimate user of the pmf via ATP synthase. If there is no ADP then ATP synthase will halt "backing up" pmf to the point where protons are no longer pumped and O<sub>2</sub> is not used for an electron transport acceptor.

KCN blocks the flow  $\text{cyt } c_{\text{red}} \rightarrow \text{O}_2$  and so halts all O<sub>2</sub> usage.

3) Oligomycin should halt O<sub>2</sub> usage for the same reason as ADP depletion above.

4) DNOC must be an uncoupler and cause a "short circuit" of H<sup>+</sup> leakage. This would be affected by KCN but not by oligomycin.

5) The short circuit increased usage of all fuels including fats, but much of the energy would be dissipated as heat (fever) because of inefficiency of ATP / H<sup>+</sup> coupling.

**Metabolism Multiple Choice (2 each). Write in letter of best answer.(40pts)**

- A** In cells, NADH serves as a carrier of which of the following?

  - Electrons
  - Protons
  - Hydroxyl groups
  - Methyl groups
  - Phosphoryl groups
- B** Which of the following best characterizes NADH and NADPH?

  - NADH and NADPH are interchangeably used for both ATP generation and biosynthesis.
  - NADH is primarily used for ATP generation, whereas NADPH is primarily used for biosynthesis.
  - NADPH is primarily used for ATP generation, whereas NADH is primarily used for biosynthesis.
  - Both ATP generation and biosynthesis preferentially use NADH over NADPH.
  - Both ATP generation and biosynthesis preferentially use NADPH over NADH.
- B** In cells, biotin serves as a carrier of which of the following?

  - Acetyl groups
  - Carboxyl groups
  - Electrons
  - Methyl groups
  - Phosphoryl groups
- B** Which of the following enzyme complexes catalyzes the reduction of oxygen to water during oxidative phosphorylation?

  - ATP synthase
  - Cytochrome *c* oxidase
  - NADH-Q oxidoreductase
  - Q-cytochrome *c* oxidoreductase
  - Succinate-Q reductase
- A** Which of the following carriers in the electron-transport chain is a protein (as opposed to a small molecule)?

  - Cytochrome *c*
  - FADH<sub>2</sub>
  - NADH
  - Succinate
  - Ubiquinone or coenzyme Q
- D** Which of the following best describes the net organic products formed during the oxidation of one acetyl group to two molecules of carbon dioxide via the citric acid cycle?

  - 1 NADH + 3 FADH<sub>2</sub> + 1 GTP
  - 2 NADH + 2 FADH<sub>2</sub> + 2 ATP
  - 3 NADH
  - 3 NADH + 1 FADH<sub>2</sub> + 1 GTP
  - 6 NADH + 6 ATP

7. E Which of the following conversions occurring in the citric acid cycle is coupled to a substrate-level phosphorylation reaction?
- Acetyl-CoA + oxaloacetate  $\rightarrow$  citrate + CoA
  - Alpha-ketoglutarate + CoA  $\rightarrow$  succinyl-CoA + carbon dioxide
  - Isocitrate  $\rightarrow$  alpha-ketoglutarate + carbon dioxide
  - Succinate  $\rightarrow$  fumarate
  - Succinyl-CoA  $\rightarrow$  succinate + CoA
8. D Which of the following best describes the net organic products formed during the oxidation of one molecule of glucose to two molecules of pyruvate via glycolysis?
- 1 ATP + 1 NADH
  - 2 ATP + 0 NADH
  - 2 ADP + 2 NAD<sup>+</sup>
  - 2 ATP + 2 NADH
  - 4 ATP + 2 NADH
9. D Why can't cells use the breakdown of glucose to pyruvate to generate energy without further conversion of pyruvate via fermentation or respiration?
- The rate of energy production by glycolysis alone is too slow for cells' biochemical reactions.
  - The amount of energy produced by glycolysis alone is insufficient to fuel cell growth.
  - The amount of inorganic phosphate available becomes limiting.
  - The electron acceptor reduced during glycolysis must be regenerated.
  - The complete breakdown of pyruvate is necessary to produce the carbon dioxide needed by cells.
10. C Which of the following is not an intermediate in the glycolytic pathway by which glucose is oxidized to pyruvate?
- 3-phosphoglycerate
  - Fructose 1,6-bisphosphate
  - Glucose 1-phosphate
  - Glyceraldehyde 3-phosphate
  - Phosphoenolpyruvate
11. D Which of the following best approximates the net yield of ATP molecules produced during cellular respiration, per molecule of glucose completely oxidized to carbon dioxide and water?
- 2
  - 6
  - 10
  - 30
  - 80
12. E Dihydroxyacetone-phosphate is
- isomerized to glyceraldehyde-3-phosphate in the cytosol
  - produced by glycerol-3-dehydrogenase in the mitochondria
  - produced by aldolase in the cytosol
  - a 3-carbon ketone
  - all of the above
13. E When intracellular ATP concentrations increase
- phosphofructose-kinase-1 activity decreases
  - pyruvate kinase activity decreases
  - pyruvate dehydrogenase activity decreases ✓
  - citrate synthase activity decreases
  - all of the above

any  
15  
ok

14. C. Electrons from glyceraldehyde-3-phosphate enter the outer mitochondrial membrane as
- dihydroxyacetone-phosphate ~~x~~
  - aspartate ~~x~~
  - malate ✓
  - carbamoyl-phosphate ~~x~~
  - all of the above
15. A. The inner mitochondrial membrane contains the following integral membrane protein(s)
- succinate dehydrogenase (Complex II)
  - cytochrome c
  - plastoquinone
  - plastocyanin
  - all of the above
16. C (D). In aerobic conditions, a pH gradient in the mitochondria can be observed between
- the extracellular space and the cytosol
  - the cytosol and the intermembrane space
  - the intermembrane space and the matrix
  - the cytosol and the matrix
  - none of the above
17. E. The standard reduction potential of NADH is less than
- the iron-sulfur center of Complex III
  - cytochrome c
  - the copper centers of Complex IV
  - ubiquinone (Q)
  - all of the above
18. A. Cyanide inhibits the electron transport chain in mitochondria and decreases the production of ATP by
- ATP synthase
  - pyruvate kinase
  - glyceraldehyde-3-phosphate dehydrogenase
  - photophosphorylation
  - glycolysis
19. C. One of the three carbons of pyruvate is oxidatively decarboxylated by
- pyruvate carboxylase
  - pyruvate kinase
  - pyruvate dehydrogenase
  - citrate synthase
  - all of the above
20. E. Pyruvate dehydrogenase complex requires the following vitamins
- pantothenic acid
  - lipoic acid
  - niacin
  - riboflavin
  - all of the above