Name: ___**Key**_____ Key_____

Chem 4512/6502 Exam 2 March 17, 2016
by Dr. A. Oyelere

Please put your name at the top of every page.

There are **6 multi-part questions** to this exam.
Please look over the whole exam before you start answering questions. The maximum points for each question are indicated. There are 100 points possible. Your whole answer **must** be written in the spaces provided. All programmable calculators must be **cleared** of any memory of previous work related to this class.

Potentially helpful information is provided

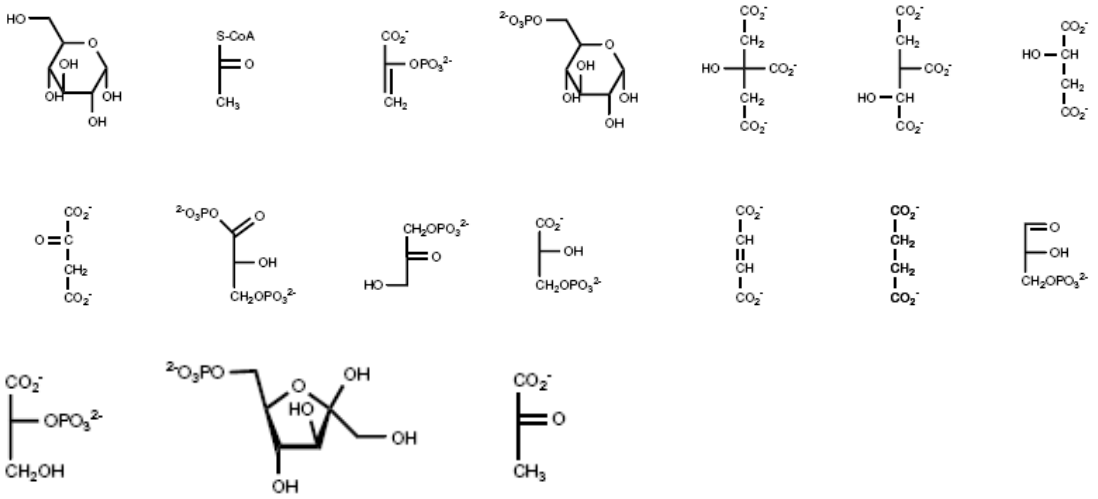
You are expected to strictly adhere to Georgia Institute of Technology Honor Code.

Georgia Institute of Technology Honor Code.

I have neither given nor received help on this work.

Signature **date**

Potentially helpful information:



Energy Charge (EC):	Change in Free Energy:
$\frac{[ATP]+0.5[ADP]}{[ATP]+[ADP]+[AMP]}$	$\Delta G' = \Delta G^{o\prime} + RT \ln \frac{[product]_{actual}}{[substrate]_{actual}}$ *At equilibrium (when $\Delta G' = 0$):* $\Delta G^{o\prime} = -RT \ln \frac{[product]_{equilibrium}}{[substrate]_{equilibrium}}$

USEFUL CONSTANTS:
R (gas constant) **= 8.315 J•mol^{-1}•Kelvin^{-1} = 8.315 x 10^{-3} kJ•mol^{-1}•Kelvin^{-1}**
F (Faraday Constant) **= 96.48 kJ•V^{-1}•mol^{-1}**

If temperature = 25 °C, absolute temperature T = 298 K (Assume this temperature unless problem states otherwise.)

Section 1: Answer <u>all</u> questions in this section. Each question is worth 2 points.

I. Below are the standard reduction potentials (E'°) for two conjugate redox pairs:
Pyruvate-/lactate- E'° = –0.185v NAD+/NADH E'° = –0.320v
Which of the following is true?

A. The pyruvate/lactate conjugate redox pair has a greater tendency to lose electrons than the NAD+/NADH redox pair.
B. Pyruvate has a greater affinity for electrons than NAD+.
C. NAD+ is a reducing agent.
D. Under standard conditions, NAD+ is more likely to be converted to NADH, than pyruvate is to be converted to lactate.
E. none of the above.

II. The chemical energy generated by mitochondrial electron transport results from which of the following?

A. Excess H^+ in the matrix
B. A H^+ gradient across the inner membrane
C. The formation of thioesters in the matrix
D. A conformational change in the inner membrane

III. In the reoxidation of QH_2 by purified ubiquinone-cytochrome *c* reductase (Complex III) from heart muscle, the overall stoichiometry of the reaction requires 2 mol of cytochrome *c* per mole of QH_2 because:

A. cytochrome *c* is a one-electron acceptor, whereas QH_2 is a two-electron donor.
B. cytochrome *c* is a two-electron acceptor, whereas QH_2 is a one-electron donor.
C. cytochrome c is water soluble and operates between the inner and outer mitochondrial membranes
D. heart muscle has a high rate of oxidative metabolism, and therefore requires twice as much cytochrome *c* as QH_2 for electron transfer to proceed normally.
E. two molecules of cytochrome *c* must first combine physically before they are catalytically active.

IV. Which of the following metal ions is not likely to directly participate in a redox reaction at the catalytic center of a redox enzyme?

A. Fe^{2+}
B. Cu^{2+}
C. Ca^{2+}
D. Mn^{2+}
E. Fe^{3+}

V. When undergoing strenuous anaerobic exercise the Cori Cycle is functioning. Which of the following compounds is being delivered to muscle tissue from the liver as part of this cycle?

A. Glucose
B. Lactate
C. Galactose
D. Pyruvate
E. Alanine

VI. At the end of glycolysis, each molecule of glucose has yielded 2 molecules of _____, 2 molecules of _____, and a net of 2 molecules of _____.

A. FAD; NAD^+; ADP
B. CO_2; NAD^+; ADP
C. Lactic acid; ethanol; CO_2
D. Pyruvate, NADH, ATP
E. H_2O; CO_2; ATP

VII. The steps of glycolysis between glyceraldehyde 3-phosphate and 3 phosphoglycerate involve all of the following except:

A. ATP synthesis.
B. Catalysis by phosphoglycerate kinase.
C. Oxidation of NADH to NAD^+.
D. The formation of 1,3-bisphosphoglycerate.
E. Utilization of Pi.

VIII. In the absence of oxygen, the primary purpose of fermentation is to:

A. Produce amino acids for protein synthesis
B. Generate a proton gradient for ATP synthesis
C. Oxidize glucose to generate reduce electron carriers
D. Generate alcohol for beverages
E. Regenerate NAD+ from NADH allowing glycolysis to continue

IX. Which of the following compounds is an important intermediate that is found in gluconeogenesis is but not formed in glycolysis?

A. Phosphoenolpyruvate
B. Glyceraldehyde-3-phosphate
C. Oxaloacetate
D. NADH

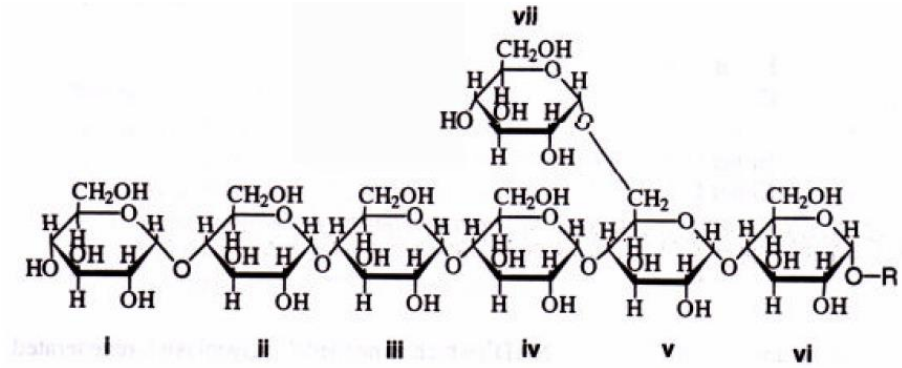
X. UDP-glucose pyrophosphorylase catalyzed reaction: G1P → UDP-Glucose ($\Delta G0' \approx 0$). The reaction is made energetically feasible by

A. The action of a pyrophosphatase that hydrolyzes pyrophosphate to release energy
B. The hydrolysis of UTP
C. ATP hydrolysis
D. The action of glycogenin

XI. A novel chemotherapeutic agent for treating cancer is found to have increased phosphoenolpyruvate (PEP) levels and decreased pyruvate levels as a side effect. Which enzyme might this chemotherapeutic agent be inadvertently targeting?

A. hexokinase
B. pyruvate dehydrogenase
C. glucose-6-phosphatase
D. Phosphoenolpyruvate Carboxykinase
E. isocitrate dehydrogenase

XII. The figure below is the structure of a partially degraded glycogen. Which of the following statements about the figure is incorrect?



A. Glycogen phosphorylase catalyzes the hydrolytic cleavage of residue vii.
B. The product of the cleavage reaction catalyzed by Glycogen phosphorylase is glucose 1 phosphate
C. In glycogen synthesis, the incoming glucose residue can be added at i or vii
D. Glycogen debranching enzyme catalyses the cleavage of α1,6 and α1,4 glycosidic bonds
E. All of the above are correct

XIII. An enzyme involved in the glycogen breakdown to glucose will be allosterically _____ in presence of excess ATP?

A. Upregulated
B. Downregulated
C. Will have no effect
D. ATP is not an allosteric regulator

XIV. Glucose is stored as the polymer glycogen:

A. So that a large amount of glucose can be stored inside cells without causing osmotic problems.
B. Because glycogen synthesis does not cost energy.
C. Because glycogen is stored extracellularly.
D. All of the above.
E. None of the above.

XV. If you injected glucagon into the blood of someone, you would expect to see:

A. a decrease in liver fructose-2,6-bisphosphate concentration.
B. a decrease in the rate of glycolysis in liver.
C. increased release of glucose to the blood from the liver.
D. all of the above.
E. None of the above

XVI. Glycogen is converted to monosaccharide units by:

A. Glucokinase.
B. Glucose-6-phosphatase
C. Glycogen phosphorylase.
D. Glycogen synthase.
E. Glycogenase.

XVII. Which is a reactant in the Krebs Cycle?

A. CO_2
B. acetyl-CoA
C. NADPH
D. $FADH_2$

XVIII. Which of the following is NOT true?

A. Glucagon causes the breakdown of glycogen in liver to yield glucose to the blood.
B. Insulin inhibits glycogenolysis in the liver.
C. Epinephrine causes the glycogen in liver to be degraded to yield glucose to the blood.
D. Insulin increases the transport of glucose into muscle cells.
E. Epinephrine inhibits glycolysis of glucose in muscle cells.

XIX. Malonate is a competitive inhibitor of succinate dehydrogenase. If malonate is added to a mitochondrial preparation, which of the following compounds would you expect to decrease in concentration?

A. Citrate
B. Fumarate
C. Isocitrate
D. Pyruvate
E. Succinate

XX. The conversion of 1 mole of **pyruvate** to 3 moles of CO_2 via the pyruvate dehydrogenase and the TCA cycle also yields ____ moles of NADH, ____ moles of $FADH_2$, and ____ moles of ATP (or GTP).

A. 3; 2; 0
B. 3; 1; 1
C. 2; 2; 2
D. 4; 2; 1
E. 4; 1; 1

Section 2: Answer <u>all</u> questions in this section.

2a.

Some standard E'° values in volts for half cells are:

NAD^+/NADH	-0.32 v	Fumarate/Succinate	0.03 v
$FAD/FADH_2$	-0.22 v	$Ferredoxin^{+3}/Ferredoxin^{+2}$	-0.43 v
Cytochrome-a	0.29 v	Oxaloacetate/Malate	-0.17 v
Cytochrome-c	0.25	½ O_2/water	0.82 v

(i). (1 pt) From the information above, which molecule can reduce NAD^+?

Ans: ferredoxin^{2+} (acceptable – writing the pair out)

(ii). (1 pt) From the information above, could succinate reduce cytochrome-c?

Ans: Yes

(iii). (2 pts) From the information above calculate the $\Delta G'^{o}$ for the malate dehydrogenase reaction at pH 7.0 when F= 96.5 kJ/V.mole at 25°C:

malate + NAD+ =====> oxaloacetate + NADH + H+

Ans: $\Delta G = -nEF = -2 \times 96.5 \times (-0.15) = 28.95$ kJ/mole.

(iv). (1 pt) Will the malate dehydrogenase reaction go as written above (in 2aii)?

Ans: No

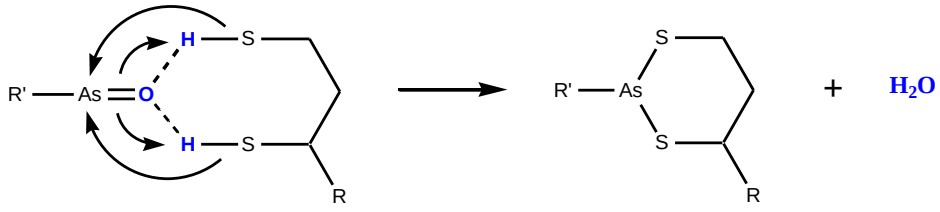
2b. (3 pts) Explain why the oxidation of a cytosolic NADH molecule which enters the respiratory chain via the Glycerol phosphate shuttle produces only 2 ATP compared to 3 ATP for the oxidation of each NADH molecule the enters the chain through the normal channel.

Ans: The NADH indirectly enters the Glycerol phosphate shuttle by reducing dihydroxyacetone phosphate to glycerol phosphate which then diffuses to a different glycerol phosphate dehydrogenase which is in the outer face of the inner mitochondrial membrane and contains an FAD prosthetic group. The mitochondrial glycerol P dehydrogenase oxidizes glycerol P back to dihydroxyacetone phosphate with the formation of FADH2, and then passes the electron pair from FADH2 to UQ in the inner membrane. *Since Complex I is bypassed, only about 2 ATPs are made per cytosolic NADH.*

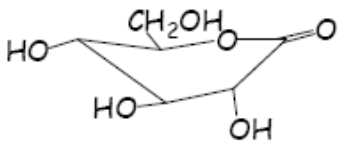
2c. (2 pts) A mutation in the a subunit of the E.coli FoF1 ATPase from Leu to Arg at position 156 may allow H^+ translocation through the membrane but not the rotation of the rings of the c subunits. How might this change affect the function of the FoF1 ATPase?

Ans: Uncoupling of the H+ translocation from ATP synthesis.

3a. (3 pts) Draw the chemical reaction that accounts for organic arsenicals (R-As=O) toxicity.

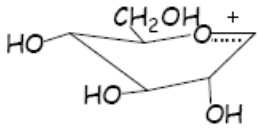


3b. (3 pts) Glycogen phosphorylase, a crucial enzyme in glycogen catabolism, is inhibited by 1,5-glucolactone (below). Justify this observation based on your understanding of the mechanism of action of glycogen phosphorylase **[appropriate structure(s) is necessary for full points]**.



1,5-Gluconolactone
(inhibitor)

1,5-Glucolactone inhibits glycogen phosphorylase reaction because it is a transition state analog of the oxonium ion intermediate developed during the cause of the reaction.



Half-chair oxonium ion
(proposed intermediate)

3c.(4 pts) Pyruvate is a key intermediate between different metabolic pathways. Give the four metabolic fates of pyruvate.

Ans: (i) Homolactic fermentation, (ii) Alcoholic fermentation, (iii) Acetyl-CoA synthesis (ultimately for TCA) and, (iv) Oxaloacetate synthesis (ultimately for gluconeogenesis).

Section 3: Questions 4 through 6. Answer <u>any two</u> questions in this section. Each question is worth 20 points

4 (20 pts).

4a

(i) (2 pts) The process of glycogen breakdown generates two products – one major and the other minor. Identify these products

Minor: **<u>Glucose</u>** Major: **<u>Glucose-1-phosphate</u>**

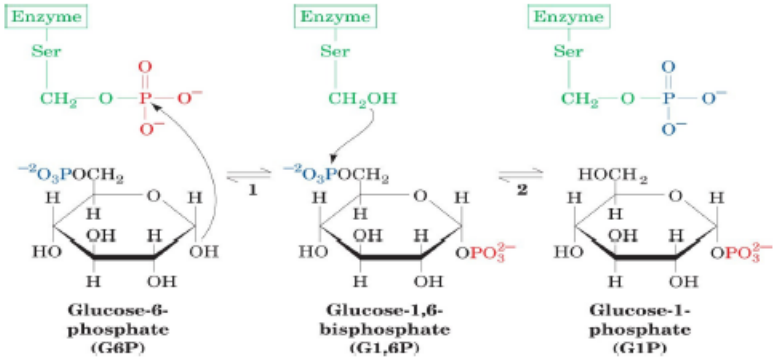
(ii) (1 pt) Glucose-1-phosphate (G1P) is the breakdown product of glycogen. But G1P is not an intermediate of glycolysis. What enzyme converts G1P to an intermediate of glycolysis?

Ans: Phosphoglucomutase

(iii) (2 pts) Provide plausible mechanism of the conversion of G1P to glycolysis intermediate by the enzyme identified above

- *Phosphoglucomutase*

glucose-1-phosphate ↔ glucose-6-phosphate



4b

(i) (2 pts) The citric acid cycle is frequently described as the major pathway of aerobic catabolism, which means that it is an oxygen-dependent degradative process. However, none of the reactions of the cycle directly involves oxygen as a reactant. Why is the pathway oxygen-dependent?

Ans: The citric acid cycle produces NADH and $FADH_2$. If these are not reoxidized via the electron transport chain, the citric acid cycle will come to a stop, by feedback inhibition (Alternatively – NAD+ and FAD are required substrates for several reactions). Oxygen is consumed by the re-oxidation of NADH and $FADH_2$.

(ii) (1 pt) From which metabolites of the TCA cycle is the 1st CO_2 product generated from?

Ans: Oxaloacetate

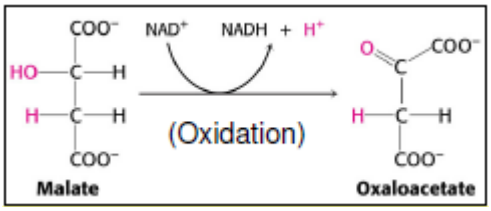
(iii) (1 pt) What enzyme of the TCA cycle catalyze the generation of the 1st CO_2 product?

Ans: Isocitrate dehydrogenase

4c

(i) (2 pts). Using chemical equation, give the last reaction of the TCA cycle.

Malate dehydrogenase catalyzed oxidation of malate to oxaloacetate



(ii) (2 pts). The reaction in 4c(i) is non-spontaneous with $\Delta G^{o}/ = + 29.7$ kJmol-1. Succinctly explain why the cycle does not grind to a halt at this step (use less than 100 words)

It is coupled to the 1st rxn of the TCA which is exergonic to give a combined transformation which is overall exergonic.

(iii) (2 pts). What enzyme catalyzes the only substrate level phosphorylation of the TCA cycle?

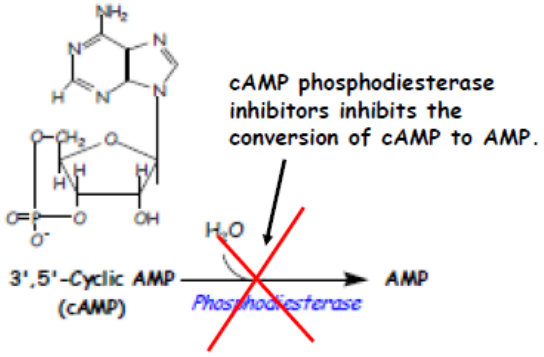
Ans: Succinyl-CoA synthase

(iv) (5 pts). What is the effect of the following conditions on the flux through the TCA cycle? [Increase or Decrease]

	Ans
(i) High [NADH]	**Decrease**
(ii) High [Succinyl-CoA]	**Decrease**
(iii) Low [ATP]	**Increase**
(iv) High [Ca^{2+}]	**Increase**
(v) High [ADP]	**Increase**

5 (20 pts)

a (5 pts). Cyclic AMP (cAMP), a second messenger, regulates the activity of protein kinase A which in turn reciprocally regulates glycolysis and gluconeogenesis through a cascade of phosphorylation events. The cellular levels of cAMP are tightly regulated by the actions of two different enzymes – adenylate cyclase and cAMP phosphodiesterase. What will be the effect of cAMP phosphodiesterase inhibitor on cAMP concentration? Justify your answer with appropriate reactions



Ans: cAMP phosphodiesterase hydrolyzes cAMP. Inhibition of cAMP phosphodiesterase will result in increase in intracellular concentration of cAMP

b (2 pts). Circle the most correct choice between each pair of underlined words in the sentence below:

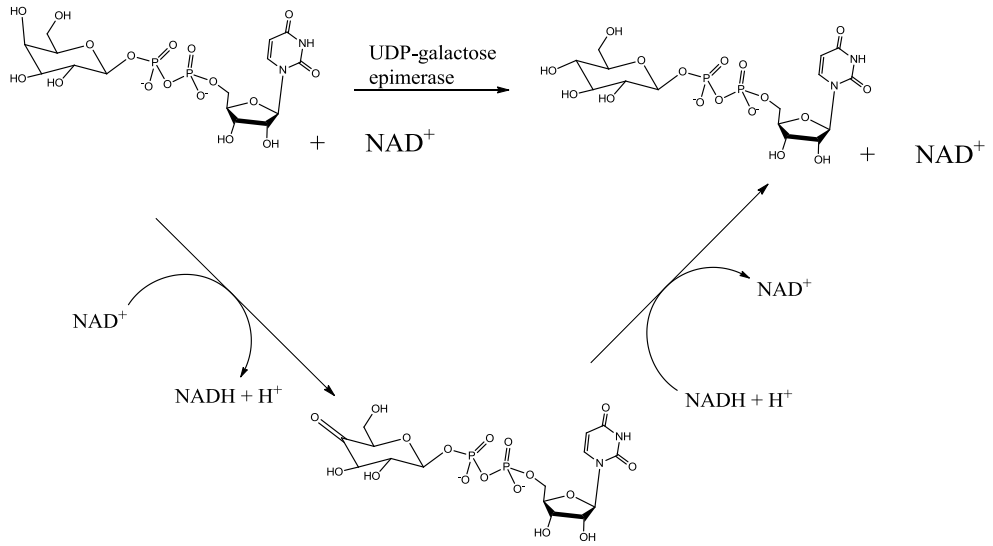
Insulin / **glucagon** stimulates an intracellular signaling pathway that results in the dephosphorylation of F-2,6-BP leading to increased flux through the glycolytic / **gluconeogenic** pathway.

c (2 pts). What is the effect of phosphorylation on the activities of the following enzymes? **(Looking for – Up-regulation or Down-regulation)**

Glycogen Phosphorylase – up regulation

Glycogen Synthase – down regulation

d (5 pts) UDP-Galactose-4-epimerase plays a key role in the entrance of galactose into the glycolytic pathway. Give the reaction catalyzed by UDP-Galactose-4-epimerase and propose a plausible mechanism for this reaction (Useful Hint: UDP-Galactose-4-epimerase is a NAD+ dependent enzyme)



(e) . The reaction of F-6-P + ATP $\rightarrow$ F-1,6 -BP + ADP has an Keq=247 at 37C.

i (2 pts). What is the standard free energy change (ΔG^o) for the reaction at 37C?

Ans:
ΔG^o = -RTlnK
$\Delta G° = -(310K)\ (8.315 \times 10^{-3}\ kJ\ mol^{-1}\ K^{-1})\ \ln 247 = -14.2\ kJ\ mol^{-1}$

ii (2 pts). Is the reaction endergonic or exergonic at cellular conditions?

Ans: Exergonic

iii (2 pts). If in the cell the ratio of [ADP]/[ATP] = 0.075 and the free energy (ΔG) is -21.6 kJ/mol. What is the ratio of [F-1,6-BP]/[F-6-P]?

Ans:
$\Delta G = \Delta G^{o} + RT\ln K$
$e^{((\Delta G - \Delta G°) / RT)} = ([ADP]/[ATP]) ([F\text{-}1,6\text{-}BP]/ [F\text{-}6\text{-}P])$
$e^{((-21.6 + 14.2) / (310\ K \times 8.315 \times 10^{-3}\ kJ\ mol^{-1} \bullet K^{-1}))} = 0.075 (([F\text{-}1,6\text{-}BP]/ [F\text{-}6\text{-}P])$
$0.0567/ 0.075 = .755$

6 (20 pts)

a. (2 pts) Fe^{++}/Fe^{+++} plays major role in the transfer electron s from one molecule to other during mitochondrial ETC and many complexes and proteins have either Fe-S centers or heme rings, but Fe^{++} in each protein has different reduction potential. Why?

Ans: Because of different electronic environments by surrounding amino acids around the F^{++} atom.

b. (2 pts) Explain the observation that rotenone, an inhibitor of the electron transport system, is able to block substrate level phosphorylation by the citrate cycle (TCA cycle) enzyme succinyl-CoA synthetase when added to a cell-free suspension of intact mitochondria.

Ans: Inhibition of the electron transport system by rotenone prevents NADH from being oxidized, leading to a decrease in mitochondrial NAD+, which is required for 3 of the citrate cycle reactions. When the citrate cycle is shut down because of lack of oxidized NAD+ for those 3 reactions, succinyl-CoA is not made and the substrate level phosphorylation reaction does not occur.

[Partial credit for inhibition of flow of electron thru complex I]

c. (3 pts) Explain why adding cyanide to a suspension of mitochondria blocks ATP synthesis.

Ans: Cyanide blocks electron transfer in the ETS at complex IV, resulting in the loss of the proton motive force (H^{+} gradient) that is required to drive ATP synthesis.

4d. One of the overall purposes of electron transport is the oxidation of NADH by O_2. The standard reduction potentials for the two half reactions are:

$NAD^{+} + H^{+} + 2e^{-} \rightarrow NADH\ \Delta E°' = -0.315\ V$

$\frac{1}{2} O_2 + 2 H^+ + 2e^- \rightarrow H_2O \quad \Delta E^{\circ\prime} = 0.815$ V

Helpful Hints:

1 Faraday = 96.5 kJ/V.mole

ATP $\rightleftharpoons$ ADP + Pi $\quad \Delta G^{o'} = -30.5$ kJ/mol

(i) (2 pts). What is the standard reduction potential for this reaction?

Ans:

$\Delta\varepsilon^{0'} = \varepsilon^{0'}$(reduction) - $\varepsilon^{0'}$(oxidation)

0.815V- (-0.315 V) = 1.130 V

(ii) (2 pts). What is the standard free energy change for this reaction?

Ans:

$\Delta G^{0'} = -n\Im\Delta\varepsilon^{0'}$

$\Delta G^{0'} = -(2)(96.5$ kJ/Vmol$)(1.13$ V$) = -218$ kJ/mol

(iii) (2 pts). Assuming standard condition and 100% conservation of energy, what is the maximum number of ATPs that can be synthesized?

Ans:

Recall,

ATP $\rightleftharpoons$ ADP + Pi $\quad \Delta G^{o'} = -30.5$ kJ/mol

The maximum number of ATP that could be synthesized under standard conditions is:

218 kJmol^{-1}/30.5 kJmol^{-1} = 7.15 mol of ATP/mol of $NADH_2$ oxidized by O_2.

e (4 pts). A male patient with acute hemolytic anemia was diagnosed with G6PD deficiency. Explain what biochemical products in the pentose phosphate pathway will be compromised in this patient. Identify whether these compounds are produced in the oxidative or non-oxidative stages of the pathway.

Ans:

Primary products of the PPP pathway are:

Ribose 5-phosphate (R5P) for synthesis of nucleotides and nucleic acids **(non-oxidative stage) (Also acceptable are Ribulose 5-phosphate and xylulose 5-phosphate)**

NADPH for reductive biosynthesis reactions within cells **(oxidative stage)**

f (3 pts). Why will a diet of fava beans exacerbate the condition of the patient in **6e** above?

Ans: Fava beans contain toxic compounds that must be reduced by glutathione in red blood cells to prevent cell damage. Without sufficient G6PD activity, NADPH levels produced by the pentose phosphate pathway are too low to keep glutathione in the reduced state.