

**1. A.** Name each enzyme present in the citric acid cycle and specify which of the following describes the reaction that is catalyzed when the cycle functions in the physiological direction:

carbon-carbon bond formation  
dehydration  
hydration  
decarboxylation  
oxidation–reduction  
substrate level phosphorylation  
isomerization

In each case show the reaction that is catalyzed and explain why you believe that it conforms to the type of reaction you propose. [Don't assume that all of the above classes of reaction are relevant. There may be more than one option for each enzyme.]

Look up all balanced eqn in the textbook or class notes.

1. Citrate synthase: C-C bond formation.
2. Aconitase-This is an isomerase rxn –but not an epimerization; more accurately it's a mutase rxn.
3. Isocitrate. D. decarboxylation & oxidation-reduction.
4.  $\alpha$ -Ketoglutarate. D. decarboxylation & oxidation-reduction. In one sense it is also a substrate level phosphorylation because the pdct is succinyl CoA which is the substrate for
5. Succinyl CoA transferase-makes GTP so is a substrate level phosphorylation.
6. Succinate D.-oxidation reduction.
7. Fumarase-hydration.
8. Malate D. oxidation-reduction.

**B.** Minced muscle that has been depleted of glycogen (and only glycogen) is incubated with pyruvate. When the incubation is conducted aerobically the pyruvate is quantitatively converted to  $\text{CO}_2$ . However when the reaction is conducted anaerobically there is a brief initial evolution of  $\text{CO}_2$  and then the  $\text{CO}_2$  evolution stops leaving most of the pyruvate unmetabolized. Why?

PDH makes acetyl CoA +  $\text{CO}_2$  + NADH. In the presence of air the NADH can be recycled via the ET chain; in the absence of air it can only be metabolized to the extent that there is NAD available.

**C.** The oxidation of pyruvate by the pyruvate dehydrogenase complex consists of 5 enzyme–catalyzed steps. Only the first three involve the carbon skeleton of pyruvate. Why are the last two necessary for the proper operation of this enzyme?

A product of the first three steps is reduced lipoic acid. It is converted back to oxidized lipoic acid by the 4'th rxn but in so doing makes  $\text{FADH}_2$ . The 5'th reaction serves to restore  $\text{FADH}_2$  to FAD so that the enzyme is ready for the next cycle.

**D** You are provided with pyruvic acid radiolabeled in the  $\alpha$ -carbonyl group. You incubate this pyruvate with a preparation of enzymes competent to carry out the citric acid cycle but which lacks dialyzable cofactors. When is radioactive carbon dioxide evolved?

- (i) Immediately
- (ii) After addition of CoA
- (iii) After addition of CoA +  $\text{NAD}^+$
- (iv) After addition of CoA +  $\text{NAD}^+$  + GDP + ADP
- (v) After addition of CoA +  $\text{NAD}^+$  + GDP + ADP +  $\alpha$ -ketoglutarate.

Explain your answer. The carbonyl C of pyruvate is incorporated into citrate as the COOH "at the top" and will be released as  $\text{CO}_2$  after the first revolution of the TCA. Both of these steps require the presence of oxalacetate which in turn requires that we have  $\alpha$ -ketoglutarate. Pyruvate is not a "sparker".

**E.** The intermediates of the citric acid cycle can be used for biosynthetic reactions and if not replenished the cycle would slowly run down. Several reactions exist to replenish these intermediates. One of them is the conversion of propionate to succinyl CoA. How is this conversion accomplished? Indicate all enzymes, intermediates and any cofactors.

The sequence is:

Propionate to propionyl CoA to D/S-methylmalonyl CoA (biotin) to L/R-methylmalonyl CoA to succinyl CoA (B12).

See class notes for names of enzymes.

**2 A.** What is the important difference between the 2-electron reaction carried out by  $\text{NAD}^+$  and that by FAD.

NAD is a substrate and serves to carry 2H between different enzymes; FAD is a prosthetic grp and serves to carry H from a donor to an acceptor in a reaction catalyzed by a single enzyme.

**B.** In "designing" a metabolic pathway you find it necessary to extend a carbon chain by 1 carbon atom. Would you select an enzyme which uses thiamin pyrophosphate as prosthetic group or one that uses biotin? Why?

TPP carries a 2-C fragment, biotin activates  $\text{CO}_2$ . I would use biotin.

**C.** In the same metabolic pathway you find it necessary to convert a carbonyl to a methylenic carbon? What sequence of reactions would you devise for this purpose? Are any prosthetic groups or any other cofactors needed for your sequence?

Focus on the two internal C atoms in the sequence represented by

Oxalacetate to malate (needs NADH) to fumarate to succinate (needs  $\text{FADH}_2$ ).

**D.** In a different pathway it is necessary to convert an  $\alpha$ -amino acid (e.g. alanine) to a  $\beta$ -amino acid (e.g.  $\beta$ -alanine). What prosthetic group would you call upon to achieve this conversion? Explain.

This interchanges substituents on 2 adjacent C atoms. This is typically catalyzed by a B12 requiring enzyme.

**3. A.** You have a colleague who has just purified a lipid from the unusual organism E. Gillis. As a favor to your colleague you agree to analyze it. The analysis reveals 1 glycerol, 1 choline, 1 phosphate and 2 myristic acid (14:0). You return to your colleague with the question " Did you isolate this organism from Old Faithful". What made you ask that question? (If you do not know what Old Faithful is ask a TA).

A PL containing saturated fatty acids would be a solid at room temperature. This would be fatal for the organism which must therefore live at a much higher temperature such as a thermal geyser such as Old Faithful.

**B.** If you were to replace either (i) the natural lipid of the cell membrane of the liver or (ii) the natural lipid of the post-synaptic membrane, by the lipid described above would the functions of the adrenaline receptor and the acetyl choline receptor be

- (a) Equally affected.
- (b) The adrenaline receptor be more affected
- (c) The acetyl choline receptor be more affected.

Explain your choice!

(b). The acetyl choline receptor does not itself depend upon membrane fluidity (tho the release of acetyl CoA from the presynaptic membrane does because of the exocytosis of the vesicles containing acetyl CoA). The adrenaline receptor needs to move to find the G-protein. If the student says (a) and gives the qualifier above that's OK.

**C.** Given that the diffusion constant of a membrane phospholipid is 100x larger than a typical intrinsic protein and that it takes a phospholipid about 1 second to have a net movement of 1  $\mu$ meter, how long would it take the protein to travel the same distance:

- (a) 0.01 sec.
- (b) 0.1 sec.
- (c) 1 sec.
- (d) 10 sec.
- (e) 100 sec.

Justify your choice.

(e).  $s_2 = 4Dt$ .  $S_2$  is the same so  $Dt$  must be the same

**D.** There is an emergency at Methodist hospital and some Type O blood is needed in a hurry! The attending physician rushes to the refrigerator and finds that only Type AB blood is available.

Fortunately the physician took Bios 302, so he dashes to his lab for an enzyme which he then incubates with the Type AB blood. What activity do you think this enzyme has? Why

A glycosidase that can hydrolyze a sugar<sub>1</sub> to sugar<sub>2</sub> 1 $\alpha$   $\rightarrow$  3 glycosidic bond. It can be non-specific to either sugar 1 or sugar 2 or it can be absolutely specific to sugar 2 and accept either Gal or N-acetyl Gal for sugar 1.

**4 A.** The Red Blood Cell (RBC) imports glucose for glycolysis. How do we know that this occurs by facilitated diffusion?

1. It exhibits a plot of Flux vs external glucose that is hyperbolic (hence a carrier) **and** 2. There is no requirement for metabolic energy.

**B.** How would you modify the RBC so that it could accumulate glucose (without modification) to a concentration much larger than that present in the medium? Explain your answer!

I would incorporate a V-type ATPase in the membrane so that ATP hydrolysis generates a proton gradient (acid inside) and modify the existing glucose transporter so that it performs glucose-proton antiport. Other logical answers are acceptable.

**C.** Describe the proton motive force in one-or-two sentences. Present the simple equation that Mitchell employed in defining it explaining the meaning of each term.

The proton motive force is an electrochemical gradient across a membrane that is produced by generating a proton gradient across the membrane.

$$P = \quad -60 \text{ pH (millivolts).}$$

The first term is the combined contribution of the membrane potential created because the proton is a cation (the second term) and the osmotic contribution because the proton is a solute (the second term). (If the proton were uncharged only the second term would exist.)

**D.** How is the proton-motive force exploited in the active transport of:

- 1) chloride
- 2) malate
- 3) sucrose

1. Electrophoretic antiport into the domain of high proton conc.-
2. Electroneutral symport down the pH gradient.- pH
3. Electrogenic symport down the pH gradient.- P

Does the presence of dinitrophenol (DNP) have an affect on the transport of any of the above compounds? Why?

It aborts all transport because it abolished P.

**E.** When chloroplasts are incubated with tetra-phenyl boron ( $\text{TPP}^-$ , a lipophilic anion) it is found that this anion is accumulated within the chloroplast to a level 1000x that present in the external medium. What can you deduce from this observation? The inside of the chloroplast is positive with respect to the outside.

Is this experiment sufficient to quantify the proton-motive force? Why?

No-it only allows one to calculate .