Biological Sciences 103
Summer, 2026
K. Hilt

Answer Key

Homework #1

Learn the structures, enzyme names, cofactor/coenzyme requirements, and regulation of glycolysis and alcoholic fermentation (see page 3). Know which reactions are reversible and irreversible.

Be able to draw the structure of a triglyceride (triacylglycerol, fat) and the phospholipids phosphatidylcholine and phosphatidylserine with any of the five fatty acids attached: 16:0, 18:0, 18:1(9), 18:2(9,12), and 18:3(9,12,15); palmitic, stearic, oleic, linoleic, and α-linolenic, respectively.

Assigned problems in *Biochemical Calculations* (Segel):

1. Weak acids/bases 29, 38 (a, d, j), 40, and 41 on pages 92 and 93
2. Beer's Law calculations: example 5-4 on page 332; #4 on page 352
3. K'_{eq} and ΔG calculations: examples 3-3, 3-4, and 3-5 on pages 161 – 164

Comments on ***Biochemical Calculations*** problems:

1. In #29, do not forget that the addition of 0.06 moles of H^+ generates 0.06 moles of NH_4^+. We also have 0.02 moles of NH_3 remaining. Use the Henderson-Hasselbalch equation to solve.
2. In #38a, do not forget that you need one equivalent of OH^- to change all of the H_3PO_4 into $H_2PO_4^{-1}$. Then use the Henderson-Hasselbalch equation to solve for "b", using the pKa of 7.21.
3. In #40, addition of H^+ causes a decrease in the pH. You will need two Henderson-Hasselbalch equations to solve this. The first equation yields the [b] and [a] at time zero. The second equation essentially works backwards: the new H-H equation is pH = 7.21 + log {[b] – [x]}/{[a] + [x]}. We know [b] and [a] from the first H-H equation and x = 0.004 M.

1. Learn the laboratory steps needed to conduct a DNA microarray experiment. Go to http://learn.genetics.utah.edu/content/labs/microarray/ . Click on the microarray image and go through the three chapters that are listed. Answer the questions that they have at the end of their chapter 3. The whole interactive laboratory exercise should take 30 minutes or less to complete and will strengthen your understanding of DNA microarray experiments. (Your computer will need the program "Adobe Flash" to run the laboratory exercise. You may already have this program. If not, download it for free from the internet).

2. A microcalorimetry experiment was done on spinach plants, following the same protocol that we discussed in lecture. Several spinach plants were "control" plants, i.e. nothing unusual was done to them. However, several plants were treated with a particular pesticide for several days.

Leaves from the control plants were placed into ampule #1. Leaves from the pesticide treated plants were placed into ampule #2. The microcalorimetry experiment was performed just as we discussed in class: in part "A" 40 µl of water was placed in the well; in part "B" 40 µl of 0.4 M NaOH was placed in the well; in part "C" 40 µl of water was placed in the well.

Now let's plot the results of this experiment. Make a graph; label each axis. Draw in carefully the results from ampule #1: a heat rate of 75 µW/mg in part "A"; a 20% increase in heat rate when 0.4 M NaOH is added to the interior well in the ampule. What should part "C" look like for ampule #1?

What would the plot look like for ampule #2 if the same mass of spinach leaves treated with pesticide gave a 30% decrease in heat rate/mg in part "A" (relative to part A for ampule #1); and the same heat rate/mg in part "B" as was seen in part "A" for ampule #2? What should part "C" look like for ampule #2?

What do you conclude about the effects of the pesticide on the metabolism of the spinach leaves?
Refer to your notes on how to make the above plots. The pesticide is reducing the heat rate of the plant (seen in part "A"); not a good thing. Part "B" shows that CO_2 production has been reduced to non-detectable levels; also not a good thing.

3. A student runs an enzyme assay using the anaerobic glycolytic enzyme, lactate dehydrogenase (LDH). Their 1 ml assay is:

900 µl	200 mM Tris, pH 7.3
35 µl	30 mM pyruvate (in H_2O)
35 µl	6.6 mM NADH (in H_2O)
30 µl	of their LDH solution, in 200 mM Tris, pH 7.3

They mix everything gently together in a cuvette and then measure the $\Delta A_{340}/\Delta$time using a spectrophotometer. Their measured ΔA_{340}/30 sec. was "-0.0311".

a) On the enzyme kinetic graph, what is plotted on the x and y axes? Refer to your notes.
b) What does the "-" in "-0.0311" tell you? The compound that you are measuring is being consumed.
c) How many I.U.'s of LDH were present in the assay? $\varepsilon_{NADH,\ 340\ nm} = 6220\ M^{-1}cm^{-1}$. 0.01 I.U.
d) Let's say that the total time of your LDH assay was 2 minutes. Did the pH of the assay change much during that time? Show a calculation. (Model your calculation after questions #40 and #41 on page 93 of *Biochemical Calculations*). The pK_a of Tris is 8.21.
No, the pH remained at 7.3. Use two Henderson-Hasselbalch equations.

e) If the pH changed in part "d" above, what would you do to minimize the pH change? Give three different answers. 1) Run the assay for a shorter period of time. 2) Use a higher molarity buffer. 3) Use a buffer with a pKa such that there is more "a" than "b" at time zero.

f) You need to make up 500 ml of 200 mM Tris buffer, pH 7.3, in order to run your LDH assays. You have three solutions already made up in the lab: 1.0 M Tris solution, pH 9; 1.0 M HCl; and 2.0 M NaOH. You also have water. How will you make up the buffer? (Note: you do not need to use all of these solutions).
100 ml 1.0 M Tris, pH 9 + 75 ml 1.0 M HCl + 325 ml water

g) To check whether Tris interferes in the enzyme assay, you decide to run the enzyme assay using 200 mM phosphate buffer, pH 7.3. How will you make up 500 ml of this buffer, using 1.0 M H_3PO_4, 2.0 M NaOH, and water? The pK_a 's of phosphoric acid are 2.12, 7.21, and 12.32. How will you decide whether to use Tris or phosphate in your future LDH assays?
100 ml 1M H_3PO_4 + 77.6 ml 2M NaOH + water to 500 ml.

Which buffer to use? Run the enzyme in each buffer. If they give the same v_o, then use either buffer. If one buffer gives a much faster v_o, then either one buffer is activating the enzyme or the other is inhibiting it. Make up a third buffer, using a man-made compound, such as HEPES. Assay the enzyme and see if this buffer matches one of the other two. Usually go with the man-made buffer.

h) Why do you need a buffer in an enzyme assay, even for assays where no H^+ is a substrate or product? Explain. If there is more than one reason, then give them all.
One reason is that most substrates have charges and/or hydrogen bonds that are necessary for proper binding to the enzyme active site. Another reason is that enzymes require hydrogen bonds for their correct secondary, tertiary and quaternary structure. Enzymes also require ionic bonds for their correct tertiary and quaternary structure. Both hydrogen bonds and ionic bonds are affected by changes in pH.

i) Bovine LDH has a native MW of 140,000. It is comprised of four equal sized subunits. When purified from steak, LDH can have either an M subunit or an H subunit (M = muscle; H = heart), with the native LDH enzyme being either M_4, H_4, or any variation in between, i.e. MH_3, etc. The pI of the M subunit is 8.2, while that of the H subunit is 6.0. What will the banding patterns look like for bovine LDH (containing all of the isozymes) run on the following gels: a) an IEF gel; b) a native PAGE gel; c) an SDS-PAGE gel; and d) a 2D IEF/SDS-PAGE gel? Draw your gels. Label each band on each gel with its name and MW. Label your electrodes. Assume that each gel was stained with Coomassie Blue and then destained after electrophoresis.

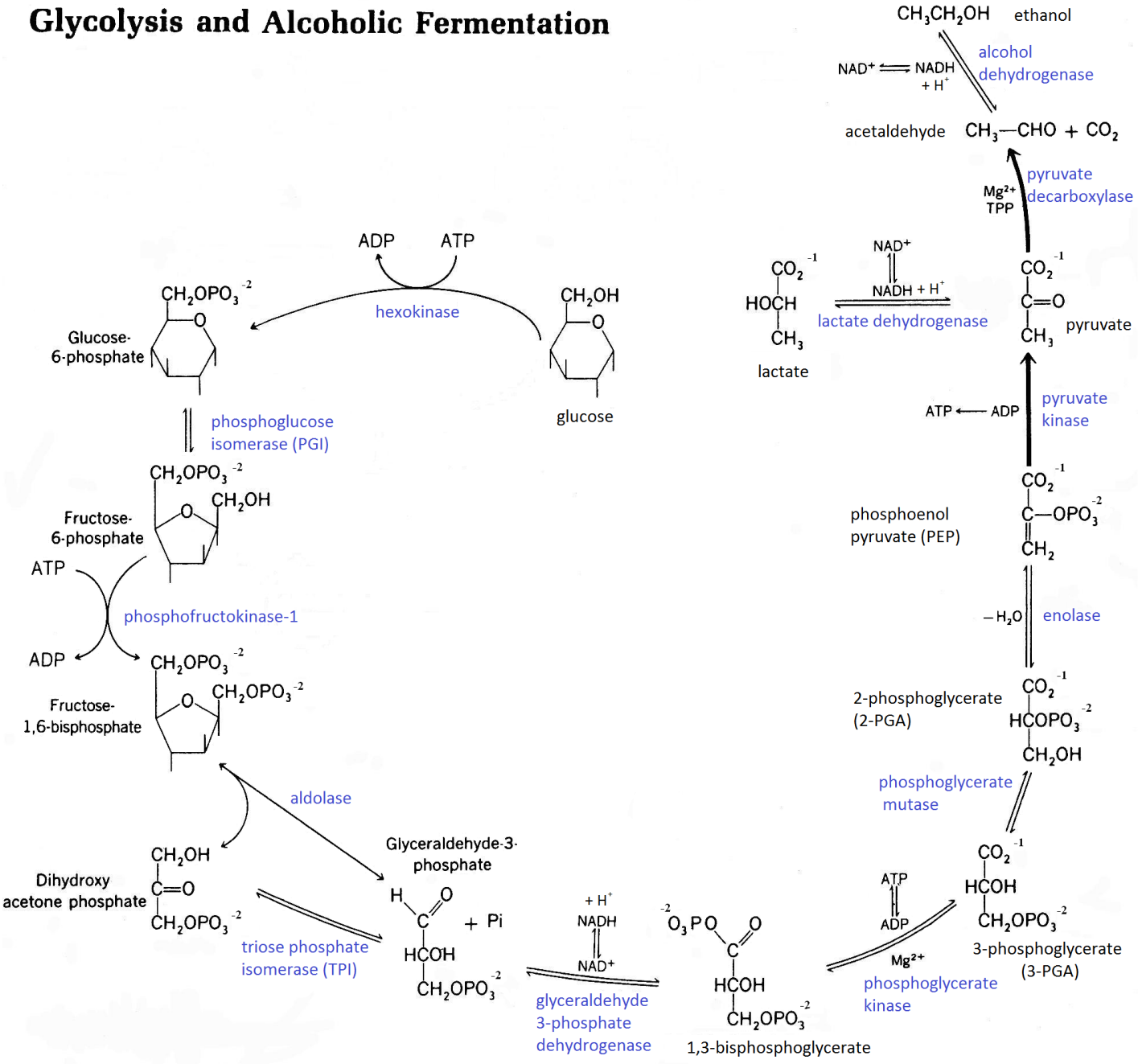
The IEF gel will give five bands; each band with a MW of 140,000. H_4 will band closest to the "+" electrode.

The native PAGE gel will give five bands, with H_4 running the fastest.

An SDS-PAGE gel will give one band with a MW of 35,000.

The 2D IEF/SDS-PAGE gel will give a single band of 35,000 MW beneath each position in the IEF gel where there was initially a protein band.

Glycolysis and Alcoholic Fermentation



Note: the vertical lines on glucose, glucose-6-P, fructose-6-P, and fructose-1,6-bisphosphate are OH groups.

The above diagram is adapted from *Outlines of Biochemistry*, 3rd ed. by Eric E. Conn and P.K. Stumpf. Professors Conn and Stumpf founded the biochemistry department at UC Davis.