Janani Sundaresan
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Question 1 (10 points)
What are the possible sources for acetyl-CoA that can feed into Krebs' cycle?

Fats, Carbohydrates (glucose), Proteins (amino acid).

Question 2 (10 points)
Suppose you found an overly high level of pyruvate in a patient's blood and urine.

a) One possible cause is a genetic defect in the enzyme pyruvate dehydrogenase. Describe the enzymes, cofactors, intermediates, and products of the pyruvate dehydrogenase complex.

PDC has 3 enzymes: E1=pyruvate dehydrogenase, E2=dihydrolipoamide acetyltransferase, E3= dihydrolipoamide dehydrogenase. All are involved in the conversion of pyruvate to acetyl-CoA. Pyruvate loses CO2 and transferred to TPP to make Hydroxyethyl TPP (HTPP). The hydroxyethyl group is oxidized to an acetyl group and transferred to lipoamide. The oxidizing agent is the oxidized form of lipoic acid which becomes reduced. Then the Acetyl group is transferred to CoASH, fully reducing lipoamide. Then the lipoamide is reoxidized (NAD+ is oxidant).

Enzymes: pyruvate dehydrogenase (E1), dihydrolipoyl transcetylase (E2), and dihydrolipoyl dehydrogenase (E3)
Cofactors: TPP, CoA, lipoic acid, FAD/FADH2, NAD+/NADH
Intermediates: HETPP, acetyl dihydrolipoamide
Products: acetyl-CoA, disulfide form of dihydrolipoamide

b) Another plausible cause for the high level of pyruvate in the patient's blood and urine is a specific vitamin deficiency. Which human disease is known for the deficiency of that vitamin in diet? Explain why the deficiency of that vitamin would account for high levels of pyruvate to be excreted in the urine.

The disease BeriBeri is known for Thiamine (Vitamin B1) deficiency. Thiamine is required for the synthesis of Thiamine Pyrophosphate (TPP) which is the prosthetic group of the enzyme pyruvate dehydrogenase. Pyruvate dehydrogenase helps covert pyruvate to Acetyl-CoA (catalyzes decarboxylations of alpha-ketos). Without Thiamine, there would be a lack of TPP, which would mean that pyruvate dehydrogenase could not function properly which would lead to an excess amount of pyruvate that would be excreted in the urine.

c) How would you determine which explanation, faulty/deficient enzyme or vitamin deficiency, is correct?

By trial and error. You would give the patient Vitamin B1 and see if they get better. If they do get better, then they had a vitamin deficiency. If they don't get better, then you know that you probably have a faulty enzyme.

Question 3 (10 points)
The citric acid cycle is described as the major pathway of aerobic catabolism, which 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?

The pathway is indirectly dependent on oxygen because the TCA cycle produces NADH that goes to the ETC to be reoxidized. The ETC is oxygen dependent and reduces 02----> H20.

Question 4 (10 points)
Explain in quantitative terms the circumstances under which the following reaction can proceed.

$$\text{L-Malate} + NAD^+ \rightarrow \text{oxaloacetate} + NADH + H^+ \qquad \Delta G'^{\circ} = +29.7 \text{ kJ/mol}$$

The given ΔG is positive which means the reaction won't go forward unless it's either 1. couple to a reaction with a big -ΔG or 2. the equilibrium is maintained.

1. By looking at the cycle, we can see that this reaction is not coupled to anything which leaves option 2

2. We can maintain the equilibrium by keeping the product, Oxaloacatate (OAA), concentration low because it will push the reactants and reaction forward. Ac-CoA will then convert the OAA to Citrate, so there should always be a low concentration of OAA.

Question 5 (10 points)
You are in charge of genetically engineering a new bacterium that will derive all of its ATP from sunlight by photosynthesis. Will you put the enzymes of the citric acid cycle in this organism? Briefly explain why or why not.

Yes I will because many of the intermediates on the TCA cycle are precursors for important biomolecules that are used in photosynthesis, so that will help the bacterium.

**Net Reduction Potential: ΔG=-nFΔE
where ΔE= Σred-Σoxd

Question 6 (10 points)
A recently discovered bacterium carries out ATP synthesis coupled to the flow of electrons through a chain of carriers to some electron acceptor. The components of its electron transfer chain differ from those found in mitochondria; they are listed below with their standard reduction potentials.

+=likely to be reduced
-=likely to be oxidized

Electron carriers in the newly discovered bacterium:

Oxidant	Reductant	Electrons transferred	E'° (V) reduction potential
NAD^+	NADH	2	–0.32
flavoprotein *b* (FP_b) (oxidized)	flavoprotein *b* (reduced)	2	–0.62
cytochrome *c* (Fe^{3+})	cytochrome *c* (Fe^{2+})	1	+0.22
Fe-S protein (oxidized)	Fe-S protein (reduced)	2	+0.89
flavoprotein *a* (FP_a) (oxidized)	flavoprotein *a* (reduced)	2	+0.77

a) Place the electron carriers in the order in which they are most likely to act in carrying electrons.

to have electron flow, you need +ΔG which means you need a -ΔE

Order (most likely--> least likely): Flavoprotein b, NAD+, Cytochrome C, Flavoprotein a, Fe-S

b) Is it likely that O_2 (for which E'° = 0.82 V) is the final electron acceptor in this organism? Why or why not?

No, the terminal acceptor would be Fe-S because it has a higher reduction potential. O2 would be the 2nd best final electron acceptor.
New Order:
Flavoprotein b, NAD+, Cytochrome C, Flavoprotein a, O2, Fe-S

c) How would you calculate the maximum number of ATP molecules that could theoretically be synthesized, under standard conditions, per pair of electrons transfered through this chain of carriers? (The Faraday constant, $\mathfrak{F}$, is 96.48 kJ/V·mol.) $\Delta G'^{\circ}$ for ATP synthesis is +30.5 kJ/mol.

**Net Reduction Potential: ΔG=-nFΔE
where ΔE= Σred-Σoxd [this is a state equation, so for ΔE, you just need to plug in the starting and ending points]
ΔE=.89-(-.62)=1.51V
F=96.48 kJ/Vmol
n=number electrons =2
ΔG= -(2)*(96.48)*(1.51)= -291 kJ/2e-

To find number of ATP molecules made, divide -291 by the ΔG of ATP synthesis to find number of ATP made
=> -291/30.5= 9.5= 9 mol ATP/2e- [you can't have half an ATP, so you must round down]

9 ATP molecules will be synthesized (theoretically).

concentration and electric gradient
are pushing H+ out of the intermem space to matrix which produces a big E field

Question 7 (10 points)
Mitochondria carrying out oxidative phosphorylation consume oxygen. **(10 points)**

a) What happens to oxygen in mitochondria?

Oxygen gets reduced to H20 and the transfer of electrons is coupled to pushing H+ into the inter-membrane space of the mitochondria which will later be used to form a gradient that will create ATP.

b) Describe the effect of an uncoupling agent such as 2,4-dinitrophenol on the rate of oxygen consumption. Assume there is a sufficient supply of oxidizable substrate, ADP, and P_i.

If there is an uncoupler, you will lose the gradient and the Hydrogens will no longer want to push out into the matrix. This will stop ATP production which the the body will try to fix by increasing the reduction of oxygen (increase oxygen consumption).

c) It was observed that if a mitochondrial ATP synthesis inhibitor "compound X" is added to the cell, the NAD^+/NADH ratio decreases. Would you expect compound X to be an uncoupling agent or an inhibitor of respiratory electron transfer? Explain briefly.

NADH is used in ETC and if the ratio decreases, then NADH is increasing, which means that something is not allowing the transport chain to work to the extent that it should (inhibiting).

efficient energy conversion
elec-->mech-->chem [transduction of energy]

Question 8 (10 points)
What are the three central elements of chemiosmotic theory for coupling oxidation to phosphorylation in mitochondria?

1. The first element is when O2-->H20 couples transfer of electrons to ETC to push Hydrogens into the inter-membrane space
2. The second element is the creation of an electric gradient (proton motive force) caused by the movement of hydrogens
3. The third element is the ATP synthase that converts ADP + pi = ATP

in summary:
1. make energy source [chemical]
2. transduce to mech energy
3. transduce that to chem energy

Question 9 (10 points)
Explain why anything that makes the mitochondrial membrane leaky stops ATP synthesis in the mitochondria.

Anything that makes the mitochondrial membrane would not allow the concentration gradient to exist so the cell won't be able to transduce energy and produce ATP. The gradient is the energy source for ATP production, so if you mess up the gradient, you mess up ATP production.

Question 10 (10 points)
Sudden addition of oxygen (O_2) to a previously anaerobic culture of yeast fermenting grape juice results in a dramatic decrease in the rate of glucose consumption. This is known as "Pasteur effect".

a) Why would the yeast cells consume less glucose in the presence of oxygen? Can you estimate how much less glucose the culture would use?

The addition of oxygen concentration increases ETC action which would create more ATP. If you produce lots of ATP, you decrease the need for glucose which thereby decreases the need for glycolysis.
Without Oxygen= 2ATP per glucose
With Oxygen= 38 ATP per glucose
==> Glucose consumption is 19 times less

b) Pasteur effect can be counteracted by the addition of 2,4-dinitrophenol (DNP), an uncoupler of oxidative phosphorylation. Why would DNP counteract or prevent the Pasteur effect?

DNP would counteract the Pasteur effect ad decrease ATP yield which would stimulate and increase glycolysis and increase glucose consumption.