Universidade de São Paulo - Instituto de Química
Admission exam for the graduate program in Biochemistry
February 2013

Name: _____

Answer each question in their own page. Do not answer more than one question per individual page because they will split among the reviewers for correction. This page may be used as a draft for all questions. Remember to write down your name in all pages of the exam.

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1. In the laboratory you need to perform the cultivation of hydroponic plants. For this purpose you need to prepare a culture medium that, in addition to various nutrients, need a strict control of pH.

The plant you want to cultivate grows only under pH values between 5 and 6. You are the only one biochemist in the laboratory and have a task to prepare 1 L of a 0.1 M buffer solution to be used in the cultivation of plants. Unfortunately the pH meter is not working properly. The solutions available are in the laboratory are:
- 2 M acetic acid, pKa = 4,5
- 2 M phosphoric acid, pKa1 = 2,13 pKa2 = 7,21 pKa3 = 12,32
- 2 M HCl
- 2 M NaOH
- Ultrapure H_2O

Note: Henderson-Hasselbalch equation

$$\mathrm{pH} = \mathrm{p}K_\mathrm{a} + \log \frac{[\mathrm{A}^-]}{[\mathrm{HA}]}$$

a) Describe how you would proceed for the preparation of the buffer, indicating the solutions used and the respective amounts.
b) What are the factors that affect the efficiency of a buffer? Justify.
c) Draw a graph of pH as a function of equivalents of OH-, showing the buffering region of the buffer you have prepared.
d) Could you use the same buffer for the cultivation of another plant whose pH optimum is between 6 and 7? Justify.

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2. Hemoglobin and myoglobin are globular metalloproteins containing prosthetic groups (heme and iron). Both are able to tightly bind oxygen. Myoglobin contains only one polypeptide chain while hemoglobin is a multimeric protein, containing two alpha and two beta chains. In adults, myoglobin is found exclusively in muscle and hemoglobin in erythrocytes.

a) Define, what are: 1) globular and fibrillar proteins; 2) prosthetic groups; 3) methalloproteins.

b) What is the importance of multiple polypeptide chains in hemoglobin compared to a single chain in myoglobin? How does this structural feature affect their function in the organism?

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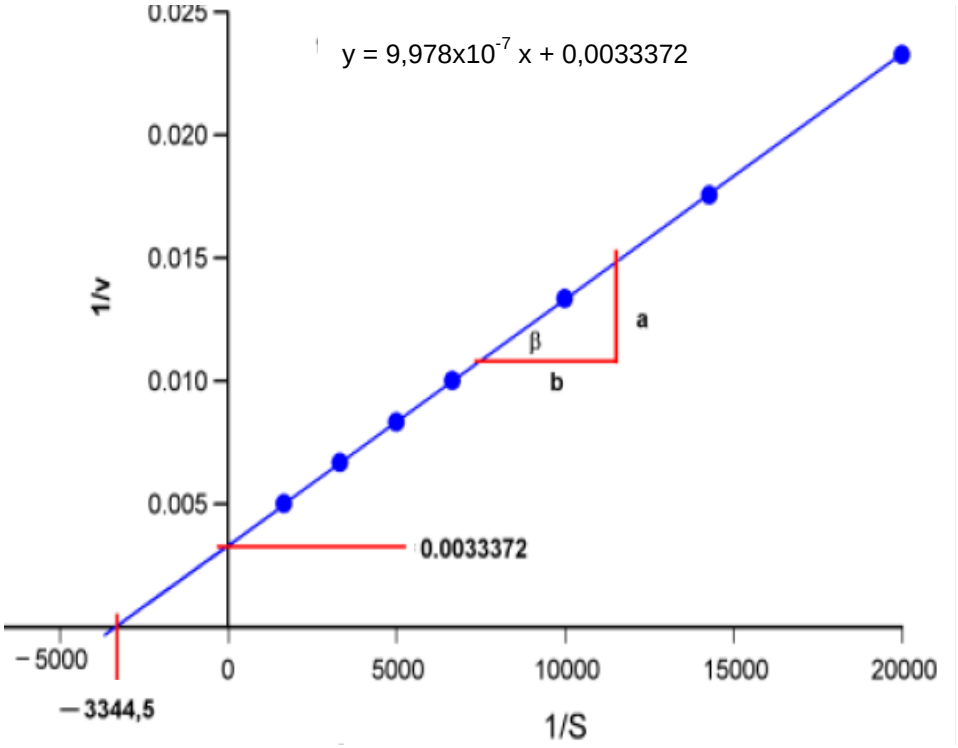
3. In liver injury, an enzyme named E_{1A} escapes from hepatocytes to blood plasma. Likewise, after intense exercise an isoenzyme from muscle, E_{1B}, is released from to plasma serum. E_{1A} and E_{1B} possess different K_M. The muscle enzyme has greater affinity by the substrate and presents a K_M 2 x 10^{-5} M. Studies in enzyme kinetics from blood plasma presented the results depicted in the graph (see below). From these results, conclude if the patient is undergoing a liver injury or if he has worked out too much. Justify your answer.



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4. One of the central hypotheses in the study of membrane lipids is that the membrane must be fluid (as opposed to "freezed") so that the membrane can perform its functions. A support for this hypothesis is provided by the observation that membrane fatty acid composition can be changed by conditions under which bacteria grows. For example, if the bacterium is growing at temperatures lower than normal, the observed quantities of unsaturated fatty acids (relative to the content of saturated fatty acid) are above normal. Conversely, if the bacterium is growing in a temperature above normal the quantities of unsaturated fatty acids (relative to the content of saturated fatty acids) found in the membrane is below normal.

a) Explain why the bacterial membrane lipid composition must be fluid for the membrane to operate properly.

b) Explain how the changes observed in the levels of unsaturated fatty acids relative to the saturated fatty acids at different growth temperatures supports the hypothesis of membrane fluidity.

c) Draw the structure of a saturated fatty acid with 18 carbons (C18:0, stearic acid acid) and an unsaturated fatty acid with 18 carbons with one unsaturation (C18: 1 Δ^{9}, oleic acid). Why the presence of unsaturation causes the membrane to be more fluid?

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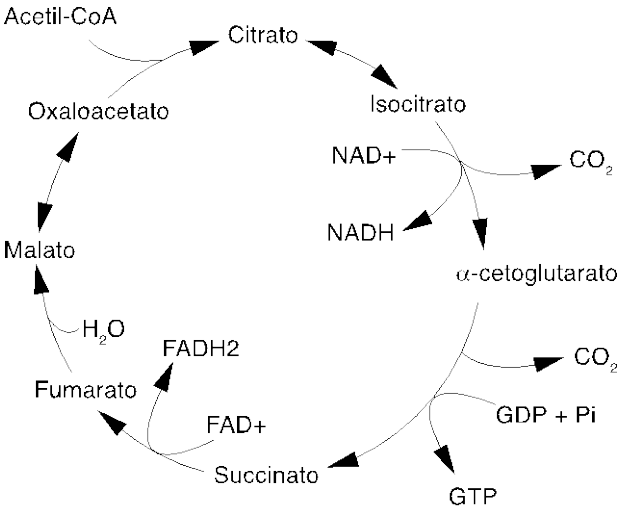
5. A suspension of mitochondria was dialyzed and incubated in isosomotic buffer containing 100 mmols acetyl-CoA, 2 mmols oxaloacetate, 5 mmols NAD^+, 30 mmols GDP, 20 mmols Pi (inorganic phosphate) and excess 2,4-dinitrophenol (DNP). Assuming that mitochondria is permeable to all these compounds, determine which compounds and their concentrations will be present at the end of the reaction. And what would happen if DNP were omitted from the reaction? Justify your answer.



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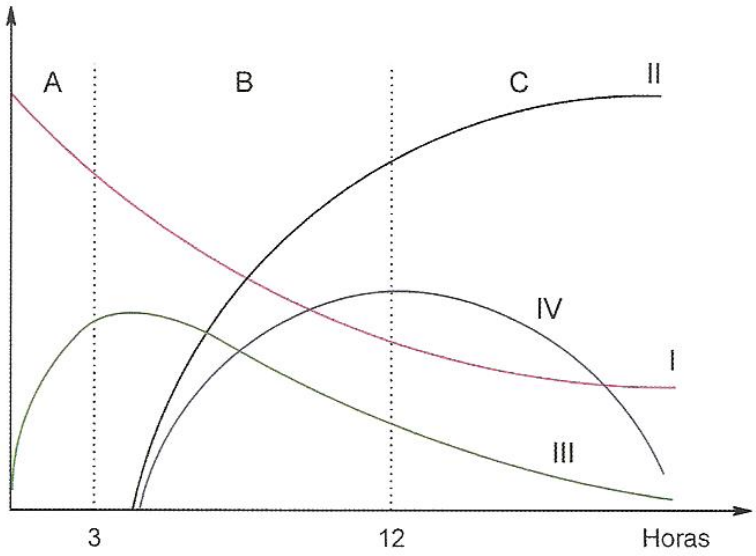
6. Observe the lines in the graph below. The data were collected after a meal (zero time in the graph). Analyze the sentences below and indicate with a T if it is a true sentence or with a F if it is a false one. JUSTIFY your answers. Only justified sentences will be considered.



a) Line I represents the concentration of insulin in blood plasma
b) Line I represents the intensity (degree) of gluconeogenesis
c) Line II represents glycolysis activity in adipocytes
d) Line III represents the consume of glucose from exogenous source
e) Line III represents the intensity of protein synthesis
f) Line IV represents the liver glycogen consume
g) The synthesis of adenosine monophosphate cyclic (AMPc)
h) The ratio glycolysis/gluconeogenesis is greater in B than in A
i) The Krebs Cycle has its maximal activity in C

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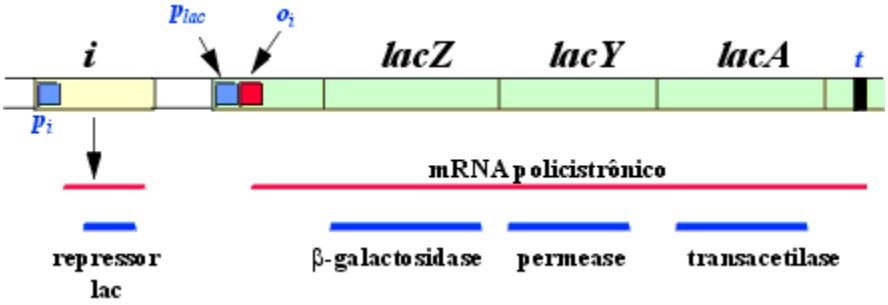
7. The figure below is a representation of the operon Lac from E.coli. Use it to answer the following questions:

a) Define promoter, operator and repressor.

b) Explain how the expression of the genes lacZ, lacY and lacA is controlled in response to available carbohydrates present in the growth medium.

c) The dissociation constant for the complex repressor/operator is approximately 0.1 pM. Explain the importance of such high affinity constant for the function of the operon.



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8 The mechanisms of DNA replication have benefited from studies that utilize bacterial strains in which the gene (or genes) that code for one of more enzymes believe to participate in this process have been removed from the genome. In the hypothetical laboratory "A", researchers generated a bacterial strain that express a functional DNA ligase only when the bacterial is cultured at 25^{o}C. If the bacteria is cultured at 37^{o}C, the DNA ligase is not functional. This bacterial mutant was then grown, in different tubes, at 25^{o}C or at 37^{o}C in the presence of ^{3}H-thymidine for 1 hour (the replication time of E.coli is about 20 minutes). At the end of the incubation time, the genomic DNA was isolated from bacteria grown at each temperature, analyzed by agarose gel and exposed to X-ray films. Describe the radioactive pattern you expect to see on the films in each condition. Justify your answer.