July 17, 2000

ID number _________

Biochemistry Cumulative Exam

Isotopic labeling is a very important tool in biochemical and biophysical analysis. It has been used in the past to delineate metabolic pathways, and is still being used for this purpose today. Recently, it has also been used in biophysical analysis to understand the origin of specific spectroscopic signals in biological molecules.

As a biochemist, you will need to be aware of and understand the major metabolic pathways, so that you can predict the results of an experiment. You will use that knowledge in this exam to answer questions about both kinds of experiments described in the preceding paragraph.

You may not use any textbooks or other materials on this exam, except for those given to you.

For this exam, showing me your reasoning is even more important than getting the right answer. (*i.e.* If you simply guess correctly, it will not count for much. If you demonstrate that you know the pathway, but make an error, you will get some credit.) You must demonstrate to me that you both understand the pathways and that you can think the problems through for a passing grade.

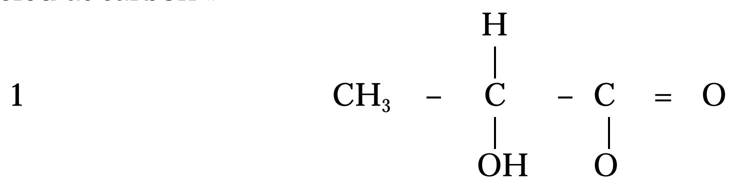
Pass or Fail: _________

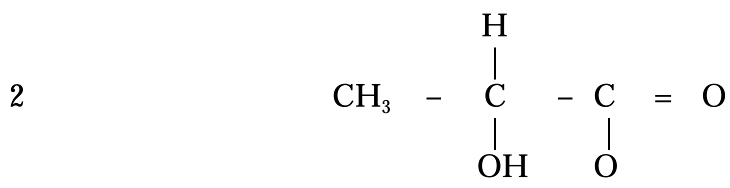
1. You make a cell-free system using pigeon muscle pulp (*i.e.* cytosol) as your source of enzymes and use this to study glycolysis. If you add glucose to the system, you get lactate (CH_3-CHOH-CO_2^-) as the fermentation end product. (The TCA cycle is not operative in this extract.)

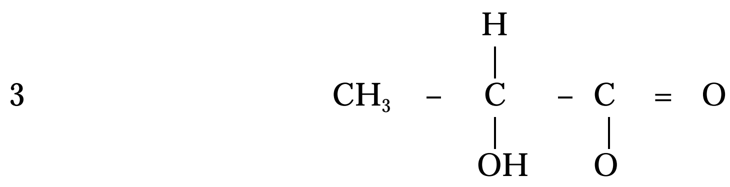
a) If you add glucose labeled with ^{14}C as the C-1 position, where would you expect to find the ^{14}C label in the product? Show how the label gets there explicitly with all intermediates drawn.

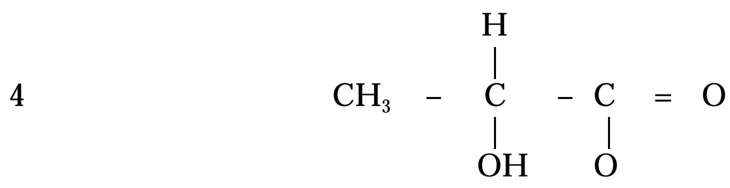
b) You go through the whole series of the positions on the glucose molecule (2 through 6), with each carbon specifically labeled as ^{14}C. Show on this structure of lactate which carbon(s) would be labeled after glycolysis of the indicated ^{14}C-glucose:

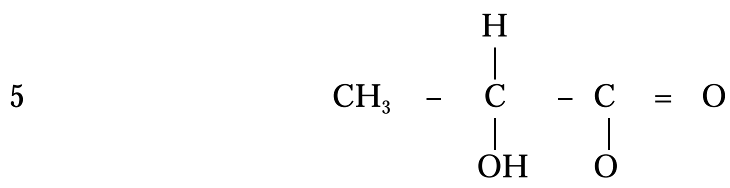
Glucose labeled at carbon #

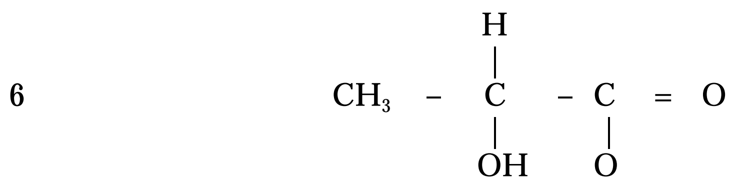












2. For another experiment, you need to produce large amounts of glutamate labeled with ^{18}O on the γ-carboxylate. For various reasons, it is cheaper to feed a bacterium with ^{18}O-labeled glucose and then isolate the glutamate from the bacterium than to simply synthesize the ^{18}O-glutamate directly. Also, your company happens to have a lot of ^{18}O-labeled glucose lying around. However, the person in charge of the isotope stocks has three different bottles with ^{18}O-glucose labeled at C-2, C-3, or C-6. He says that one of them should work to label glutamate, you just have to work out how glucose feeds into glycolysis and the Krebs (TCA) cycle up to the precursor of glutamate to figure out which one to use.

   Which one would you use? Justify your answer.

3. Researchers working on the bacterium *Bacillus gogeticus* discover a novel cytochrome containing heme *a*. At one point, they measure the FTIR (Fourier transform infrared) spectrum of the reduced and oxidized cytochromes and calculate a difference spectrum, which can identify those vibrational modes that are specific to the heme or nearby amino acid residues (*i.e.* those that will experience the change in charge). The FTIR spectroscopist finds a prominent band at 1720 cm^{-1} that is upshifted to 1735 cm^{-1} upon oxidation and tentatively assigns it to the C=O of the formal group attached to heme *a*. In order to test this assignment, he would like a sample isotopically labeled (with ^{13}C) at that carbon. It is your job to produce such a sample.

   On the next page is shown the pathway from succinyl-CoA and glycine to δ-aminolevulinate, its subsequent condensation into porphobilinogen is shown below, as well as porphobilinogen's condensation ultimately to uroporphyrinogen III; also shown are the structures of protoheme and heme *a*.

   Unfortunately, *B. gogeticus* will not take up either glycine or succinyl-CoA. However, it will import serine and a-ketoglutarate, and it possesses a serine hydroxymethyl transferase and all of the TCA (Krebs) cycle enzymes. Using what you know and the information given here, devise a strategy to label the formyl carbon of heme *a* with ^{13}C. (*i.e.* tell me which carbon of which molecule you will label and feed to the bug). Show how you expect it to be incorporated.

   Will the formyl carbon be the only atom of heme *a* labeled? If not, which other atoms will be labeled using your strategy? And would they pose a problem for the spectroscopy? Explain why or why not.

   If the FTIR spectroscopist was right about his assignment of the 1720/1735 cm^{-1} band, and you succeed in making a sample with the formyl group labeled with ^{13}C, what sort of effect would you expect the this to have upon the FTIR difference spectrum?