Introductory Biochemistry BIOC*2580
Final Examination
Winter 2011
Wednesday, April 20, 2011, 8:30 – 10:30 a.m.

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PLEASE PRINT YOUR NAME HERE

SURNAME: **___________________________________**

given NAME: **_________________________________**

Student number: ________________________

Remember to enter your name and student number on the "scantron" card! (Your e-mail address is not required.)

Instructors: Dr. F.J. Sharom, Dr. E. P. Wijekoon
Time allotted = 2 hours (120 minutes).
Note: You are not allowed to leave during the last 15 minutes of the exam.
This is a closed-book exam: no notes or aids of any kind (other than a calculator with no stored information) may be consulted. **This booklet has 13 pages, plus the metabolic chart, attached at the end.**

Total marks for this paper = 100.
This examination determines **40%** of the final course grade.
Multiple-choice questions. 30 questions; 2 marks per question; 60 marks total; no marks will be deducted for incorrect answers. Use a soft pencil to mark your answers *on the test-scoring card*.
Written answer questions. 40 marks total. You can use either pen or pencil to answer the questions. DO NOT USE RED PEN.

(Do not write below this line)

1	2	3	4	5	6	7	8	Bonus	TOTAL
2	4.5	5	6	10	4	3.5	5	4	40

Name:____________________ **Student Number:_____________**

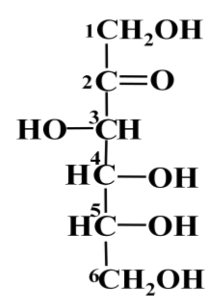
Multiple-choice questions. Two (2) marks each × thirty (30) questions = 60 marks total.
For each question, choose the best answer from among the possible answers given. Enter your answers on the Scantron card.

1. Which of the following acids is not a saturated fatty acid?
   a) Lauric.
   b) Myristic.
   c) Palmitic.
   d) Linoleic.

2. Which of the following fatty acids is likely to have the lowest melting temperature?
   a) $CH_3(CH_2)_{18}COOH$.
   b) $CH_3(CH_2)_{20}$ COOH.
   c) $CH_3(CH_2)_7CH{=}CH(CH_2)_7COOH$.
   d) $CH_3(CH_2)_4CH{=}CH$ $CH_2CH{=}CH(CH_2)_7COOH$.

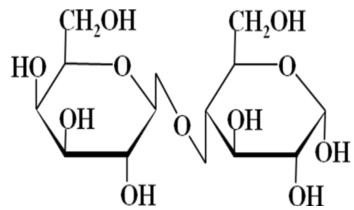
3. The complete hydrolysis of one mole of phosphatidylcholine yields the components glycerol, fatty acid, phosphate, and choline, in which of the following respective molar ratios?
   a) 2 :1:1:1
   b) 1:2:1:1
   c) 1:1:2:1
   d) 1:2:1:2

4. In cyclization of the sugar molecule (D-fructose) shown on the right, the O atom of the -OH group at C-5 acts as the nucleophile. Which of the carbon atoms (labeled 1-6) acts as the electrophile in this reaction?
   a) 1
   b) 2
   c) 3
   d) 4
   e) 6



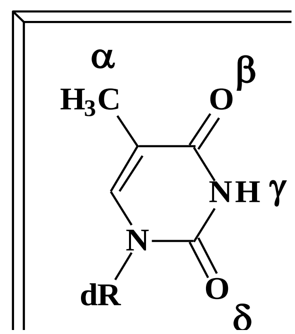
5. In contrast to nucleic acids and polypeptides, many polysaccharides are highly branched. This is a reflection of the fact that…..
   a) monosaccharide building blocks themselves are branched.
   b) monosaccharide building blocks have multiple anomeric carbon atoms.
   c) monosaccharide building blocks have multiple –OH groups.
   d) polysaccharides can be built from more than one type of monosaccharide building block.
   e) unlike nucleic acids and polypeptides, polysaccharides are assembled by spontaneous chemical processes, without requirement for enzymatic catalysis.

6. The disaccharide lactose is shown at right. The sugar on the left is galactose and the sugar on the right is glucose. Lactose is best described as:
    a) Galactofuranosyl (β1 → 3) glucofuranose.
    b) Galactopyranosyl (α1 → 3) gluopyranose.
    c) Galactofuranosyl (α1 → 4) glucofuranose.
    d) Galactopyranosyl (β1 → 4) glucopyranose.



7. Deoxyribose (the sugar in DNA) differs from ribose (the sugar in RNA) by the absence of the hydroxyl group at position:
    a) 1
    b) 2
    c) 3
    d) 4
    e) 5

8. The structure of a thymidine residue of a DNA double helix is shown at right. dR indicates the position of the deoxyribose sugar. The Watson-Crick base-pairing hydrogen bonds to adenine would be found at the positions labelled at…
    a) α, β and γ.
    b) β and γ.
    c) β, γ and δ.
    d) α, β and δ.
    e) α and β.



9. Coenzyme A is….
    a) a thiol.
    b) derived from the vitamin pantothenic acid.
    c) an adenosine-containing cofactor.
    d) named for its role in acetylation reactions.
    e) all of the above are correct.

10. Consider that the following four compounds are completely oxidized to CO_2.

    (i) CH_3-CH_3 (ii) CH_3CHO (iii) CH_3CH_2OH (iv) CH_3COOH

    The relative quantities of free energy released under standard conditions are:

    a) (i)>(iii)>(ii)>(iv)
    b) (iv)>(ii)>(iii)>(i)
    c) (ii)>(iii)>(i)>(iv)
    d) (iii)>(ii)>(i)>(iv)

11. Within the cell, ATP exists mainly as a complex with:
    a) K^{+}
    b) Cationic proteins
    c) Ca^{+2}
    d) Mg^{+2}
    e) Fe^{+3}

12. Each of the following molecules (or classes of molecules) is transported across the inner mitochondrial membrane of a eukaryotic cell by a specific protein carrier, except:
    a) fatty acids.
    b) pyruvate.
    c) malate.
    d) ATP.
    e) NADH.

13. Which one of the following statements about redox reactions in biochemistry is **false**?
    a) O_2 is the strongest oxidant commonly encountered in biochemical processes.
    b) Most biological oxidations involve reactions between an organic substrate and O_2.
    c) NADH is a stronger reducing agent than $FADH_2$.
    d) Reduction reactions in anabolic metabolic pathways usually use NADPH, not NADH.

14. Which of the following tissues must always be supplied with a supply of glucose?
    a) brain and skeletal muscle.
    b) erythrocytes and skeletal muscle.
    c) brain and cardiac muscle.
    d) brain and erythrocytes.

15. Acetyl CoA is supplied to the citric acid cycle by..
    a) glycolysis (via pyruvate).
    b) beta oxidation of fatty acids.
    c) amino acid degradation.
    d) all three of the above processes.
    e) glycolysis and beta oxidation, but not amino acid degradation.

16. According to the mechanism of ATP synthase catalysis proposed by Paul Boyer, the energy released by the proton motive force is used mainly to promote the…..
    a) binding of the alpha subunit to the beta subunit.
    b) binding of ADP to the enzyme.
    c) condensation of ADP with inorganic phosphate to form ATP.
    d) release of ATP from the enzyme.
    e) none of the above.

17. Oxidative phosphorylation and electron transport are said to be "coupled". By this, we mean that, in the mitochondrion:
    a) neither process can occur without the other.
    b) oxidative phosphorylation requires electron transport, but not vice versa.
    c) electron transport requires oxidative phosphorylation, but not vice versa.
    d) the same proteins catalyze both processes.
    e) addition of chemical "uncouplers" stops both oxidative phosphorylation and electron transport.

18. The mechanism of rotation of the catalytic sites of the ATP synthase enzyme with respect to the inner mitochondrial membrane depends on the making/breaking of an ionic interaction ("salt bridge") between positively and negatively charged functional groups found in the…
    a) c subunit and the phosphate head groups of the membrane phospholipids.
    b) a and c subunits.
    c) α and β subunits.
    d) α and γ subunits.
    e) ATP and ADP binding sites.

19. Inorganic fluoride ion (F^-) inhibits the enzyme enolase. In an anaerobic system that is metabolizing glucose as a substrate, which of the following compounds would you expect to accumulate, following addition of fluoride?
    a) glucose-6-phosphate.
    b) phospho-enol-pyruvate.
    c) pyruvate.
    d) 2-phosphoglycerate.
    e) glyceraldehyde.

20. Which statement fits the glycerol-3-phosphate shuttle most accurately?
    a) operates primarily in skeletal muscle and brain.
    b) is responsible for transferring NADH reducing equivalents from the cytosol into the mitochondria.
    c) results in production of 1.5 ATP for each NADH oxidized.
    d) all of the above statements are correct.

21. Identify the ***incorrect*** answer. Coenzyme Q (ubiquinone):
    a) is small and hydrophobic and therefore easily diffuses within the inner mitochondrial membrane.
    b) acts as a collection point for reducing equivalents from several different sources.
    c) can accept either one or two electrons and therefore can act at the junction between a two electron donor and a one electron acceptor.
    d) contains a tightly bound heme co-factor which acts as the redox active center in the molecule.

22. According to Peter Mitchell's chemiosmotic theory, electron transport in the respiratory chain is accompanied by transfer of protons across the inner mitochondrial membrane by the respiratory complexes. How many protons are translocated by complex IV?
    a) 4
    b) 2
    c) 0
    d) 6

23. A molecule of acetyl CoA enters the Krebs cycle; the cycle proceeds until oxaloacetate is formed, but no further. The result is that,
    a) no CO_2 is produced, one GDP (or ADP) is converted to GTP (or ATP).
    b) two molecules of CO_2 are produced, and there is net synthesis of one molecule of oxaloacetate.
    c) two molecules of CO_2 are produced, three molecules of NAD^+ and one molecule of FAD are reduced, and one molecule of GDP (or ADP) is converted to GTP (or ATP).
    d) no CO_2 is produced, two molecules of NAD^+ and one molecule of FAD are reduced, and one molecule of GDP (or ADP) is converted to GTP (or ATP).
    e) no CO_2 is produced, three molecules of NAD^+ and one molecule of FAD are reduced, and one molecule of GDP (or ADP) is converted to GTP (or ATP).

24. The mechanism of the reaction catalyzed by the beta-oxidation enzyme enoyl-CoA hydratase is similar to that of the citric acid cycle enzyme ...
    a) malate dehydrogenase.
    b) α-ketoglutarate dehydrogenase.
    c) aconitase.
    d) fumarase.
    e) citrate synthase.

25. The majority of ATP is synthesized in the mitochondria while the majority of ATP usage occurs in the cytosol. Choose the **most appropriate answer** with regards to ATP transport across the inner mitochondrial membrane.
    a) ATP once formed in the mitochondrion will diffuse down its concentration gradient into the cytosol.
    b) ATP will leave the mitochondrion in exchange for a molecule of ADP that comes into the mitochondrion through the adenine nucleotide translocase antiporter.
    c) ATP will leave the mitochondrion along with an inorganic phosphate molecule through the phosphate translocase symporter.
    d) ATP will cross the mitochondrial membrane by going through the F_o pore of ATP synthase.

Note: ATP transport was not covered this semester. So don't worry about this question.

26. Electron transport chains…
    a) allow the indirect oxidation of reduced cofactors by molecular oxygen, preventing an otherwise wasteful release of energy.
    b) are formed by arranging electron carriers in order of increasing reduction potential.
    c) conserve the energy of oxidation by generating a proton motive force across the inner mitochondrial membrane.
    d) all of the above statements are correct.

27. Experimental measurements of the chemical potential difference (Δμ) and electrical potential difference (Δψ) across the inner membrane of an intact mitochondrion show that ...
    a) only Δψ contributes significantly to the proton-motive force.
    b) only Δμ contributes significantly to the proton-motive force.
    c) both Δψ and Δμ contribute significantly to the proton-motive force.
    d) Δψ contributes to the proton-motive force, whereas Δμ reduces it.

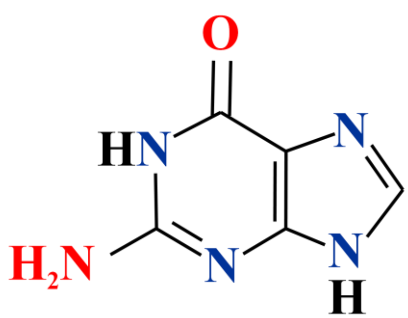
28. Enzymes that catalyze reactions in which a phosphate group is transferred from ATP to a substrate are known as:
    a) synthases.
    b) ATPases.
    c) synthetases.
    d) phosphatases.
    e) kinases.

29. Which of the following statements about glucose is **false**?
    a) The concentration of glucose in the blood of a well-nourished human is about 5 mM.
    b) Glucose enters most human cells readily, by passive diffusion across the cell membrane.
    c) Upon entry into most cells, glucose is rapidly phosphorylated.
    d) Glucose can be catabolized under either aerobic or anaerobic conditions.

30. The acute toxicity of carbon monoxide (CO) to mammals is primarily caused by its ...
    a) binding to myoglobin.
    b) action as an uncoupler of oxidative phosphorylation.
    c) inhibition of the mitochondrial ATP synthase.
    d) inhibition of cytochrome oxidase.
    e) all of the above are correct.

Written-answer questions. Answer all questions. All answers should be written in this booklet. Please DO NOT use red pen.

1. **Draw the complete structure of the nucleotide base guanine. (2 marks)**



2. Adenosine triphosphate

i. Indicate two (2) reasons for the large and negative $\Delta G^{0'}$ associated with ATP hydrolysis. (1 mark each)

i. Charge repulsion associated with the negatively charged components of phosphoanhydrides.
ii. Resonance stabilization of the product inorganic phosphate (P_i).
iii. Ionization of the ADP product.

ii. Some reactions are driven by the hydrolysis of ATP to ADP + P_i, whereas some others are driven by the hydrolysis of ATP to AMP + PP_i. Explain the advantage of the second form of hydrolysis over the first, if any. (2 marks)

Hydrolysis of ATP to ADP and Pi results in the hydrolysis of only one phosphoanhydride linkage of ATP. Hydrolysis of ATP to AMP + PP_i initially hydrolyzes one phosphoanhydride bond; however, due to the action of the enzyme *inorganic pyrophosphatase*, the remaining phosphoanhydride bond of PPi is also hydrolyzed to give two moles of Pi. The end result is the hydrolysis of both phosphoanhydride linkages of ATP. Therefore, the free energy change for the hydrolysis of ATP to AMP + PPi is twice as much as the free energy change for the hydrolysis of ATP to ADP + Pi.

$$\text{ATP} \rightarrow \text{ADP} + \text{P}_i$$

$$\text{ATP} \rightarrow \text{AMP} + \text{PP}_i \text{ (pyrophosphate)}$$

$$\mathbf{PP}_i \xrightarrow[\textit{pyrophosphatase}]{} \mathbf{2\ P}_i$$

iii. What is the chemical mechanism which allows ATP to drive energetically unfavourable reactions forward? (Naming the mechanism is adequate). (0.5 marks)

Group Transfer.

(ATP provides the energy to drive energetically unfavourable reactions by transferring a group from its structure (phosphoryl, pyrophosphoryl or an adenylate moiety) to a substrate of the reaction).

3. **Choose the clue which best describes each term given in column 1. Write the letter corresponding to the correct term in the last column, next to each clue. (1 mark each)**

Term	*Clue*	*Term*
A. Glycoside	The product of a reaction between an aldehyde and an alcohol	B
B. Hemiacetal	Stereoisomer resulting from cyclization of a sugar	D
C. Hemiketal	The product of a reaction between a ketone and an alcohol	C
D. Anomer	A pair of sugars that are identical except for the chirality at one carbon atom	E
E. Epimer	The product of condensation of an alcohol with the anomeric carbon of a sugar	A

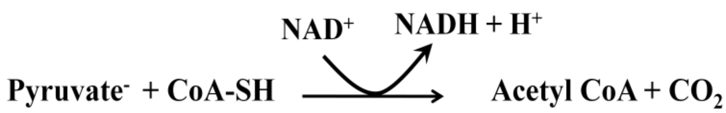
4. **Match the enzyme with its catalytic mechanism. Write the letter corresponding to each enzyme in the last column, in front of the catalytic mechanism through which it works. (1 mark each)** ***** The mechanism of thiolase was not discussed this semester. Hence, you will not be tested on it.***

Enzyme	*Catalytic mechanism*	*Enzyme*
A. Acyl CoA synthetase	Deprotonates the –SH group of CoA and steers it towards the electrophilic beta carbonyl carbon atom.	**B**
B. Thiolase**	Reversibly hydrates an alkene.	**D**
C. Triose phosphate isomerase	A histidine residue at the active site accepts a phosphate to form a phosphoenzyme intermediate.	**F**
D. Aconitase	Catalyzes the formation of an enzyme bound ene-diol intermediate.	**C**
E. Citrate synthase	Catalyzes the formation of an acyl-adenylate intermediate.	**A**

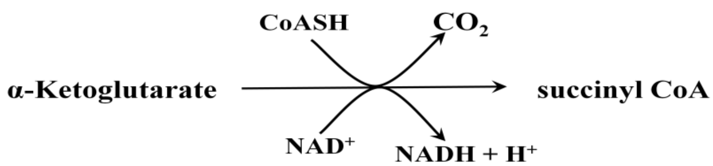
5. **For each of the following questions, write a complete balanced equation for the chosen reaction *(in words, structures not necessary)*, indicating the enzyme that catalyzes it. The same example cannot be used more than once. (2 marks each)**

Example: Glutamate + ATP + NH_3 $\xrightarrow{\textit{Glutamine synthetase}}$ Glutamine + ADP + P_i

i. Any enzymatic reaction which utilizes thiamine pyrophosphate (derived from vitamin B1) as a cofactor.

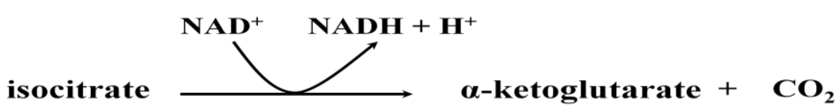


Pyruvate Dehydrogenase complex

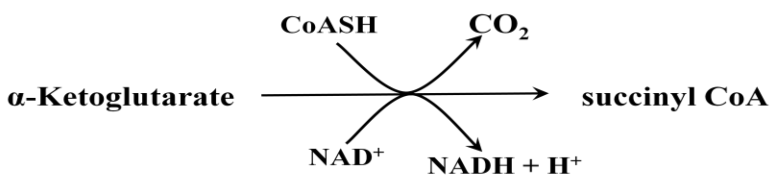


α-Ketoglutarate dehydrogenase

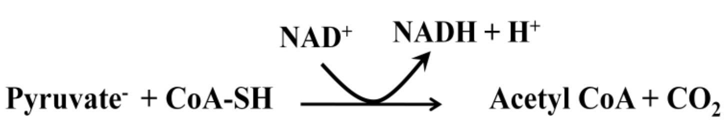
ii. Any enzymatic reaction in which an oxidative decarboxylation occurs.



Isocitrate dehydrogenase

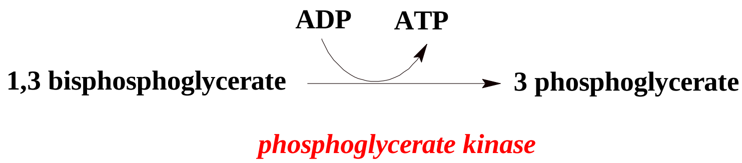


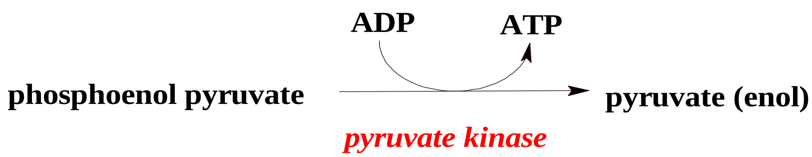
α-Ketoglutarate dehydrogenase

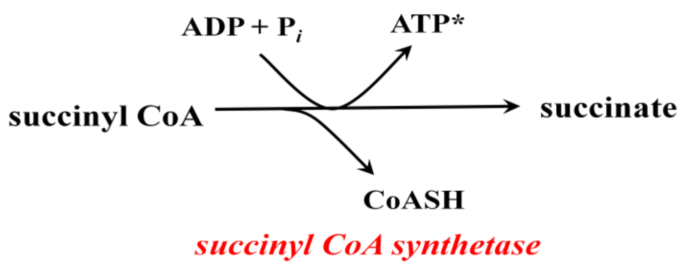


Pyruvate Dehydrogenase complex

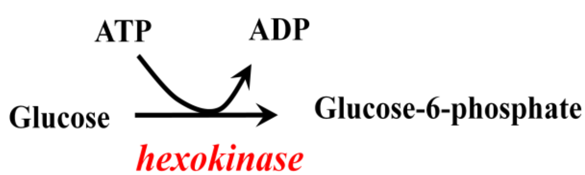
iii. **Any enzymatic reaction in which a "substrate level phosphorylation" occurs.**

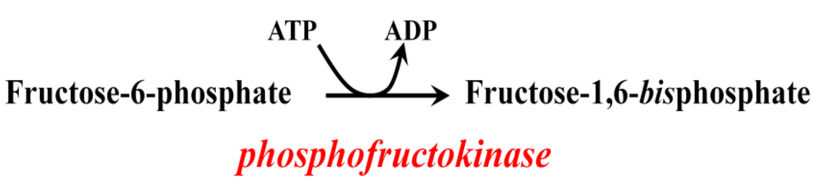




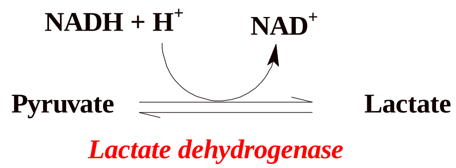


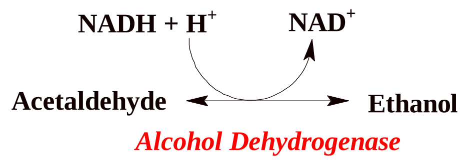
iv. **Any enzymatic reaction which uses ATP as a phosphoryl donor to phosphorylate a molecule.**





v. **Any enzymatic reaction which is involved in oxidation of NADH to NAD^+ in the cytosol.**





Cytosolic malate dehydrogenase (Malate-aspartate shuttle) and cytosolic glycerol 3-phosphate dehydrogenase (glycerol 3-phosphate shuttle) are also correct.

6. **Answer the following questions in the space provided.**
   i. **Free palmitate is activated to its coenzyme A derivative in the cytosol before it can be oxidized in the mitochondria. If palmitate and radioactive [^{14}C] coenzyme A are added to a liver homogenate, palmitoyl-CoA isolated from the cytosolic fraction is radioactive, but that isolated from the mitochondrial fraction is not. Explain the reason for this. (2 marks)**

Palmitate reacts with the added [^{14}C] coenzyme A in the cytosol to produce radioactive palmitoyl-CoA.

$$\text{Palmitate}^{-} + {}^{14}\text{CoA} + \text{ATP} \xrightarrow{\textit{Acyl-CoA synthetase}} {}^{14}\text{Palmitoyl-CoA} + \text{AMP} + 2\ \text{Pi}$$

For beta oxidation to occur, the palmitoyl-CoA thus produced needs to be transported to the mitochondrial matrix.

However, palmitoyl-CoA cannot cross the inner mitochondrial membrane and therefore needs to be converted to palmitoyl-carnitine in order to be carried across the inner mitochondrial membrane.

This reaction removes the radioactive CoA and replaces it with a molecule of carnitine.

Once in the matrix, carnitine is removed from palmitoyl-carnitine and is replaced with a new coenzyme A molecule from the mitochondria to reform palmitoyl-CoA.

Since this CoA is not radioactive, the palmitoyl CoA produced is also non radioactive.

   ii. ***Pyruvate kinase deficiency* (PKD) is a rare human genetic disease, affecting about one person in 20,000. Individuals with PKD have unusually low levels of lactate in their blood even after exercise. Briefly explain the reason for this. (2 marks)**

Pyruvate kinase catalyzes the conversion of phosphoenolpyruvate to pyruvate in the cytosol during glycolysis. People with PKD would be unable to carry out this reaction and therefore would not be able to synthesize pyruvate.

Lactate is formed by reduction of pyruvate in anaerobic glycolysis especially during strenuous exercise.

Since individuals with PKD do not produce any pyruvate their blood lactate levels would be minimal.

7. **Complete the table below to show the total yield of ATP from oxidation of one mole of stearoyl-CoA (18 carbon saturated fatty acid). (0.25 marks each)**

Enzyme	**Number and type of reduced cofactor**	**Number of ATP**
acyl-CoA dehydrogenase	8 $FADH_2$	12
β-hydroxyacyl-CoA dehydrogenase	8 NADH	20
isocitrate dehydrogenase	9 NADH	22.5
α-ketoglutarate dehydrogenase	9 NADH	22.5
succinyl-CoA synthetase	-	9
succinate dehydrogenase	9 $FADH_2$	13.5
malate dehydrogenase	9 NADH	22.5
Total number of ATP	-	**122**

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8. All of the dehydrogenases of glycolysis and the citric acid cycle use NAD^+ as the electron acceptor except succinate dehydrogenase, which uses covalently bound FAD. The $E_0{}'$ values for NAD^+/NADH, covalently bound FAD/$FADH_2$ and fumarate/succinate are given below.

$NAD^+ + 2H^+ + 2e^- \longrightarrow NADH + H^+$ $E_0{}' = -0.32$ V

$FAD + 2H^+ + 2e^- \longrightarrow FADH_2$ $E_0{}' = +0.05$ V

$Fumarate + 2H^+ + 2e^- \longrightarrow Succinate$ $E_0{}' = +0.031$ V

i. Calculate $\Delta E_0{}'$ for the oxidation of succinate by NAD^+. (2 marks)

$Succinate + NAD^+ \longrightarrow Fumarate + NADH$

$NAD^+ + 2H^+ + 2e^- \longrightarrow NADH + H^+$ $E_0{}' = -0.32$ V

$Succinate \longrightarrow Fumarate + 2H^+ + 2e^-$ $E_0{}' = -0.031$ V

$\Delta E_0{}'$ = -0.32 + -0.031

= -0.351 V

ii. Calculate $\Delta E_0{}'$ for the oxidation of succinate by covalently bound FAD. (2 marks)

$Succinate + FAD \longrightarrow Fumarate + FADH_2$

$FAD + 2H^+ + 2e^- \longrightarrow FADH_2$ $E_0{}' = +0.05$ V

$Succinate \longrightarrow Fumarate + 2H^+ + 2e^-$ $E_0{}' = -0.031$ V

$\Delta E_0{}'$ = +0.05 V + -0.031 V

= 0.019 V

iii. Based on the values obtained in questions i and ii, explain why FAD is a more appropriate electron acceptor than NAD^+ in the dehydrogenation of succinate to fumarate. (1 mark)

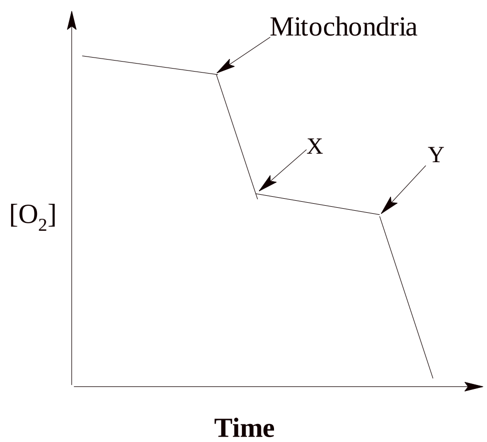
For a reaction to proceed spontaneously in a given direction, the $\Delta E_0{}'$ has to be >0. The $\Delta E_0{}'$ for the first reaction is less than zero and therefore, it would not proceed as written. NAD^+ would be unable to oxidize succinate. (In fact, this reaction would proceed in the opposite direction). The $\Delta E_0{}'$ for the oxidation of succinate by FAD is >0 and the reaction would proceed in the direction shown.

Bonus Question (4 marks)

Mitochondria from Brown Adipose Tissue are suspended in a buffered medium with succinate as the substrate. The oxygen consumption by these mitochondria is measured with an oxygen electrode apparatus. The following changes were observed in the oxygen consumption when mitochondria and the substances X and Y were added to the medium. Identify the nature of X and Y from the following list.

(Note that the substances are added consecutively, so that when "Y" is added "X" is still present in the medium)

A. An uncoupler
B. An inhibitor of succinate dehydrogenase
C. An inhibitor of thermogenin
D. An inhibitor of the electron transport chain
E. An inhibitor of ATP synthase



X - "B" an inhibitor of thermogenin

Y- "A" an uncoupler

A little note to explain what's going on here….

Brown Adipose tissue contains an uncoupling agent called thermogenin. Since respiration occurs with the addition of mitochondria without the presence of ADP and Pi, you can assume that the protons are coming back to the mitochondrial matrix through this uncoupler and not through ATP synthase. Therefore, X has to be an inhibitor of thermogenin. It cannot be an inhibitor of the ETC because addition of Y is able to resume respiration. Since the substances are added consecutively X is still present when Y is added. If the ETC is inhibited nothing can overcome that to resume oxidation again. Hence, X has to an inhibitor of thermogenin and Y has to be another uncoupler, able to overcome the inhibition of thermogenin by providing an alternate route for proton transfer across the inner mitochondrial membrane.

NOTE: we did not discuss thermogenin in this series of lectures. Therefore an explanation about thermogenin and brown adipose tissue would have preceded this question if asked from this class.