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B.M.S. College of Engineering, Bengaluru-560019

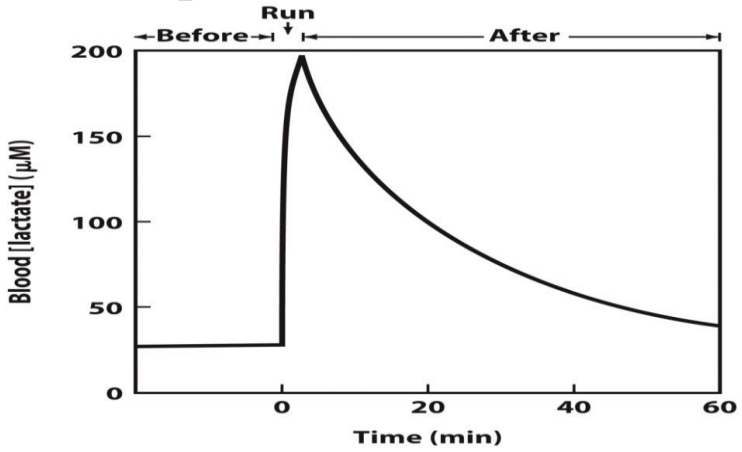
Autonomous Institute Affiliated to VTU

August 2024 Semester End Main Examinations

Programme: B.E. **Semester: IV**
Branch: Biotechnology **Duration: 3 hrs.**
Course Code: 23BT4PCBAB **Max Marks: 100**
Course: Biochemistry and Bioenergetics

Instructions: 1. Answer any FIVE full questions, choosing one full question from each unit.
2. Missing data, if any, may be suitably assumed.

		UNIT - I	CO	PO	Marks
1	a)	Considering the following reaction Acetaldehyde + NADH + H^+ → Ethanol + NAD^+ with $E'^0_{NAD^+/NADH}$ = -0.32 V and $E'^o_{Acetaldehyde/Ethanol}$ ← = -0.197 V. i. Which redox pair has the greater tendency to lose electrons? Explain. ii. Which pair is the stronger oxidizing agent? Explain. iii. Beginning with 1 M concentrations of each reactant and product at pH 7 and 25 °C, in which direction will the above reaction proceed and why? iv. What is the standard free-energy change ($\Delta G'^0$) for the reaction? v. What is the free-energy change (ΔG) for the reaction at 37 °C if [acetaldehyde] and NADH are 1.0 M, and [ethanol] and [NAD^+] are 0.1 M? vi. What is the standard equilibrium constant (K'^0_{eq}) for this reaction?	CO2	PO1	15 (2+2+1+2+5+3)
	b)	The phosphorylation of glucose in the cell is coupled to the hydrolysis of ATP; that is, part of the free energy of ATP hydrolysis is used to phosphorylate glucose: (1) Glucose + Pi ⟶ Glucose 6-phosphate + H_2O $\Delta G'^0$ = 13.8 kJ/mol (2) ATP + H_2O ⟶ ADP + Pi $\Delta G'^0$ = − 30.5 kJ/mol Sum: Glucose + ATP ⟶ Glucose 6-phosphate + ADP Calculate K'^0_{eq} at 37 °C for the overall reaction. For the ATP-	CO 2	PO 1	05

			dependent phosphorylation of glucose, what concentration of glucose is needed to achieve a 250 µM intracellular concentration of glucose 6-phosphate when the concentration of ATP and ADP are 3.38 mM and 1.32 mM, respectively? Does this coupling process provide a feasible route, at least in principle, for the phosphorylation of glucose in the cell? Explain.			
			UNIT - II			
	2	a)	The degree of reduction of each carrier in the respiratory chain is determined by conditions in the mitochondrion. For example, when NADH and O_2 are abundant, the steady-state degree of reduction of the carriers decreases as electrons pass from the substrate to O_2. When electron transfer is blocked, the carriers before the block become more reduced and those beyond the block become more oxidized. For each of the conditions below, predict the state of oxidation of ubiquinone and cytochrome b, c_1, c, and a + a_3. i. Abundant NADH and O_2, but cyanide added ii. Abundant NADH and O_2 exhausted iii. Abundant O_2, but NADH exhausted iv. Abundant NADH and O_2	***CO*** 3	***PO*** *2*	**08 (2x4)**
		b)	Why would it be disadvantageous to the organism to have Glycolysis and Gluconeogenesis operating simultaneously within a cell? Briefly describe one example of reciprocal regulation of Glycolysis and Gluconeogenesis, involving an allosteric regulator. For the example chosen, write out the reaction catalyzed by the enzyme in each pathway, and indicate the nature of the effect of the regulator (e.g., inhibition or activation).	***CO*** 5	***PO*** *2*	**07**
		c)	The concentrations of lactate in blood plasma before, during, and after a 400 m sprint are shown in the graph.  i. What causes the rapid rise in lactate concentration? ii. What causes the decline in lactate in lactate concentration after completion of the sprint? Why does the decline occur more slowly than the increase? iii. Why is the concentration of lactate not zero during the resting state?	***CO*** ***4***	***PO*** ***2***	**05 (1+2+ 2)**
			OR			

	3	a)	Refer the figure 3a given below as a guide to answer the following questions.	*CO 4*	*PO 2*	**10 (2+1+ 2+2+3)**

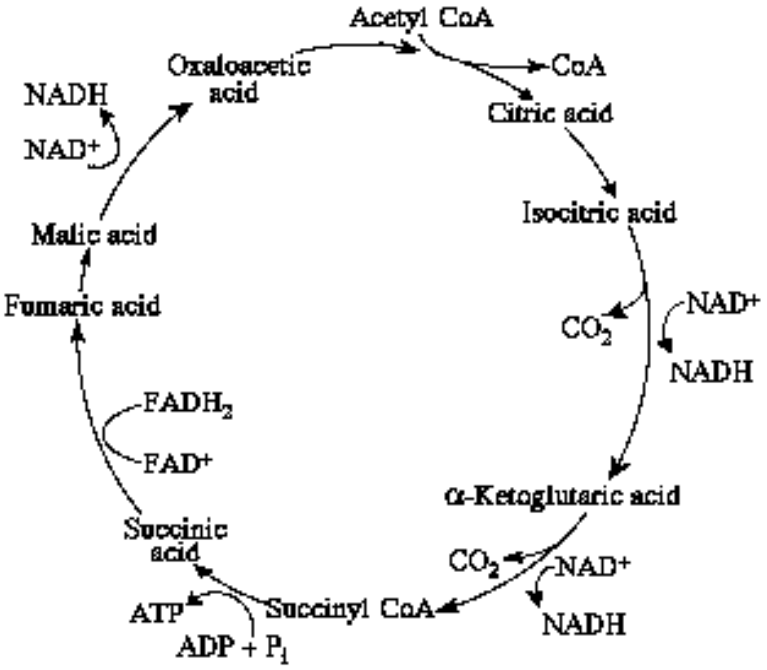


Figure 3a

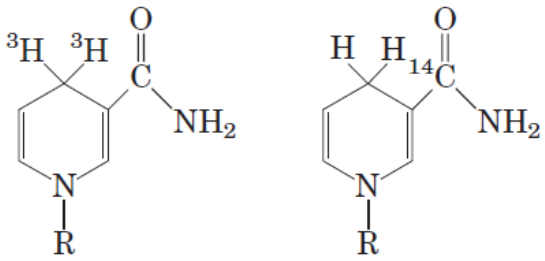
i. What is substrate-level phosphorylation? Starting with citric acid and ending with fumeric acid in Figure 3a, what is the maximum number of ATP molecules that could be made through substrate-level phosphorylation?

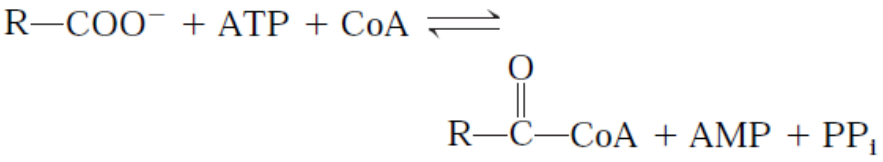
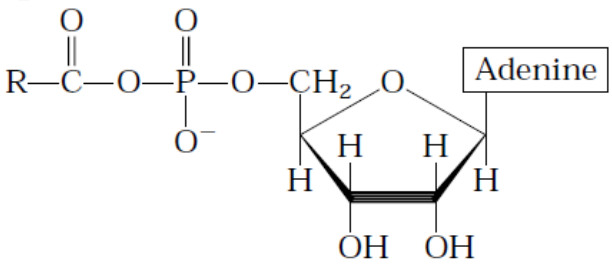
ii. Refer to Figure 3a. Carbon skeletons for amino acid biosynthesis are supplied by intermediates of the Krebs cycle. Which intermediate would supply the carbon skeleton for synthesis of a 5-carbon amino acid?

iii. Refer to Figure 3a. Starting with citric acid and ending with oxaloacetic acid, how many ATP molecules can be formed from oxidative phosphorylation?

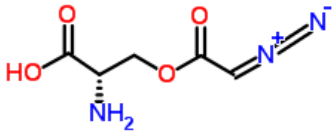
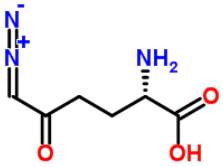
iv. How many ATP molecules can be made through substrate-level phosphorylation and oxidative phosphorylation if you started with succinyl CoA and ended with oxaloacetate?

v. What are anaplerotic reactions? Write any two

		b)	Under aerobic conditions, extramitochondrial NADH must be oxidized by the mitochondrial electron-transfer chain. Consider a preparation of rat hepatocytes containing mitochondria and all the cytosolic enzymes. If [4-^{3}H]NADH is introduced, radioactivity soon appears in the mitochondrial matrix. However, if [7-^{14}C]NADH is introduced, no radioactivity appears in the matrix. What do these observations reveal about the oxidation of extra mitochondrial NADH by the electron-transfer chain?  [4-^{3}H]NADH [7-^{14}C]NADH	***CO 4***	***PO 2***	**05**
		c)	If the oxidation of glucose 6-phosphate via the pentose phosphate pathway were being used primarily to generate NADPH for biosynthesis, the other product, ribise 5-phosphate, would accumulate. What problems might this cause?	CO 4	*PO 2*	**03**
		d)	Chemical compound 2, 4-dinitrophenol (DNP), when added to intact mitochodria can uncouple oxidation from phosphorylation. What seems the reason for this uncoupling?	PO 4	*PO 2*	**02**
			UNIT - III			
	4	a)	Outline the reductive pentose phosphate pathway giving names and structures of substrates and products, and the name of each enzyme.	***CO 1***	***PO 1***	**10**
		b)	In typical C_4 plants, the initial capture of CO_2 occurs in one cell type, and the Calvin cycle reactions occur in another. Voznesenskaya and colleagues have described a plant, *Bienertia cycloptera*-which grows in salty depressions of semidesert in Central Asia-that shows the biochemical properties of a C_4 plant but unlike typical C_4 plants does not segregate the reactions of CO_2 fixation into two cell types. PEP carboxylase and rubisco are present in the same cell. However, the cells have two types of chloroplasts, which are localized differently, as shown in the micrograph. One type, relatively poor in grana (thylakoids), is confined to the periphery; the more typical chloroplasts are clustered in the center of the cell, separated from the peripheral chloroplasts by large vacuoles. Thin cytosolic bridges pass through the vacuoles connecting the peripheral and central cytosol.	***CO 4***	***PO 2***	**04**

			 In this plant, where would you expect to find (i) PEP carboxylase, and (ii) Rubisco? Explain your answers for CO_2 fixation in these C_4 cells.			
		c)	The light used by vascular plants for photosynthesis has a wavelength of about 600 nm. Calculate the energy in a "mole" of photon (an einstein) of light of this wavelength, and compare this with the energy needed to synthesize a mole of ATP. (Given h= 6.626 X 10^{-34} J . s)	***CO 2***	***PO 1***	**03**
		d)	The extent to which an electron carrier is oxidized or reduced during photosynthetic electron transfer can sometimes be observed directly with a spectrophotometer. When chloroplasts are illuminated with 700 nm light, cytochrome *f*, plastocyanin, and plastoquinone are oxidized. When chloroplasts are illuminated with 680 nm light, however, these electron carriers are reduced. Explain.	***CO 3***	***PO 2***	**03**
			UNIT - IV			
	5	a)	Fatty acids are converted to their coenzyme A esters in a reversible reaction catalyzed by acyl-CoA synthetase:  $R—COO^- + ATP + CoA \rightleftharpoons R—C(=O)—CoA + AMP + PP_i$	***CO*** 1	***PO 1***	**10 (2+2+ 6)**
			The enzyme-bound intermediate in this reaction has been identified as the mixed anhydride of the fatty acid and adenosine monophosphate (AMP), acyl-AMP: 			

			i. Write two equations corresponding to the two steps of the reaction catalyzed by acyl-CoA synthetase. ii. The acyl-CoA synthetase reaction is readily reversible, with an equilibrium constant near 1. How can this reaction be made to favor formation of fatty acyl–CoA? iii. Describe four basic steps of β-oxidation of saturated fatty acid.			
	b)	Using your knowledge of fatty acid biosynthesis, provide an explanation for the following experimental observations: i. Addition of uniformly labeled [^{14}C]acetyl-CoA to a soluble liver fraction yields palmitate uniformly labeled with ^{14}C. ii. However, addition of a *trace* of uniformly labeled [^{14}C]acetyl-CoA in the presence of an excess of unlabeled malonyl-CoA to a soluble liver fraction yields palmitate labeled with ^{14}C only in C-15 and C-16.	***CO*** 4	***PO 2***	**05 (2+3)**
	c)	During the biosynthesis of fatty acid, the acetyl group of acetyl-CoA, produced by the oxidative decarboxylation of pyruvate in the mitochondrion, is transferred to the cytosol by the acetyl group shuttle. i. Write the overall equation for the transfer of one acetyl group from the mitochondrion to the cytosol. ii. What is the cost of this process in ATPs per acetyl group?	***CO 4***	***PO 2***	**05 (3+2)**
		OR			
6	a)	In biochemistry, fatty acid synthesis is the creation of fatty acids from acetyl-CoA and NADPH through the action of enzymes called fatty acid synthases. i. Give the sequence of reactions catalyzed by the fatty acid synthase during the biosynthesis of a molecule of Laurate. ii. Write the net equation for the biosynthesis of a molecule of Laurate in rat liver, starting from mitochondrial acetyl-CoA and cytosolic NADPH, ATP and CO_2.	***CO 1***	***PO 1***	**10 (07 + 03)**
	b)	Cholesterol is an essential component of cell membranes and the precursor for the synthesis of steroid hormones and bile acids. i. If 2[^{14}C]acetyl-CoA is added to a rat liver homogenate that is synthesizing cholesterol, where will the ^{14}C label appear in Δ^3-isopentenyl pyrophosphate, the activated form of an isoprene unit? ii. Sketch the de novo biosynthetic pathway of cholesterol in the organism and identify the main regulatory enzymes involved in this process.	***CO*** 4, 5	***PO 2***	**10 (03 + 07)**

			UNIT-5			
	7	a)	In a study conducted some years ago, cats were fasted overnight then given a single meal complete in all amino acids except arginine. Within 2 hours, blood ammonia levels increased from a normal level of 18 µg/L to 140 µg/L, and the cats showed the clinical symptoms of ammonia toxicity. A control group fed with a complete amino acid diet or an amino acid diet in which arginine was replaced by ornithine showed no unusual clinical symptoms. i. What was the role of fasting in the experiment? ii. What caused the ammonia levels to rise in the experimental group? iii. Why did the absence of arginine lead to ammonia toxicity? iv. Is arginine an essential amino acid in cats? Why or why not? v. Why can ornithine be substituted for arginine?	***CO*** 4	***PO*** *2*	**10 (2x5)**
		b)	Azaserine (O-diazoacetyl-L-serine) and 6-diazo-5-oxo-L-norleucine (DON) are glutamine analogs.   **Azaserine** **6-diazo-5-oxo-L-norleucine** They form covalent bonds to nucleophiles at the active sites enzymes that bind glutamine, thereby irreversibly inactivating these enzymes. Identify any three of these enzymes involved in the nucleotide biosynthesis and intermediates that accumulate in the presence of either of these glutamine antagonists.	***CO*** 5	***PO*** *2*	**06 (2x3)**
		c)	How is the rate of pyrimidine nucleotide synthesis regulated through aspartate transcarbamoylase (ATCase)? Explain briefly.	***CO5***	***PO 2***	**04**

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