CHEM-342 Introduction to Biochemistry **Group Members**
Final Examination - Group (Part II)
Thursday, 24 May 2012 _____
5:30 – 6:30 PM
H. B. White – Instructor _____

30 Points _____

N = 7 groups _____
Range = 16-25
Mean ± SD = 20.5 ± 3.1 _____

Important - Please read this before you turn the page.

- You must sign your name on this page to receive the group grade.

- You may refer to your notes, course reader, handouts, or graded homework assignments.

- In CHEM-342, hemoglobin is a vehicle for learning how to learn by asking questions and pursuing answers to those questions. Undoubtedly you have learned a lot about hemoglobin in the process but you also should be developing habits of mind that will enable you to solve problems in other courses and throughout your life. This part of the final examination provides an opportunity for you and the other members of your group to display problem-solving skills as a team. It is extremely unlikely that anyone in your group or in the class has encountered the information on the following pages. Your answers should display your collective:

- breadth of knowledge (not limited to hemoglobin or biochemistry)

- ability to analyze, make connections, and ask probing questions

- sense of logic and organization

- skill at generating models (testable hypotheses)

1. (12 points) In 1946, Shemin and Rittenberg discovered by accident that glycine was a direct precursor of the heme group of hemoglobin. Shemin consumed a total of 66 g of the ^{15}N-labeled glycine at hourly intervals for almost 3 days. Then he removed samples of his own blood periodically for more than 7 months and had the globin and heme separately analyzed for incorporation of ^{15}N. In related study, he analyzed stercobilin, an excreted product of heme (hemin) breakdown derived from bilirubin, as shown below [*J. Biol. Chem*, **184**, 351 (1950)].
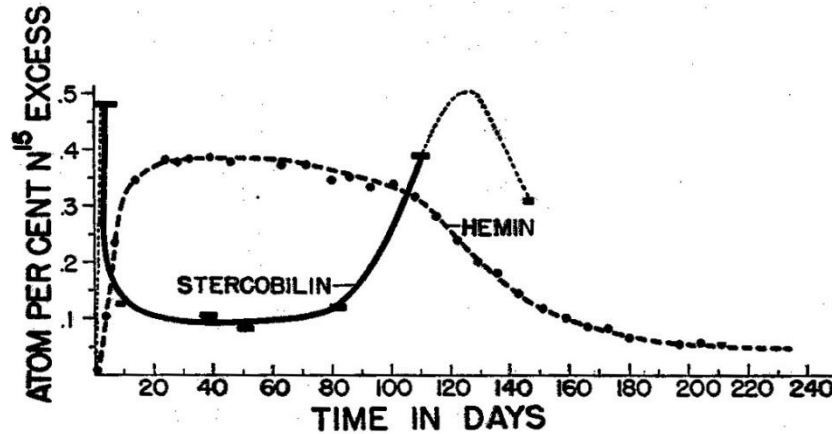

Fig. 1. ^{15}N concentration in heme and stercobilin of a normal man after the start of feeding ^{15}N-labeled glycine for 2 days.

   a. (6 points) Based on the information in Shemin & Rittenberg (1946) and information you have gained from other sources, construct a model (draw a diagram) of the relationship of heme, globin, bilirubin, and stercobilin to glycine as revealed by pulse-case experiments with ^{15}N from glycine.

   b. (6 points) Explain how it is possible for the atom percent ^{15}N excess in stercobilin between 110 and 140 days is greater than it ever was in heme isolated from hemoglobin.

2. (10 points) Pauling et al. (1949) observed that there was a small amount of hemoglobin that moved with a different mobility than HbA or HbS. This most likely was HbA$_{1c}$, a post-translationally modified HbA in which the N-terminal valine of the beta chain has reacted with glucose reversibly and then undergone an Amadori rearrangement. This is a nonenzymatic reaction that depends on the concentration of glucose. Consequently, levels of HbA$_{1c}$ are often monitored in people with diabetes.

Bunn et al. (1976) [*J. Clin. Invest.* **57**,1652-1659] studied the synthesis of HbA$_{1c}$ in two healthy volunteers who were injected with 20 μCi of ^{59}Fe transferrin. Thereafter at approximately 2 week intervals for 100 days, 40 ml of blood were taken, the hemoglobin HbA and HbA$_{1c}$ separated from red blood cells, and the radioactivity measured. Their results are shown in the graph below.
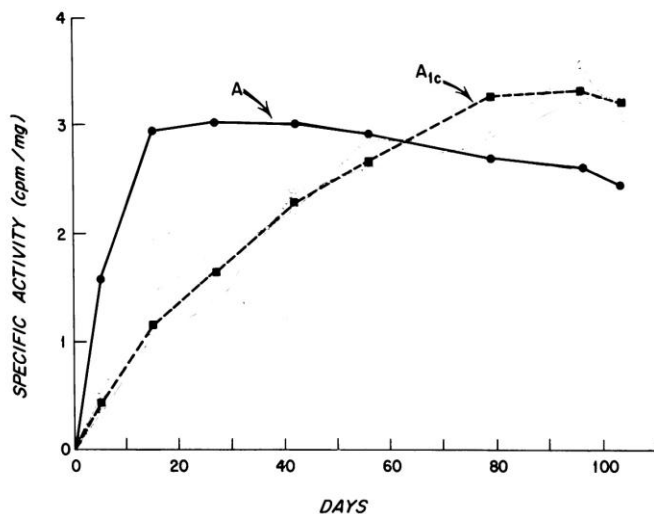

Extend the x-axis to 150 days and predict how each of the lines would look. Explain your reasoning. A diagram showing the relationships of the precursors to intermediates and products would be helpful.

3. (8 Points) Bohr et al. (1904) and many others who measured the oxygen binding curve for hemoglobin used whole red blood cells. When the same studies were done on purified "stripped" hemoglobin, the oxygen binding curve shifted to unphysiologically low oxygen concentrations. More than 60 years later, Reinhold and Ruth Benesch [*Nature* **221**, 618 (1969)] showed that 2,3 diphosphoglycerate (DPG), a modified intermediate of glycolysis, occurred at high (5mM) concentrations in red blood cells and that it bound stoichiometrically to hemoglobin and restored the physiologically normal oxygen binding curve found in red blood cells.
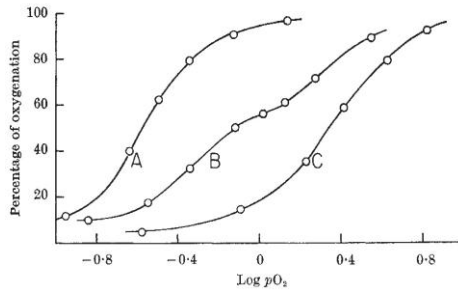
Oxygenation curves for 0.8% Hb in 10mM NaCl at 10ºC and pH 7.0.
A = "stripped" Hb
B = "stripped" Hb = 0.5 mole DPG/mole Hb
C = "stripped" Hb = 1.0 mole DPG/mole Hb
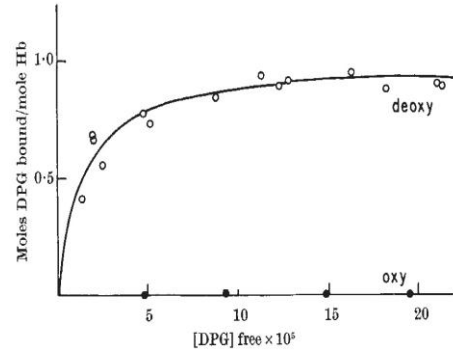
Binding of DPG to hemoglobin in 0.1M NaCl, pH 7.3, at 22±2ºC.

We know that hemoglobin has four heme binding sites. Hemoglobin with its $\alpha_2\beta_2$ structure has C_2 symmetry, which means you can rotate it 180º around its two-fold axis of symmetry and it will look the same. With that structure, it is easy to imagine there would be two DPG binding sites, but one? The figure below represents hemoglobin with its two-fold axis of symmetry orientated vertically in the plane of the paper. The beta subunits are shaded. Given the information, draw on the picture below where you predict DPG would bind? Why?