Respiratory Physiology Lab

I. OXYHEMOGLOBIN SATURATION

MATERIALS:
1. Graduated cylinder and a 1-cc syringe
2. Test tube and distilled water
3. Spectrophotometer
4. Sodium dithionite (hydrosulfite), 1.0 g per 100 mL
5. A lancet for preparing fingertip blood (students provide their own)

OBJECTIVES:
1. Define the term *percent saturation*.
2. Explain the clinical significance of the percent oxyhemoglobin measurement.
3. Explain the clinical significance of the percent carboxyhemoglobin measurement.
4. Describe how an absorption spectrum is obtained, and explain how the absorption spectra of the different forms of hemoglobin are used to determine the percent saturation.

BACKGROUND:
The ability of the blood to carry oxygen depends on (1) ventilation; (2) gas exchange across the alveoli of the lungs; (3) the red blood cell count and hemoglobin concentration; and (3) the chemical form of the hemoglobin.

There are two chemical forms of normal hemoglobin. Normal hemoglobin without oxygen is called deoxyhemoglobin; after it binds to oxygen, it is called oxyhemoglobin. If a person suffers from carbon monoxide poisoning, however, the abnormal hemoglobin called carboxyhemoglobin (hemoglobin bound to carbon monoxide) causes the blood to carry a lower amount of oxygen. This is because the carbon monoxide displaces oxygen and binds to hemoglobin with a higher affinity than does oxygen. Therefore, when health professionals need to determine the oxygen carrying capacity of the blood, they need to learn the relative proportion of each hemoglobin type as well as the total hemoglobin concentration.

The relative proportion of each type of hemoglobin is given as its percent saturation. The percent oxyhemoglobin saturation, for example, is the proportion of hemoglobin bound to oxygen. Normally, this value is approximately 97% in arterial blood and 75% in venous blood. This determination of percent oxyhemoglobin saturation is a very sensitive means of assessing the effectiveness of pulmonary function. When pulmonary function and blood hemoglobin are normal, the arterial blood has a percent oxyhemoglobin saturation of about 97%. Even when pulmonary function is normal, the percent oxyhemoglobin saturation can be decreased by carbon monoxide poisoning. When a person’s blood has a carboxyhemoglobin saturation of 6.9%, for example (obtained in one study using cigarette-smoking New York taxicab drivers), the percent oxyhemoglobin saturation is decreased accordingly.

Another abnormal form of hemoglobin is methemoglobin, which is oxidized hemoglobin that lacks the electron needed to bond with oxygen, and therefore cannot participate in oxygen transport. Notice that these impairments in oxygen transport cannot be detected by standard measurement of red blood cell count, hematocrit, or total blood hemoglobin.

The percent saturation of the different types of hemoglobins is measured by comparing the absorption spectrum of an unknown sample of blood with the absorption spectra of pure oxyhemoglobin, pure reduced hemoglobin, and pure carboxyhemoglobin. Since these hemoglobins have different colors, they absorb different amounts of light at each wavelength.

The absorption spectrum of an unknown sample of blood will display some combination of these three absorption spectra, since the blood contains all three types of hemoglobins. The relative contribution of each hemoglobin type to the absorption spectrum is proportional to the relative amount of each type in the blood.

In this exercise, you will construct an absorption spectrum for 100% oxyhemoglobin and 100% reduced hemoglobin. By bubbling air into a flask containing blood until the blood is in equilibrium with the air, 100% oxyhemoglobin may be obtained. This process essentially duplicates the process that occurs in the capillaries surrounding the lung alveoli. A simpler (but less accurate) method is to obtain a sample of blood from the fingertip, which has a high percent oxyhemoglobin saturation. The sample of 100% reduced hemoglobin may be obtained by adding sodium hydrosulfite to a second sample of blood. The sodium hydrosulfite (also called sodium dithionite) removes oxygen from oxyhemoglobin.

PROCEDURE

1. Add 8.0 mL of distilled water to a test tube. Obtain a large drop of blood by wiping the fingertip with 70% alcohol, let dry, and puncturing it with a sterile lancet. Then, mix this blood with the distilled water by inverting the test tube over the punctured finger.

2. Transfer half the contents of the test tube (4.0 mL) to a second tube.

3. Add 0.20 mL of 1.0% sodium dithionite solution to the second test tube and mix thoroughly.

Note: The dithionite solution should be freshly prepared just prior to use, and the absorbance values of the two tubes should be determined within 5 minutes of the time the dithionite is added to the second tube.

4. Transfer the two solutions to two cuvettes. Make 2 blank cuvettes (1 with 4.0 mL distilled water and 1 with 3.8 mL distilled water and 0.2 mL sodium dithionite solution).

5. Record the absorbances of solutions 1 and 2 and at 500nm, then change the wavelength by increments of 10 nm until you reach 600 nm (you must blank the spec each time you change the wavelength). Record and graph the absorbances of the two solutions in your lab notebook with ABS on the y axis and wavelength on the x axis.

II. RESPIRATION AND ACID-BASE BALANCE

MATERIALS:
1. pH paper (wide and narrow range), droppers, beakers, straws
2. Buffer, pH = 7.000
3. Concentrated HCl and concentrated NaOH
4. Phenolphthalein solution (saturated)

OVERVIEW:
Carbon dioxide in plasma can combine with water to produce carbonic acid, which in turn dissociates to produce protons (H^+) and bicarbonate ions (HCO_3^-). Ventilation regulates the carbon dioxide concentration of the plasma and has an important role in acid-base balance.

OBJECTIVES
1. Describe the pH scale and define terms acid and base.
2. Explain how carbonic acid and bicarbonate are formed in the blood and describe their functions.
3. Define the terms acidosis and alkalosis and explain how these conditions relate to hypoventilation and hyperventilation.
4. Explain how ventilation is adjusted to help maintain acid-base balance.

BACKGROUND
Ventilation has two different but related functions: (1) oxygenation of the blood, accomplished by bringing new air into the alveoli during the inhalation phase, and (2) elimination of carbon dioxide from the blood, accomplished by the diffusion of CO_2 from the blood into the alveoli and the extrusion of this CO_2 by exhalation. The first function serves to maintain aerobic cell respiration; the second serves to maintain the normal pH of the blood.

The pH indicates the concentration of H+ (hydrogen ion) in a solution and is defined by the following formula:

$$pH = \log 1/[H+]$$

Recall that an acid has the ability to donate H+ ions to solution, a base donates OH- ions and a buffer prevents pH change of a solution.

To maintain homeostasis, the pH of the blood is carefully regulated by the respiratory system and the renal system. Bicarbonate serves as the major buffer of the blood, helping to stabilize the pH of plasma despite the continuous influx of H^+ from molecules of lactic acid, fatty acids, ketone bodies, and other metabolic products. The H^+ released by these acids is prevented from lowering the blood pH because it is combined with bicarbonate. When this system fails, blood pH may fall below 7.35 or to rise above 7.45. These conditions are called acidosis and alkalosis, respectively.

Normally, the rate of ventilation is matched to the rate of CO_2 production by the tissues, so that the carbonic acid, bicarbonate, and H^+ concentrations in the blood remain within the normal range. What happens when a patient hyper- or hypoventilates?

PROCEDURE
We will be using pH paper to measure pH of solutions; use the wide-range (0 - 14) paper first, then select the appropriate narrow-range paper to make the measurement. Please conserve the pH paper and use tweezers to place the paper in the solutions.

Set up the following conditions and measure the pH of each (mix solutions before taking pH):

Beaker 1: pH of distilled water, then add 1 drop of HCl and measure pH again.

Beaker 2: pH of distilled water, then add 1 drop of NaOH and measure pH again.

Beaker 3. pH of buffer, then add 1 drop HCl and measure pH again. Add another drop and measure and then finally a 3rd drop and measure.

Beaker 4: pH of buffer, then add 1 drop NaOH and measure pH again. Add another drop and measure and finally add a 3rd drop and measure.

Rinse and dry all materials and return.

III. EFFECT OF EXERCISE ON THE RATE OF CO_2 PRODUCTION

Increased muscle metabolism during exercise results in an increase in CO_2 production. Despite this, the CO_2 levels and pH of arterial blood do not normally change significantly during exercise; this is because the increased rate of CO_2 production is matched by an increase in the rate of its elimination through ventilation. The mechanisms responsible for exercise hyperpnea (increased breathing) are complex and incompletely understood.

PROCEDURE
1. Fill a beaker with 200 mL of distilled water and add 5.0 mL of 0.10N NaOH and a few drops of phenolphthalein indicator. This indicator is pink in alkaline solutions and clear in neutral or acidic solutions. Divide this solution into two beakers.

2. While sitting quietly, exhale through a glass tube or straw (or double straws) into the solution in the first beaker. Carefully record the time required to turn the solution from pink to clear in your laboratory report.

3. Exercise vigorously for 2 to 5 minutes by running up and down stairs or by doing jumping jacks. Exhale through a glass tube or straw (or double straws) into the second beaker, and again record the time it takes to clear the pink solution.

IV. ROLE OF CARBON DIOXIDE IN THE REGULATION OF VENTILATION

The carbon dioxide concentration of the blood reflects a balance between the rate of its production (by aerobic cell respiration) and the rate of its elimination through the lungs. When a person consciously holds his or her breath for a sufficiently long time, the carbon dioxide level rises (and the pH falls) to such an extent that reflex breathing occurs. On the other hand, during hyperventilation, the pH of the blood can rise to the point that the desire to breathe is eliminated until the amount of carbon dioxide in the blood again rises above the critical point.

PROCEDURE

1. Count the number of breaths you take in 1 minute of relaxed, unforced breathing. Enter this number in your laboratory report.

2. Force yourself to hyperventilate for about 10 seconds; stop if you begin to feel dizzy.

3. Immediately after hyperventilation, count the number of breaths you take in 1 minute of relaxed, unforced breathing.