

Oxidative phosphorylation

Question 1 and 2 are about the TCA cycle. The rest are about oxidative phosphorylation.

1. How many NADH molecules would be produced after two rounds of the TCA cycle:
A) 2
B) 3
C) 4
D) 6
E) 8
2. Which of the following enzymes is regulated through covalent modification:
A) Malate dehydrogenase
B) Alpha-ketoglutarate dehydrogenase complex
C) Pyruvate dehydrogenase complex
D) Aconitase
E) B + C
3. Mitochondria were isolated from a sample of cells and placed in multiple test tubes, each containing a unique set of conditions as displayed in the following table. In which test tube would you most expect to detect ATP production: (Note: All test tubes contain ADP + Pi)

Test tube number	Oxygen supply	Substances or substrates present in test tube	Other comments
Test tube 1	Yes	NADH and Rotenone	—
Test tube 2	Yes	Succinate	—
Test tube 3	No	—	Mitochondria suspended in low-pH solution
Test tube 4	Yes	Glucose	—

- A) Test tube 1 only
- B) Test tube 2 only
- C) Test tubes 1 and 2
- D) Test tubes 2 and 3
- E) Test tube 4 only

4. Choose the CORRECT statement regarding cytochromes of the ETC:

- A) Cytochromes can be found in all complexes (1-4) of the ETC
- B) *Cytochrome c* is an immobile electron carrier
- C) Complex 3 receives its electrons from *cytochrome c*
- D) *Cytochrome a* has greater reduction potential than *cytochrome c1*
- E) None of the above

5. Which of the following is FALSE regarding Complex 1 of the ETC:

- A) Contains Flavin mononucleotide
- B) Contains Iron-sulfur centers
- C) Contains Copper center
- D) Does not contain any cytochromes
- E) None of the above

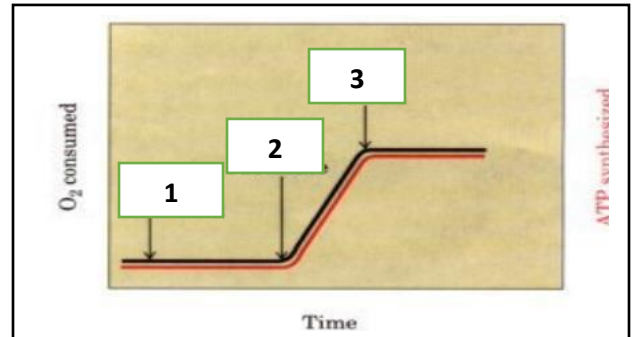
6. Which of the following electron carriers would you suspect to find in the oxidized form after treatment of mitochondria with Antimycin A:

- A) FMN
- B) CoQ
- C) Cytochrome a
- D) Iron-sulfur centers of Complex 1
- E) Iron-sulfur centers of Complex 2

7. The adjacent graph shows oxygen consumption and ATP production by mitochondria when different substrates/substances are gradually introduced. Numbers 1,2 and 3 represent (respectively):

- A) Succinate, ADP+Pi, DNP
- B) NAD⁺, ADP+Pi, Oligomycin
- C) ADP+Pi, Succinate, NADH
- D) NADH, ADP+Pi, CN⁻
- E) None of the above

(*DNP: 2,4-Dinitrophenol)



8. Choose the FALSE statement:

- A) The F1 portion of ATP synthase faces the matrix of the mitochondria.
- B) Blocking the F0 portion of ATP synthase would prevent oxidative phosphorylation from proceeding.
- C) The cytochrome oxidase components that form bonds with O₂ are: Fe of Fe-S center and CuB
- D) 4 reduced *cytochrome c* molecules are required to completely reduce one O₂ molecule
- E) B+C

9. All complexes (1-4) of the ETC contain iron.

- A) True
- B) False

10. Out of complexes (1-4) of the ETC, complex 2 is the only one that does not span the membrane.

- A) True
- B) False

Answers

1. D	2. C	3. D	4. D	5. C
6. C	7. D	8. C	9. True	10. True

1. D

Explanation: We know that one round of the TCA cycle yields 3 NADH molecules. Two rounds would simply yield twice as much. So, 6 NADH molecules.

2. C

Explanation: Pyruvate dehydrogenase complex (PDHC) is regulated through phosphorylation (covalent modification). Other options provided are either allosterically regulated or not regulated at all.

3. D

Explanation:

(The question may or may not lack scientific credibility/accuracy.

Nevertheless, the underlying general concepts are what really matter)

- **Test tube 1:** Despite the supply of O₂ and NADH, the additional presence of Rotenone (ETC inhibitor at complex 1) prevents oxidative phosphorylation from occurring → No H⁺ gradient → No ATP synthesis.
- **Test tube 2:** Succinate is oxidized at complex 2 of the ETC chain, and the resulting electrons can travel down the ETC and reach O₂ (which is present in this test tube) → Generation of H⁺ gradient → ATP synthesis.
- **Test tube 3:** Although no substrate or O₂ is present, the low pH environment (high H⁺ concentration) in which the mitochondria is suspended provides the necessary H⁺ gradient for ATP synthesis.
- **Test tube 4:** Glucose cannot be used directly by mitochondria for energy production, it must first go through glycolysis in the cytosol, which is absent in this case → No ATP production.
- We conclude that test tubes 2 and 3 are the ones that produce ATP.

4. D

Explanation:

- Option A: cytochromes are not present in complexes 1 and 2, so this option is false.
- Option B: *cytochrome c* is mobile as it transfers electrons from complex 3 to 4, so this option is false.
- Option C: *Complex 3* receives electrons from CoQ not *cytochrome c*, so this option is false.
- Option D: As we proceed towards the end of the ETC, the reduction potential of the carriers increases (electrons travel from carriers with lower reduction potential to carriers with higher reduction potential). Therefore, cytochrome a has higher reduction potential than cytochrome c1. This is a true statement.

5. C

Explanation: Copper centers are found in complex 4, not complex 1. The rest of the options are true.

6. C

Explanation: Anything that precedes the point of inhibition in the ETC will be reduced (because it would not be able to donate its electrons to the next carrier), while anything that is present after the point of inhibition in the ETC sequence would be oxidized as it is not receiving electrons from preceding donors. Antimycin A inhibits the ETC before cytochrome a (refer to slides), therefore, cytochrome a would be oxidized.

7. D

Explanation:

- Option A: DNP is an uncoupler, it destroys the H^+ gradient. Therefore, ATP synthesis would stop, however, O_2 consumption would continue. So, the O_2 consumption line (black) should continue to rise, while the ATP synthesis line (red) would stop rising.
- Option B: NAD^+ cannot be used in oxidative phosphorylation, as it is already oxidized. We need the reduced form, which is: NADH.

- Option C: We need some sort of inhibitor at step 3 to stop both O₂ consumption and ATP synthesis. NADH is not an inhibitor. In fact, it undergoes oxidation itself to produce ATP and consume O₂.
- Option D: By first adding NADH, nothing occurs due to the absence of ADP and Pi. When we add ADP and Pi, both lines rise, as the presence of NADH causes oxidative phosphorylation to occur (consuming O₂), and the presence of ADP and Pi allows for ATP synthesis. Finally, addition of CN⁻ (an ETC inhibitor) would prevent oxidative phosphorylation from occurring. Thus, there is no O₂ consumption → and No H⁺ gradient → No ATP synthesis because they are coupled. Therefore, both O₂ consumption and ATP synthesis stop. Correct answer.

8. C

Explanation: O₂ binds to Fe of heme a₃ (not Fe-S) and to CuB. Therefore, statement C is false.

9. True.

Explanation: Complex I : Fe in Fe-S centers

Complex II: Fe in Fe-S centers

Complex III: Fe in heme groups of cytochromes and Fe-S centers.

Complex IV: Fe in heme groups of cytochromes

10. True.

Explanation: Refer to slides. (Complex II does not pump H⁺ because it does not span the membrane).

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