THESE ANSWERS TO DR. MUSHYNSKI'S REVIEW QUESTIONS ARE OF UNKNOWN SOURCE ! We cannot assure you that all answers are correct

1. The DG°' for the malate hydrogenase reaction is +29.7 kJ/mol. What does this tell you about the concentration of oxaloacetate in mitochondria? Why does citrate synthase manage to produce sufficient amounts of citrate under these conditions?

The $\Delta G^{o'}$ indicates that the concentration of oxaloacetate is very low. Citrate synthase manages to produce sufficient amounts of citrate because the $\Delta G^{o'}$ has of –31.5 kJ/mol. This is enough to drive the production of citrate even at low concentrations of oxaloacetate.

2. Explain why the two carboxymethyl groups in citrate behave as though they are not equivalent.

They act as though they are not equivalent because two chemically identical substituents to an other wise chiral tetrahedral center are geometrically distinct; that is, the center has no rotational symmetry so that it can be unambiguously assigned left and right sides. (Voet & Voet p.66)

3. List the three major functions of the citric acid cycle.

1- Pathways utilize CAC intermediates
   a) Glucose biosynthesis utilizes oxaloacetate (transported across the mitochondrial membrane through a temporary conversion to malate).
   b) Lipid biosynthesis requires acetyl-CoA (transported across the mitochondrial memebrane as citrate).
   c) Amino acid biosynthesis utilizes the intermediates α-Ketoglutarate to synthesize glutamate and oxaloacetate to synthesize aspartate.

2- Its reduced products, NADH and $FADH_2$, are reoxidized by the electron-transport chain during oxidative phosphorylation and the free energy released is coupled to the biosynthesis of ATP.

3- It can act as a reservoir since it can ship out its intermediates to make amino acids or, it can take up intermediates that are in excess in the cytosol to burn them to produce energy. (NTC set 6, Oct 13, p. 1 or 4)

4. Mitochondria are incubated in excess phosphate buffer in an oxygen electrode along with 20 mmoles of ADP, rotenone and substrate X. Oxygen consumption levels off after 5 mmoles of oxygen are consumed. What is the P/O ratio and what is the probable identity of X?

P/O = (20 μmoles of ADP phosphorylated) / (2 x 5 μmoles of O_2) = 2

Substrate X is most likely $FADH_2$

5. In the reaction catalyzed by succinlyl CoA synthetase, the free energy transfer from succinyl CoA to GTP is mediated by two intermiediates. Do these intermediates share any properties with the precursor and end product of the reaction?

In the intermediates, succinyl phosphate and 3-phospho-His residue of the enzyme, the high energy succinyl-CoA's free energy of hydrolysis are conserved in the successive formation of high energy compounds with the final product being GTP. The process is reminiscent of passing a hot potato. (Voet & Voet p.553)

6. Name the location of succinate dehydrogenase in mitochondria and provide an explanation for its location.

Succinate dehydrogenase contains an FAD (covalently bound), the reaction's electron receptor. The oxidation of succinate to fumarate is sufficiently exogernic to reduce FAD to $FADH_2$. Since the $FADH_2$ is permanently linked to the enzyme, this prosthetic group cannot function as a metabolite as does NADH. Rather, succinate dehydrogenase is reoxidized by the electron-transport chain. This rationalizes why succinate dehydrogenase, which is embedded in the inner mitochondrial membrane, is the only membrane-bound citric acid cycle enzyme. (Voet & Voet p. 554)

7. Name a conpetitive inhibitor of succinate dehydrogenase and explain how this inhibitor was used to elucidate the cyclical nature of the CAC.

Malonate is potent inhibitor of succinate dehydrogenase. Succinate is formed from fumarate, malate, or oxaloacetate in the presence of the metabolic inhibitor malonate. Since malonate inhibits the direct reduction of fumarate to succinate, the succinate must be formed by an oxidative cycle. (Voet & Voet p. 541)

8. Name the three CAC enzymes that catalyze rate-determining steps and list the types of mechanisms involved in their regulation. How do increases in the NADH/NAD^+ ratio affect the activities of these enzymes?

Citrate synthase, α-Ketoglutarate dehydrogenase, and isocitriate dehydrogenase are the three CAC enzymes that catalyze rate-determining steps. These enzymes are controlled by: (1) substrate availability, (2) product inhibition, and (3) competitive feedback.

Citrate synthase: citrate is a competitive inhibitor of oxaloacetate for citrate sythase (product inhibition); the fall in [citrate] caused by increased isocitrate dehydrogenase activity (i.e. larger [NADH] /[NAD^+]) increases the rate of citrate formation.

α-Ketoglutarate dehydrogenase is also strongly inhibited by its products, NADH and succinyl-CoA. Its activity therefore increases when [NADH]/[NAD^+] decreases.

Isocitriate dehydrogenase is also inhibited by a rise in [NADH]/[NAD^+] the ratio. Succinyl-CoA also competes with acetyl-CoA in the citrate synthase reaction (competitive feedback inhibition).

(Voet & Voet p. 558)

9. Why is the measured voltage difference between two half cells directly related to the free energy of a redox reaction? What is the free energy change for a redox reaction in which the electron acceptor and electron donor have standard redox potentials of 0 and –0.30V, respectively?

Any redox reaction can be divided into its component half-reactions. In an electrochemical cell, each half reaction takes place in its separate half-cell. These half reactions can be assigned reduction potentials, $\mathscr{E}_A$ and $\mathscr{E}_B$, in accordance with the Nernst equation:

$$\mathscr{E}_A = \mathscr{E}_A^{o} - \frac{RT}{n\mathscr{F}} \ln\left(\frac{[A_{red}]}{[A_{ox}^{n+}]}\right)$$

$$\mathscr{E}_B = \mathscr{E}_B^{o} - \frac{RT}{n\mathscr{F}} \ln\left(\frac{[B_{red}]}{[B_{ox}^{n+}]}\right)$$

For the redox reaction of any two half reactions, the difference in reduction potentials is described as follows:

$$\Delta\mathscr{E}^{o} = \mathscr{E}^{o}_{(\text{e–acceptor})} - \mathscr{E}^{o}_{(\text{e-donor})}$$

Thus, when the reaction proceeds with A as the electron acceptor and B as the electron donor, $\Delta\mathscr{E}^{o} = \mathscr{E}_A^{o} - \mathscr{E}_B^{o}$ and similarly with $\ddot{A}\mathscr{E}$.

For a reaction in which the electron acceptor and electron donor have standard redox potentials of 0 and –0.30V, respectively, and supposing that the number of electrons transferred is 1, the free energy change can be found as follows:

$$\ddot{A}G^{o\prime} = -n\mathscr{F}\Delta\mathscr{E}^{o\prime}$$

$$\Delta G^{o\prime} = -1(96\,494\ \text{J V}^{-1}\text{mol}^{-1})(30\text{V})$$

$$\Delta G^{o\prime} = -2894\ \text{kJ mol}^{-1}$$

(Voet & Voet p. 435)

10. Which property of CoQ enables it to serve as a conduit for electron transport between NADH and cytb_L?

CoQ's hydrophobic tail makes it soluble in the inner mitochondrial membrane's lipid bilayer. (Voet & Voet p. 575)

11. Contrast the effects of rotenone, antimycin A and CN^- on electron transport.

Rotenone inhibits NAD^+-linked oxidation. Antimycin A inhibits FAD-linked oxidation. CN^- completely inhibits oxidation (i.e. after the third point of entry of electrons). (Voet & Voet p. 570)

12. How does cytochrome c differ from other proteins components of the electron transport chain?

Unlike other proteins of the electron transport chain, cytochrome c is a peripheral membrane protein that is loosely bound to the outer surface of the inner mitochondrial membrane. It alternately binds to cytochrome c_1 of Complex III and to cytochrome c oxidase (Complex IV) and thereby functions to shuttle electrons between them.

13. List the various types of evidence that support the chemiosmotic hypothesis.

1- Oxidative phosphorylation requires an intact inner mitochondrial membrane.

2- The inner mitochondrial membrane is impermeable to ions such as H^+, OH^-, K^+, and Cl^-, whose free diffusion would discharge an electrochemical gradient.

3- Compounds that increase the permeablility of the inner mitochondrial membrane to protons, and thereby dissipate the electrochemical gradient, allow electron transport (from NADH and succinate oxidation) to continue by inhibit ATP synthesis; that is, they "uncouple" electron transport from oxidative phosphorylation. Conversly, increasing the acidity outside the inner mitochondrial membrane stimulates ATP synthesis.

(Voet & Voet p. 583)

14. In a tightly coupled mitochondrial preparation, what effect would depletion of ADP have on electron transport? What type of compound might be added to allow electron transport to resume under these conditions?

The electrochemical gradient would build until the free energy required to transport H^+ balances the free energy of electron transport. Any compound that can dissipate the electrochemical gradient would allow electron transport to continue.

15. What condition must be satisfied for the redox loop mechanism to transport protons out of mitochondria? In realistic terms, which component(s) of the electron transport chain satisfies this requirement?

The redox loop mechanism requires that the first redox carrier contain more hydrogen atoms in its reduced state than in its oxidized state and that the second redox carrier have no difference in its hydrogen atom content between its reduced and oxidized states. FMN and CoQ, in fact, contain more hydrogen atoms in their reduced state than in their oxidized state and thus can qualify as proton carriers as well as electron carriers. If these centers were spatially alternated with pure electron carriers (cytochromes and iron-sulfur clusters), such a mechanism could well be accommodated. (Voet & Voet p. 584)

16. What is the role ofg-subunit in the proposed model for ATP synthesis by the proton translocation ATP synthase?

The conformational differences between F_1's three catalytic sites appear to be correlated with the position of the γ subunit. Apparently the γ subunit, which is thought to rotate within the $\alpha_3\beta_3$ assembly, acts as a molecular cam shaft in linking the proton gradient-driven rotational motor to the conformational changes in the catalytic sites of F_1. (Voet & Voet p. 590)

17. Outline the general process of amino aci d degradation and how it is regulated (i.e. transamination and glutamate deyhydrogenase). How is net flux in the direction of amino acid catabolism maintained even when the energy charge of the cell is high?

Most amino acids are deaminated by transaminat ion, the transfer of their amino group to an α-keto acid of the orignal amino acid and a new amino acid, in reactions catalyzed by transaminases. The predominant amino group acceptor is α-ketoglutarate, producing glutamate as the new amino acid:

$$\text{Amino acid} + \alpha\text{-ketoglutarate} \rightleftharpoons \alpha\text{-keto acid} + \text{glutamate}$$

Glutamate's amino group, in turn, is transferred to oxaloacetate in a second transamination reaction, yielding aspartate:

$$\text{Glutamate} + \text{oxaloacetate} \rightleftharpoons \alpha\text{-ketoglutarate} + \text{aspartate}$$

Transamination, of course, does not result in any net deamination. Deamination occurs largely through the oxidative deamination of glutamate by glutamate dehydrogenase (GDH), yielding ammonia. The reaction requires NAD^+ or $NADP^+$ as an oxidizing agent and regenerates α-ketoglutarate for use in additional transamination reactions:

$$\text{Glutamate} + NAD(P)^+ + H_2O \rightleftharpoons \alpha\text{-ketoglutarate} + NH_4^+ + NAD(P)H$$

(Voet & Voet p. 728)

Regulation: GDH is activated by GDP and ADP and inhibited by GTP and ATP.

18. List the enzymes, precursors and products of the three ammonia assimilation reactions.

1. Glutamine synthetase
   - Enzymes: glutamine synthetase
   - Precursors: glutamate, ATP, NH_3, and γ-glutamylphosphate (intermediate)
   - Products: glutamine, ADP and P_i

2. Glutamate dehydrogenase
   - Enzymes: glutamate dehydrogenase
   - Precursors: α-ketoglutarate, NH_4^+, NAD(P)H and α-iminoglutarate (intermediate)
   - Products: glutamate, $NAD(P)^+$ and H_2O

3. CPS1
   - Enzymes: carbomoyl phosphate synthtase 1
   - Precursors: 2ATP, HCO_3^-, NH_3
   - Products: carbamoyl phosphate, 2ADP and P_i

19. How is urea synthesis regulated?

Carbamonyl phosphate synthetase I, the mitochondrial enzyme that catalyzes the first committed step of the urea cycle, is allosterically acitivated by *N*-acetylglutamate. This metabolite is synthesized from glutamate and acetyl-CoA by *N*-acetylglutamate synthase and hydrolyzed by a specific hydolase. The rate of urea production by the liver is, in fact, correlated with the *N*-acetylglutamate concentration. Increased urea synthesis is required when amino acid breakdown rates increase, generating excess nitrogen that must be excreted. Increases in these breakdown rates are signaled by an increase in glutamate concentration through transamination reactions. This situation, in turn, causes an increase in *N*-acetylglutamate synthesis, stimulation carbamoyl phosphate synthetase and thus the entire urea cycle. The remaining enzymes of the urea cycle are controlled by the concentration of their substrates. (Voet & Voet p. 734)

20. Briefly describe alternative pathways for excreting ammonia in patients with a defect in urea synthesis.

1. Sodium Benzoate:
   Benzoate combines in an ATP dependant reaction with glycine to yield an activated CoA intermediate. This intermediate reacts with a second glycine to form hippuric acid. This can then be efficiently excreted in the urine in the form of hippurate.

2. Phenyl Acetate
   Phenyl acetate reacts with glutamine, using CoA intermediate and using ATP, to make phenylacetylglutamine. This is then excreted in the urine.

(NTC set 8, Oct 30 p. 5)

21. List three features of the total body pool of free amino acids.

1. Pool is dynamic: constant flux of amino acids in and out of the pool.
   300g in by degradation of proteins,
   100g in from the diet,
   300g out by protein synthesis

2. All amino acids in the free pool are kinetically indistinguishable.

3. The size of the pool falls within narrow limits.

22. What effect might one expect on nitrogen balance if one of the essential amino acids is lacking in the diet?

If an essential amino acid is absent in the diet, the subject will go into negative nitrogen balance (NTC set 8, Oct 23, p.1)

23. Name the two key components of pyrodoxal phosphate that enable it to be such a versatile coenzyme in amino acid metabolism.

1- Examination of the amino acid-PLP Schiff base's structure reveals why this system is called "an electron-pusher's delight." Cleavage of any of the amino acid C_α atom's three bonds (labeled a, b, and c) produces a resonance-stabilized C_α carbanion whose electrons are delocalized all the way to the coenzyme's protonated pyridinum nitrogen atom; that is, PLP functions as an electron sink. (Voet & Voet p. 730)

2- For electrons to be withdrawn into the conjugated ring system of PLP, the π-orbital system of PLP must overlap with the bonding orbital containing the electron pair being delocalized. This is possible only if the bond being broken lies in the plane perpendicular to the plane of the PLP π-orbital system. Different bonds to C_α can be placed in this plane by rotation about the C_α—N bond. Evidently, each enzyme specifically cleaves its corresponding bond because the enzyme binds the amino acid-PLP Schiff base adduct with this bond in the plane perpendicular to that of the PLP ring. (Voet & Voet p. 738)

24. Know the mechanism for the transamination reaction.

See Voet & Voet p. 729

25. Using a diagram and descriptive terms, explain the term stereochemical control as it pertains to pyrodoxal phosphate-containing enzymes.

See question 23 (2) and figure 24-12 in Voet & Voet.

26. Contrast the mechanism for the normal decarboxylation of glutamate with that involving an unforced error. What effect does the latter have on glutamate decarboxylase?

In the normal sequence, there is a shift of electrons to give an external aldenine linkage and a proton is added. The net result is the replacement of the carboxyl group with a hydrogen. Hydrolysis of the molecule regenerates the PLP and produces GABA.

In the unforced error, an amino group is added to C_α instead of a hydrogen. After hydrolysis, an inactive aldehyde is produced and the PLP is not regenerated but rather PMP is produced.

PMP dissociates from glutamate decarboxylase rendering it inactive (glutamate decarboxylase is PLP dependent).

(NTC set 8, Oct 27, p. 2)

27. Why is there compartmentation of urea cycle reactions?

There is compartmentation because carbamoyl phosphate is the end product of 2 different reactions and used for two different purposes: in the mitochondrial matrix it is used for the urea cycle, in the cytosol it is used for pyrimadine synthesis. The compartmentation greatly increases the efficiency of the UC system.

28. Know the CPS1 reaction mechanism. What ensures that the reaction is irreversible?

See figure 24-5 in Voet & Voet.

The reaction is irreversible because 2 ATPs are used thereby making the reverse reaction nearly impossible.

29. Contrast the metabolic fates of glucogenic and ketogenic amino acids.

Glucogenic amino acids' carbon skeletons are degraded to pyruvate, α-ketoglutarate, succinyl CoA, fumarate, or oxaloacetate and are therefore glucose precursors.

Ketogenic amino acids' carbon skeletons are broken down to acetyl-CoA or acetoacetate and can thus be converted to fatty acids or ketone bodies.

30. An inherited defect in cystathionine-b-synthase gives rise to a condition known as homocystinuria. Why can certain patients harboring this defect be treated with high levels of vitamin B6?

To be determined…