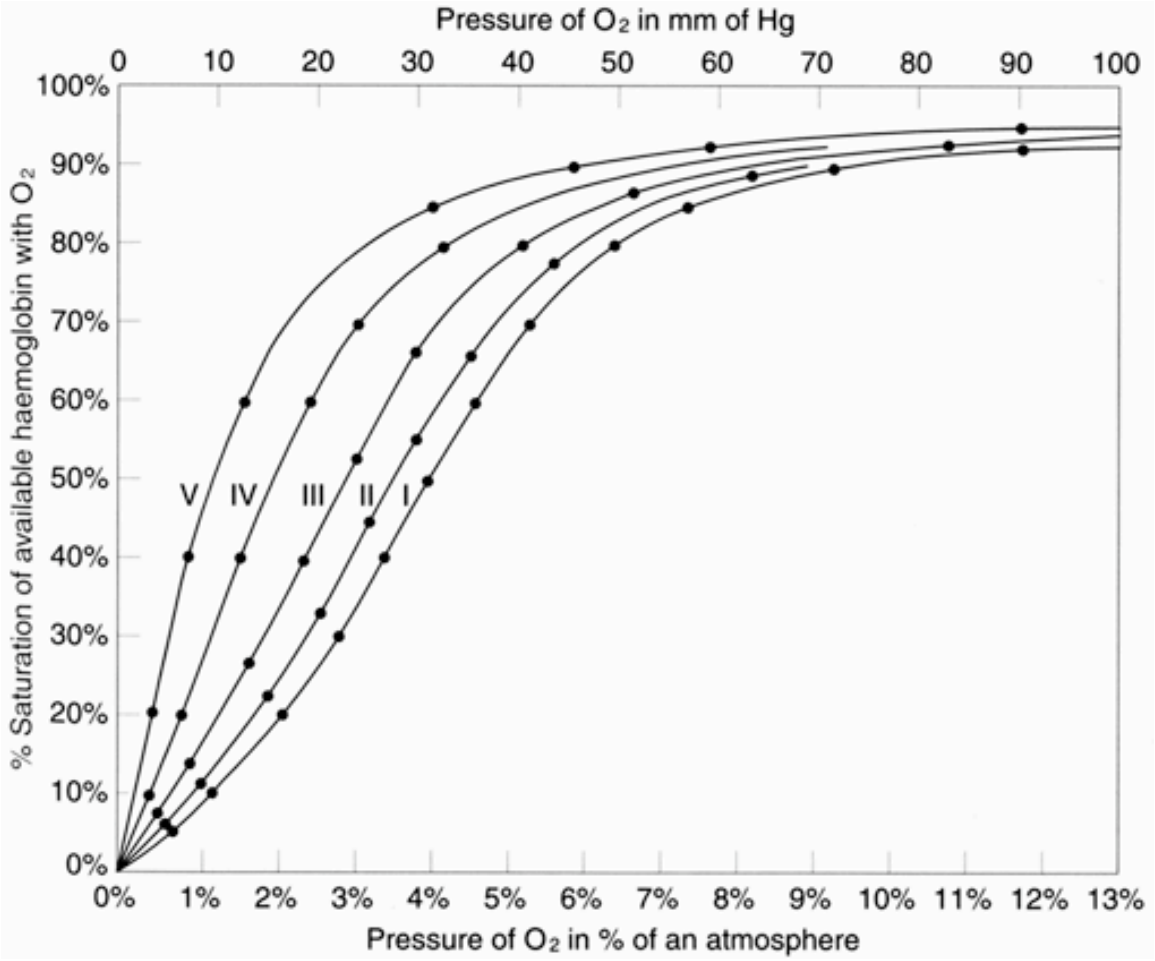
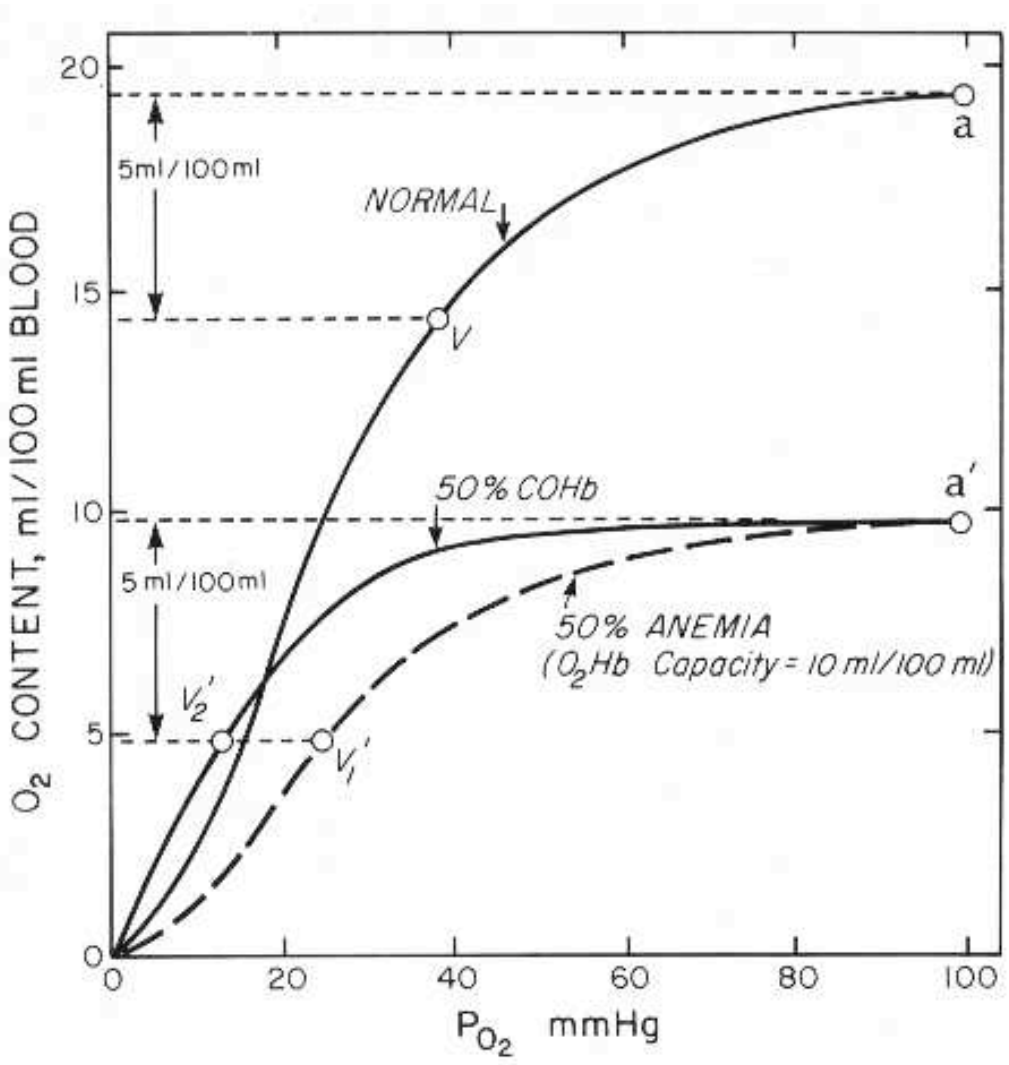
Question 1

Carbon monoxide (CO) is a potentially fatal poison. Amongst other things, CO exposure causes anoxemia, a reduced oxygen concentration in blood. CO binds avidly to hemoglobin (Hb), to form carboxyhemoglobin, COHb. For answering these questions, you can assume that if a sample of blood or Hb is described as containing (for example) 50% COHb, this means that 50% of the total available O_2 binding sites are occupied by CO. In this case, individual Hb molecules will be bound by, **on average**, two molecules of CO.



This diagram shows oxygen-binding curves for Hb, in the presence of increasing proportions of COHb (I = 0% COHb, II = 10% COHb, III = 25% COHb, IV = 50% COHb, V = 75% COHb). Here, oxygen binding on the y-axis is expressed as the % of available O_2 binding sites in Hb that are bound by O_2 (so each curve is normalized to 100%).

(a) What is the effect of CO on the oxygen binding properties of hemoglobin? On the basis of the shapes of these curves, how could you best describe the behavior of CO with regards to its effect on O_2 binding?



This diagram shows the oxygen binding curve of hemoglobin in the absence and presence of 50% COHb (curves I and IV on the previous Figure), but now oxygen binding is expressed as the oxygen content of whole blood.

(b) What are the **two** major differences between the oxygen binding curves in the presence and absence of COHb?

(c) Can you suggest **two** reasons why CO restricts the delivery of O_2 to tissues?

(d) Is CO poisoning (to a level of 50% COHb) more or less severe than 50% anemia, in terms of the ability of blood to deliver O_2 to tissues?

Question 2

Glyceraldehyde-3-phosphate dehydrogenase catalyzes the following reaction from glycolysis:

Glyceraldehyde-3-phosphate + P_i + NAD^+ $\longrightarrow$ 1,3-bisphosphoglycerate + NADH

In a spectrophotometer, NADH absorbs light at 340 nm, NAD^+ does not.

The **Beer-Lambert Law** relates the absorbance of a solution to its concentration, and states that:

$$A = \varepsilon.c.L$$

Where A is absorbance, e is the molar extinction coefficient, c is concentration, and L is the path length of the solution in the spectrophotometer. In all the calculations that follow assume L = 1 (*ie* all measurements were made in a cuvette with a 1 cm path length), so L can effectively be disregarded (although not its unit).

The molar extinction coefficient for NADH is 6,300 litres/mol cm^{-1} [or $(mol/litre)^{-1}$ cm^{-1} or M^{-1} cm^{-1}]. The molar extinction coefficient is the absorbance of a 1 molar solution in a 1 cm path length cuvette.

The molecular weight of NADH is 663.4.

In a 1 ml cuvette, you mix 0.1 ml of 2.5 mM NADH, 0.1 ml of 0.2 M glyceraldehyde-3-phosphate, 0.6 ml of a suitable buffer, and 0.2 ml of a solution of purified glyceraldehyde-3-phosphate. You mix, place the cuvette in a spectrophotometer (with a chart recorder attached) and follow the absorbance at 340 nm. You measure a rate of increase of absorbance (ΔA) of 0.73 min^{-1}. You are told that the glyceraldehyde-3-phosphate solution you used in the assay contained 8.68 mg of protein per ml.

(a) Calculate the rate of production of NADH in this assay, in units of micromoles/min [Tip: you need to consider the reaction volume]

(b) Calculate the **specific activity** of glyceraldehyde-3-phosphate dehydrogenase in this assay, in units of micromoles NAD^+ reduced/minute/mg protein

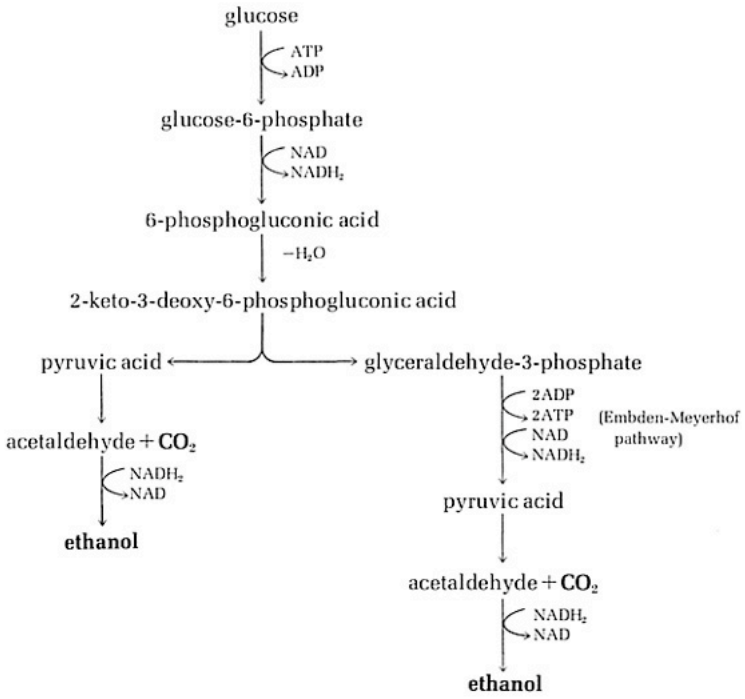
(c) Arsenate (AsO_4^{3-}) closely resembles P_i (inorganic phosphate) in structure and reactivity. In the reaction catalyzed by glyceraldehyde 3-phosphate dehydrogenase, arsenate can replace phosphate in attacking the energy-rich thioester intermediate. The product of this reaction, 1-arseno-3-phosphoglycerate, is unstable and it is rapidly and spontaneously hydrolyzed to 3-phosphoglycerate. What is the effect of arsenate on energy generation in a cell?

Question 3

(a) The lactic acid bacteria grow anaerobically converting glucose to lactate. Imagine a mutation in this organism that eliminates the activity of triose phosphate isomerase. What is the ATP yield of glycolysis in this mutant? Do you expect the mutant to be viable?

(b) Glycerol can enter glycolysis by the pathway described on pages 557-559. You might imagine that the lactic acid bacteria could grow on glycerol by converting it to lactate. What would the ATP yield of this pathway be? Draw out your proposed pathway from glycerol to lactate, and use it to explain why this pathway does not work as a means of sustaining growth.

(c) Glycolysis is not the only pathway for glucose catabolism. For example, some prokaryotes use a pathway called the Entner-Doudoroff pathway. The bacterium *Zymomonas mobilis* converts glucose to ethanol but, unlike yeast, does so using the Entner-Doudoroff pathway (fermentation by *Zymomonas* is important in the production of tequila). The ED pathway is shown below. Write an equation for the conversion of glucose to ethanol by the ED pathway and explain the major energetic difference between this pathway and glycolysis.



(d) Compounds that inhibit glycolysis are attracting interest as potential treatments for cancer. Why is this?

Question 4

Alcohol is detoxified in liver by conversion to acetaldehyde (by alcohol dehydrogenase) and then acetaldehyde is oxidized to acetate by aldehyde dehydrogenase. Both enzymes require NAD^+.

(a) Write balanced equations for the conversion of ethanol to acetate.

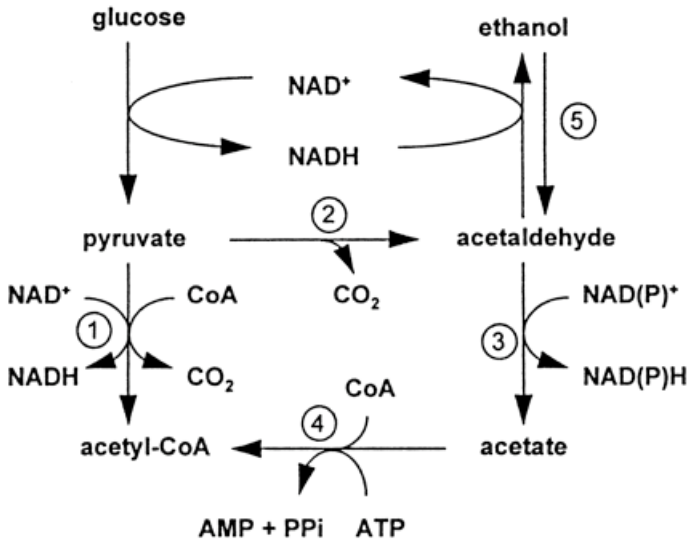
(b) Alcohol intoxication is often accompanied by the accumulation of lactate in the bloodstream. Can you suggest a biochemical explanation for why this would occur?

(c) Design an experiment to test the mechanism you suggested in (b).

(d) What are the possible metabolic fates of the acetate produced by these reactions?

(e) Ethylene glycol ($CH_2OH.CH_2.CH_2OH$) is a component of anti-freeze and a quite frequent cause of poisoning. An effective treatment for ethylene glycol poisoning is the administration of an intoxicating dose of ethanol. Explain how this treatment works.

Question 5



In most organisms, oxygen slows down glycolysis, and pyruvate is directed towards the TCA cycle rather than fermentative pathways (this is called the **Pasteur Effect**). The brewers' yeast *Saccharomyces cerevisiae* displays the opposite effect, called the **Crabtree Effect**, in which high concentrations of glucose accelerate glycolysis and the production of ethanol, even in the presence of oxygen. In other words, this organism generates ATP by fermentation even when oxygen is available. When the glucose concentration falls, the ethanol is taken back up, and it is converted to acetyl-CoA via acetaldehyde and acetate (the acetyl-CoA can then enter the TCA cycle).

(a) For each molecule of glucose that is converted to two molecules of acetyl-CoA via ethanol and acetate, what is the net yield of ATP and NADH?

(b) The inter-conversion of ethanol and acetaldehyde in *S. cerevisiae* is catalyzed by two isoenzymes of ethanol dehydrogenase. What predictions can you make about the kinetic properties of these enzymes?

(c) Energetically, it would seem more favorable to convert ethanol to acteyl-CoA via acetaldehyde and pyruvate. Why do you think this pathway does not operate?

(d) Do some research about the Crabtree effect online – can you give two possible explanations for why yeast makes ethanol under aerobic conditions?