UNIVERSITY OF SWAZILAND

FINAL EXAMINATION PAPER: MAY 2013

TITLE OF PAPER: BIOCHEMISTRY & CELL BIOLOGY

COURSE CODE: B203

TIME ALLOWED: THREE HOURS

INSTRUCTIONS:

1. ANSWER QUESTION 1 (COMPULSORY) AND ANY THREE OTHER QUESTIONS.
2. ANSWER A TOTAL OF 4 (FOUR) QUESTIONS
2. EACH QUESTION CARRIES TWENTY FIVE (25) MARKS
3. ILLUSTRATE YOUR ANSWERS WITH LARGE AND CLEARLY LABELLED DIAGRAMS WHERE APPROPRIATE

SPECIAL REQUIREMENTS:

1. GRAPH PAPER
2. CANDIDATES MAY USE CALCULATORS

THIS PAPER SHOULD NOT BE OPENED UNTIL PERMISSION HAS BEEN GRANTED BY THE INVIGILATORS

[PLEASE TURN OVER]

Section A (Compulsory)

Answer all questions in this section

Question 1 (compulsory)

(a) Explain the concept of 'cell theory'. (3 marks)

(b) Explain the difference between the following:
(i) metaphase I of meiosis and metaphase of mitosis, (1 mark)
(ii) anaphase I of meiosis and anaphase of mitosis, (1 mark)
(iii) apoptosis and necrosis, (1 mark)
(iv) tumor-suppressor gene and proto-oncogene, (1 mark)
(v) totipotent cell and pluripotent cell. (1 mark)

(c) State three points in cell cycle where the cell is checked for integrity.(3 marks)

(d) If a cell in (c) above is found to be faulty, explain its possible fates. (2 marks)

(e) Define pK_a for a weak acid in relation to its acid dissociation constant, K_a, and by reference to a titration curve for the weak acid. (1 mark)

(f) A solution is made by combining 50 mL of a 0.1 M sodium acetate solution with 150 mL of 1 M acetic acid (pK_a = 4.7). Calculate the pH of the resulting solution. (3 marks)

(g) In proteins, the amino acid histidine (His) plays an important role in many biological reactions. The pK_a for the protonation of His to form $HisH^+$ = 6.0. When pH = 7.0, determine the fraction of total histidine that will be in the protonated form ($HisH^+$). (5 marks)

(h) Explain how changes in pH can alter the conformation of a protein. (3 marks)

[TOTAL MARKS = 25]

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Section B

Answer **any three** questions from this Section.

Question 2

(a) Write notes on **any one** of the following methods for purifying proteins, highlighting the physicochemical properties employed during purification:

(i) Ion-exchange chromatography (5 marks)
(ii) Affinity chromatography (5 marks)
(iii) Gel filtration (5 marks)
(iv) Isoelectric focussing (5 marks)
(maximum of 5 marks)

(b) Temakholo wishes to determine the sequence of a protein that contains 123 amino acid residues. After breaking all of the disulphide bonds, she treated the protein with cyanogen bromide (CNBr) and generated seven conveniently short peptides , which are separated from each other. It is your turn to take over. Briefly outline the steps you would take to determine, unambiguously, the sequence of amino acid residues in the original protein. (8 marks)

(c) Prostaglandins are a class of eicosanoids, fatty acid derivatives with a variety of extremely potent actions on vertebrate tissues. They are responsible for producing fever and inflammation and its associated pain. Prostaglandins are derived from the 20-carbon fatty acid arachidonic acid in a reaction catalyzed by the enzyme prostaglandin endoperoxide synthase. This enzyme, a cyclooxygenase, uses oxygen to convert arachidonic acid to PGG_2, the immediate precursor of many different prostaglandins.

When a person has extreme pain, he/she may take ibuprofen, an inhibitor of prostaglandin endoperoxide synthase. By inhibiting the synthesis of prostaglandins, ibuprofen reduces inflammation and pain. The kinetic data given below are for the reaction catalysed by prostaglandin endoperoxide synthase in the absence and presence of ibuprofen.

[Arachidonic acid] (mM)	**Rate of formation of PGG_2 (mM/min)**	**Rate of PGG_2 with 10g/mL ibuprofen (mM/min)**
0.5	23.5	16.67
1.0	32.2	25.25
1.5	36.9	30.49
2.5	41.8	37.04
3.5	44.0	38.91

Draw the Lineweaver-burk plot to determine V_{max} and K_m in the absence and presence of ibuprofen. Hence explain the type of inhibitor ibuprofen is how it interacts with the endoperoxide synthase active site. (12 marks)

[TOTAL MARKS = 25]

Question 3

(a) (i) A solution of glucose 1-phosphate (0.1 M) at 25°C is incubated with a catalytic amount of phosphoglucomutase and is isomerized to glucose 6-phosphate. At equilibrium, the concentrations of the reaction components are:

Glucose 1-phosphate $\rightleftharpoons$ **glucose 6-phosphate**
4.5×10^{-3} M $\qquad$ 9.6×10^{-2} M

Calculate K'_{eq} and $\Delta G'^{\circ}$ for this reaction. (R = 8.315 J/mol·K.) (6 marks)

(ii) A direct measurement of the standard free-energy change associated with the hydrolysis of ATP is technically demanding because the minute amount of ATP remaining at equilibrium is difficult to measure accurately. The value of $\Delta G'^{\circ}$ can be calculated indirectly, however, from the equilibrium constants of two other enzymatic reactions having less favourable equilibrium constants:

(1) Glucose 6-phosphate + H2O → glucose + P_i, (K'_{eq} = 270)
(2) ATP + glucose → ADP + glucose 6-phosphate, (K'_{eq} = 890)

Using this information for equilibrium constants determined at 25°C, calculate the standard free energy of hydrolysis of ATP. (R = 8.315 J/mol·K.) (6 marks)

(b) Explain why it is important that gluconeogenesis is not the exact reversal of glycolysis? (5 marks)

(c) People with beriberi, a disease caused by thiamine deficiency, have elevated levels of blood pyruvate and α-ketoglutarate, especially after consuming a meal rich in glucose. With reference to the Kreb's cycle, explain how these effects related to a deficiency of thiamine. (8 marks)

[TOTAL MARKS = 25]

Question 4

(a) (i) Oxaloacetate is formed in the last step of the Citric Acid Cycle by the NAD^+-dependent oxidation of L-malate. Examine the possibility of a net synthesis of oxaloacetate from acetyl-CoA occur using only the enzymes and cofactors of the citric acid cycle, without depleting the intermediates of the cycle. Indicate how is oxaloacetate that is lost from the cycle (to biosynthetic reactions) replenished. (5 marks)

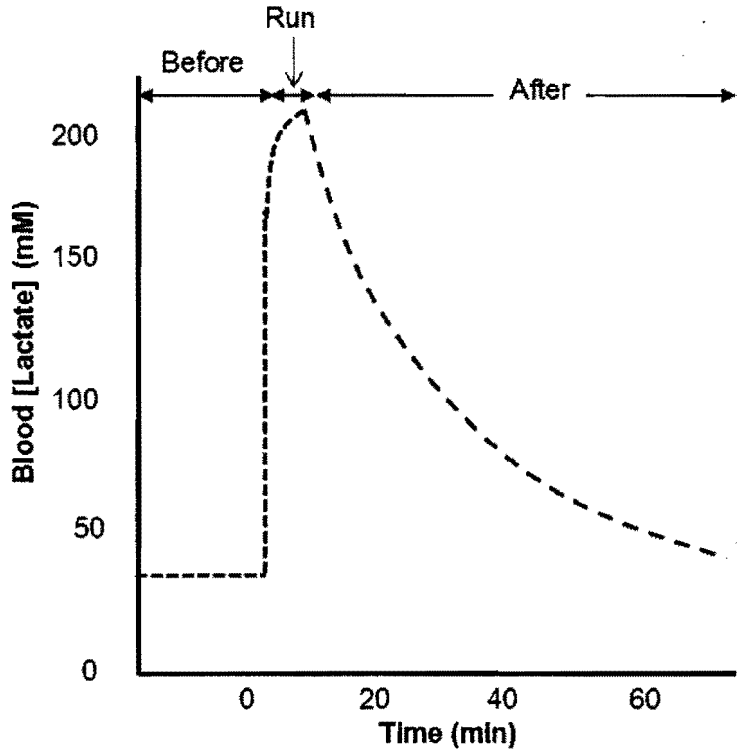
(b) Although oxygen does not participate directly in the citric acid cycle, the cycle operates only when O_2 is present. Explain this fact. (5 marks)

(c) When the acetyl-CoA produced during β-oxidation in the liver exceeds the capacity of the citric acid cycle, the excess acetyl-CoA forms ketone bodies such as acetone, acetoacetate, and D-β-hydroxybutyrate.

(i) Explain why this occurs in severe, uncontrolled diabetes. (2 marks)

(ii) Although acetyl-CoA is not toxic, the mitochondrion must divert the acetyl-CoA to ketone bodies. Explain the problem that would arise if acetyl-CoA were not converted to ketone bodies, highlighting how this diversion to ketone bodies solves the problem. (6 marks)

(d) The concentrations of lactate in blood plasma before, during, and after a 400 m sprint are shown in the graph.



(i) Explain what causes the rapid rise in lactate concentration during the run and its decline after completion of the sprint. (3 marks)

(ii) Explain why the decline occurs more slowly than the increase and why the concentration of lactate is not zero during the resting state. (4 marks)

[TOTAL MARKS = 25]

Question 5

(a) Describe and explain the chemiosmotic model for ATP synthesis. (15 marks)

(b) Discuss the effect of macronutrient malnutrition in a human being. (10 marks)

[TOTAL MARKS = 25]

Question 6

(a) The DNA below is replicated from left to right. Strands A and B are templates for which strands during DNA replication? (1 mark)

Strand A **5'-ACTTCGGATCGTTAAGGCCGCTTTCTGT-3'**
Strand B **3'-TGAAGCCTAGCAATTCCGGCGAAAGACA-5'**

(b) Write down the missing terms/phrases in the following paragraph (4 marks)

When both strands of DNA serve as templates, the mechanism of DNA replication is said to be (i) _____. DNA replication in *E. coli* begins at a site in the DNA called the (ii) _____. At the replication fork the (iii) _____ strand is synthesized continuously while the other strand is synthesized discontinuously. On the strand synthesized discontinuously, the short pieces are called (iv) _____. An RNA primer for each of the pieces above is synthesized by an enzyme called (v) _____, and this RNA primer is removed after the fragment is synthesized by the enzyme (vi) _____, using its (vii) _____. The nicks left behind in this process are sealed by the enzyme (viii) _____.

(c) (i) Describe briefly the relationship between chromatin structure and transcription in eukaryotes. (3 marks)

(ii) Explain the catabolite repression of the *lac operon*, highlighting the effect on the expression of lac genes when both lactose and glucose are available as carbon sources either individually or together. (7 marks)

(d) A given mRNA sequence might be translated in any of three reading frames. Describe how prokaryotes and eukaryotes determine the correct reading frame. (4 marks)

(e) The following DNA sequence occurs in the non-template strand of a structural gene in a bacterium (the promoter sequence is located to the left but is not shown):

5'-GAATGTCAGAACTGCCATGCTTCATA↓TGAATAGACCTCTAG-3'

(i) Give the mRNA sequence that is transcribed from this piece of DNA. Label its 5' and 3' ends (1 mark)

(ii) Use the Genetic Code and the answer to **Q6(d)** above to decipher the amino acid sequence of the polypeptide encoded by this mRNA. (2 marks)

(iii) If the nucleotide indicated by the arrow undergoes a mutation that changes **T** to **A**, give the new amino acid sequence that results following transcription and translation. (***You may refer to page 7***). (3 marks)

[TOTAL MARKS = 25]

THE END OF QUESTION PAPER

The Genetic code