How-To-Do-It

Biology, Yes. But Why Study Physics Too?

William J. Brett

Students taking biology classes often question why they should also be competent in chemistry, mathematics and physics. Occasionally, professional biologists have passed through this phase and recognized that an understanding of biology requires background in all these cognant areas. Therefore, they may underestimate the desirability of demonstrating to students, at an early stage of their scientific development, the dependency of biology on the physical sciences. A method that creates an enduring impression for students is the laboratory exercise that requires the application of the physical sciences to solve biological problems. A laboratory exercise concerned with the measurement of oxygen-carrying capacity of the blood lends itself well to this goal.

The students may be required to read any of many good physiology textbooks to obtain a sufficient understanding of blood and its role in carrying oxygen to the tissues. The amount and type of reading assigned may be determined by the depth of understanding desired from the exercise and by the ramifications of the basic objectives of the exercise. Therefore, only a brief introduction to the subject is included in this laboratory unit "Oxygen Carrying Capacity of the Human Blood."

Oxygen Carrying Capacity of the Human Blood

Blood of the average healthy person contains about 15 grams of hemoglobin (Hb) per 100ml of blood. Each gram of blood Hb can bind a maximum of 1.34ml of O_2; therefore, the Hb in 100ml of blood can combine with 20ml of O_2 when the Hb is 100 percent saturated (15 grams × 1.34ml O_2/gram = 20.1ml O_2). Under normal conditions, Hb is only 97 percent saturated and thus the total quantity of bound O_2 is 19.5ml/100ml of blood. Some O_2 does dissolve in the water of the plasma and cells in the blood, but about 97 percent of the O_2 transported from the lungs to the tissues is carried in combination with the Hb. In determining the O_2 carrying capacity of the blood, one must know the amount of Hb present. Two factors play roles in determining the total Hb: the number of red blood cells and the amount of Hb in each red blood cell.

Hb is composed of four polypeptide chains each attached to a heme molecule containing an iron atom. Each heme molecule, actually the iron, can combine with a molecule of oxygen; therefore, a molecule of Hb can combine with four molecules of O_2. In addition to Hb's role, other factors are necessary to provide an adequate supply of oxygen at the cellular level. These include a proper exchange of oxygen between the atmosphere and the individual and a properly functioning circulatory or transport system.

Under resting conditions and with an adequate amount of O_2 received by the lungs, an individual can supply 250ml of O_2/min. by breathing (alveolar ventilation); this is about 4.2 liters of air/min. During exercise, if 1,000ml of O_2 is being used each minute, the rate of breathing (alveolar ventilation) must increase four times to about 18 liters of air/min. in order for an adequate amount of O_2 to reach the lungs. The rate of O_2 transport to the tissues can be increased as much as 15 times under conditions of strenuous exercise; it may increase as much as 20 times under extreme conditions in a well-trained athlete. This increase is the result of more blood reaching the tissues per minute and an in-

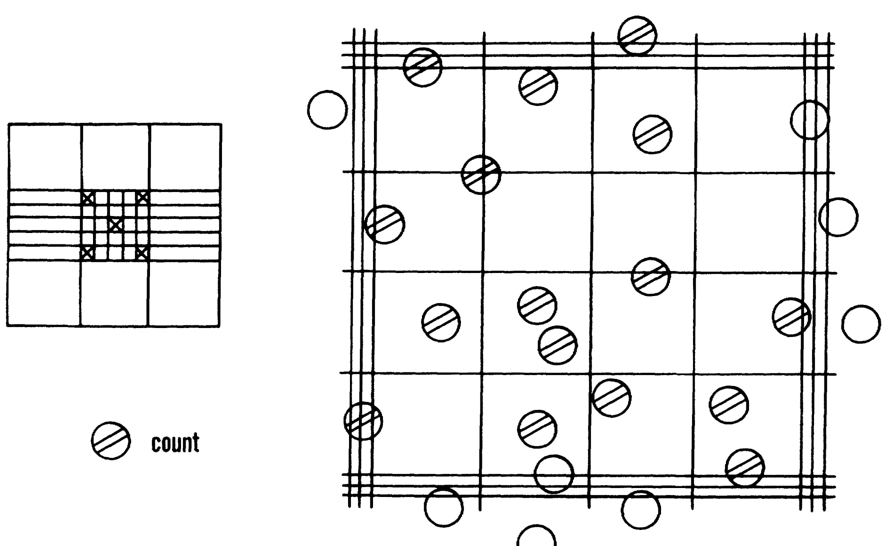

Figure 1. Showing ruled area of hemocytometer and method of determining which cells shown should be counted; A is overall view of the ruled area and B is an enlargement of one group of 16 squares.

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Table 1. Transmission Reading at Wavelength of 540 nm for Grams of Hemoglobin/100 ml of Blood.

T	*0*	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*
10										
20	22.3	21.6	21.0	20.3	19.7	19.2	18.6	18.0	17.6	17.1
30	16.6	16.1	15.7	15.3	14.9	14.5	14.1	13.7	13.4	13.0
40	12.6	12.3	12.0	11.6	11.4	11.0	10.7	10.4	10.1	9.8
50	9.5	9.3	9.0	8.7	8.5	8.2	8.0	7.7	7.5	7.2
60	7.0	6.8	6.5	6.4	6.1	5.9	5.7	5.5	5.3	5.1
70	4.9	4.7	4.5	4.3	4.1	3.9	3.8	3.6	3.4	3.2
80	3.0	2.9	2.7	2.5	2.4	2.2	2.0	1.9	1.7	1.6
90	1.4	1.3	1.1	1.0	0.8	0.7	0.5			

Source: Bausch & Lomb Spectronic 20 Clinical Methods and Calibrations Manual.

creased release of O_2 from the Hb. The result is an increase in O_2 transport to the tissues from 250ml/min. under resting conditions to 3,750ml/min. under conditions of strenuous exercise.

This introduction to some of the factors involved in supplying an adequate amount of oxygen to the organism under a range of tissue demands should enable one to better understand the significance of this laboratory exercise. The laboratory exercise requires the determination of the values that will permit calculation of oxygen-carrying capacity of the blood.

The objectives of the exercise are: 1) to learn how to determine red blood cell count; 2) to learn how to determine Hb concentration in the blood; 3) to determine the size of a red blood cell; 4) to calculate total number of red blood cells and amount of Hb in the total blood volume of an adult human; 5) to determine how many Hb molecules are contained within a single red blood cell; and 6) to determine oxygen-carrying capacity of a single blood cell and of the total blood volume.

Method for Determination of Number of RBCs in the Human Body

You will use a hemocytometer for determining the RBC count. As the term indicates (hemo-blood, cyto-cell, meter-measure), this is an instrument for counting blood cells. Place a hemocytometer on the microscope stage and examine the ruled area under low power (Figure 1A). The RBCs usually are counted in the five groups of 16 small squares indicated by Xs in the diagram. Obtain a clean RBC pipette and observe that it consists of a capillary tube leading into a small bubble-like chamber. Part way up the capillary tube there is a .5 mark and above the bubble there is a 101 mark. Procedure:

a. Clean your middle finger with a piece of cotton moistened with alcohol.
b. Using a lancet make a clean, relatively deep puncture so that the blood will flow freely. (Blood for the Hb determination should be drawn at the same time as that for the RBC count.)
c. Attach a tube with a mouth piece to the pipette and draw the blood to the .5 mark. Do this by bringing the tip of the pipette to the drop of blood and tilting toward the vertical as the capillary fills toward the desired mark. If you get blood above the .5 mark, touch the tip of the pipette to some cotton which will serve to draw some of the blood from the pipette.
d. Put the tip of the pipette into the diluting solution (Hayem's fluid) and, sucking lightly on the mouth piece, fill the pipette to the 101 mark. As the bulb fills, be ready to take the tip out of the fluid quickly or the mixture will be drawn above the 101 mark.
e. Remove the tubing while holding your middle finger over the tip of the pipette. Now place your thumb over the other end of the tube, and shake the pipette vigorously for one or two minutes.
f. Force out four or five drops of clear diluting fluid which remain in the capillary part of the pipette.
g. Cover the ruled area of the hemocytometer with the special cover slip. Place a drop of blood so that it is pulled under the cover slip. There should be no air bubbles.
h. Let the preparation stand for one or two minutes; this will allow the cells to settle to the bottom of the counting chamber.
i. Adjust the chamber under the low power and locate the area in the microscope field with the aid of Figure 1. Move to one of the indicated squares consisting of the 16 small squares and then turn to high power. Count the number of RBCs in the 16 small squares and then move to each of the other four squares (each containing 16 small squares). To avoid counting a larger area than intended, count those RBCs that touch, in any way, the middle line on the left and top slides but only those that are completely within the middle line on the right and bottom sides (Figure 1B).
j. Calculation of the number of RBCs in a cubic millimeter of blood is done as follows:

(1) the dilution is 1-200 (0.5 to 101)
(2) each of the smallest squares is 1/20mm × 1/20mm × 1/10mm or 1/4,000 cubic millimeter
(3) if you counted 80 small squares (5 × 16) as suggested, the number of RBCs in a cubic millimeter of your blood is:

$$\frac{\#\text{ of cells counted} \times \text{dilution (200)} \times 4{,}000}{\#\text{ of small squares counted (80)}} =$$

$$\frac{\#\text{ RBCs} \times 200 \times 4{,}000}{80} \text{ or}$$

$$\#\text{ RBCs} \times 10{,}000$$

Knowing the number of RBCs in a mm^3 of your blood, it is possible to estimate the total # of RBCs in your total blood volume (TBV). The TBV can be determined by the following equation: TBV = body weight in kilograms × 75ml (males) or 67ml (females). Use 2.2 pounds equal 1 kilogram to calculate your weight in kilograms. You have determined the # of RBCs in a mm^3 of your blood, and there are 1,000 mm^3 in one cm^3 (1ml); therefore, the # of RBCs in your TBV = #ml of blood × 1,000 × #RBCs/mm^3. There are _____ RBCs in your TBV.

Table 2. Values for the Basic Data.

Number of RBCs/ mm^3 male blood	5.2×10^6
Number of RBCs/ mm^3 female blood	4.7×10^6
Grams of Hb/100 ml of male blood	16.0
Grams of Hb/100 ml of female blood	14.0
Molecular weight Hb	64,458.0
Molecular weight O_2	32.0
Avogadro's number	6.02×10^{23}

Determination of Amount of Hb in 100ml of Blood, one RBC and the TBV

Prior to performing this exercise, read the instructions for the use of the Spectronic 20. If a Spectronic 20 is not available, one can use a Sahli Hb apparatus or the Visual Paper Method (this consists of placing drop of blood on a specific type of paper and comparing the color with a color chart) for determination of Hb. concentration. Obtain a clean Hb pipette and observe that the capillary tube has a 20cmm mark (20mm^3).

Procedure:

a. Using the same technique as employed in determining the # of RBCs, draw blood to the 20cmm mark.
b. Into two colorimeter tubes, pipette exactly 5ml of Drabkin's solution (use a pipette attached to a syringe as Drabkin's solution contains cyanide).
c. Into one of the tubes add the 20cmm of whole blood. Rinse the pipette several times by drawing some of the liquid into the pipette and expelling it into the tube.
d. Mix the contents of the tube by swirling.
e. The other tube serves as the "blank."
f. Allow the tubes to stand for 10 minutes.
g. Set the wavelength of the Spectronic 20 at 540 namometers and then place the "blank" tube into the instrument and set the light control so that the scale reads 100 percent transmittance.
h. Remove the "blank" and place your blood sample in the machine. Read the percent transmittance.
i. Refer for transmission reading to Table 1 and obtain the concentration of Hb/100ml of your blood.

____
grams Hb in 100ml blood

The total Hb in your blood is calculated by multiplying the amount of ml blood in your body/100 by the grams Hb/100ml of blood.

____
grams of Hb in TBV

Also calculate the amount of Hb in a single RBC. You measured the grams of Hb in 100ml of blood; therefore, dividing this amount by 100,000 gives the amount in an mm^3. _____ grams Hb/mm^3 The amount of Hb in a single RBC is obtained by dividing the amount of Hb in a mm^3 by the # of RBCs in an mm^3. _____ grams Hb/RBC

Table 3. Sample Calculations for Determination of Values

Value	*Calculation*
4.5×10^6 RBCs/mm^3	
4.5×10^9 RBCs/ml	1 ml = 1,000 mm^3 $45 \times 10^6 \times 1{,}000 = 45 \times 10^9$
150 lb. person weighs 68.18 Kg	1 Kg = 2.2 lbs $150 \div 2.2 = 6818$
TBV = 5113.5 ml	75 ml/Kg 6818 Kg $\times$ 75 ml = 5,113.5 ml
2.3×10^{13} RBCs in TBV	5113.5 ml $\times$ $(45 \times 10^9) = 2.3 \times 10^{13}$
14.5 grams Hb/100 ml	
741.53 grams Hb in TBV	$5113.5 \div 100 = 5114 \times 145 = 741.53$ grams Hb
741.53×10^9 Hb in RBCs TBV	1 g = 10^9 nanograms (ng)
0.03224 ng/RBC	$741.53 \times 10^9 \div 2.3 \times 10^{23} =$ 0.03224 ng/RBC
$6/93 \times 10^{21}$ molecules Hb TBV	$741.53 \div 64{,}458 \times (6.02 \times 10^{23}) = 6.93 \times 10^{21}$
27.72×10^{21} O_2 for TBV	$6.93 \times 10^{21} \times 4 = 27.72 \times 10^{21}$
30.11×10^7 molecules Hb/RBC	0.03224×10^{-9} g Hb $\div 64.458 \times (6.02 \times 10^{23})$
120.44×10^7 O_2/RBC	$30.11 \times 10^7 \times 4 = 120.44 \times 10^7$
1.47 grams O_2 TBV	$27.72 \times 10^{21} \div (6.02 \times 10^{23}) \times 32$
6.4×10^{-14} grams O_2/RBC	$120.44 \times 10^7 \div (6.02 \times 10^{23}) \times 32$

Table 4. Calories Consumed/hr for a 70 kg Male Eating a Reasonable Diet

Activity	*Calories/hr*
Lying in bed	77
Sitting at rest	100
Standing	105
Light exercise	170
Walking slowly 2.6 miles/hr	200
Active exercise	290
Severe exercise	450
Swimming	500
Running 5.3 miles/hr	570
Very severe exercise	600
Walking very fast 5.3 miles/hr	650
Walking upstairs	1100

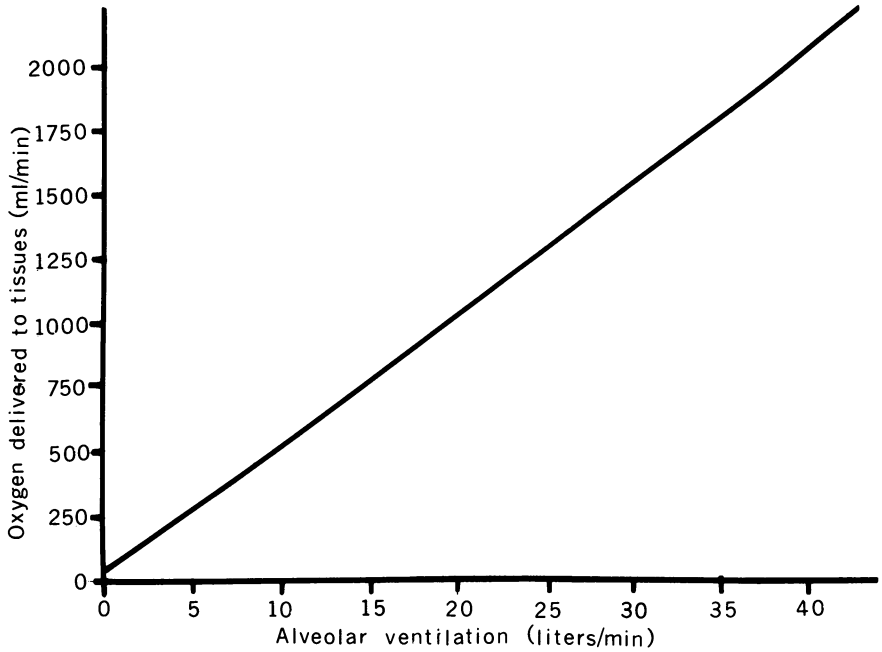
Determination of Oxygen Capacity of a Single RBC and of the TBV

A single molecule of Hb can carry four molecules of oxygen. The molecular weight of Hb is 64,458. Avogadro's number, which tells the number of molecules of a substance in one gram molecular weight of that substance, is 6.02×10^{23}. With this information, plus that from the previous exercises, you should be able to determine the number of molecules of Hb and the oxygen carrying capacity of one RBC and of your TBV. You may use the actual figure of 97 percent saturation for the Hb, or calculate the values as if the Hb were 100 percent saturated. Please indicate which percentage you use. Number of Hb molecules in a single RBC equals the grams of Hb per RBC divided by the gram molecular weight of Hb times Avogadro's number. _____ # of Hb molecules/RBC Number of Hb molecules in TBV equals grams Hb in TBV divided by gram molecular weight of Hb times Avogadro's number. _____ # Hb molecules/TBV Oxygen carrying capacity of a single RBC equals number of molecules of Hb in RBC times 4. _____ # O2/RBC Convert the number of molecules of O_2 to grams of oxygen in an RBC by dividing the number of molecules of O_2 in an RBC by Avogadro's number times the gram molecular weight of O_2. _____ grams O_2/RBC Calculate the grams of O_2 that can be carried in the TBV.

____
grams O_2/TBV

Determination of Size of RBC

Place the hemocytometer on the stage of a microscope equipped with an ocular micrometer and measure the diameter of the cell. The instructor will provide unit values for the ocular



micrometer for both low and high power. The diameter of your RBC is _____ microns. The average diameter given for the human RBC is about seven microns. The cell is about two microns at the thickest part. How does thisṕre to your measurements? _____ Human RBCs and those of other mammals do not possess nuclei. Assume that the nucleus in the RBC of other animals (amphibians, reptiles and birds) occupies 25 percent of the cell volume. Calculate what effect having a nucleus would have on the amount of Hb and amount of O_2 within a human RBC. _____ A distinct plus for this laboratory exercise is the range in amount of work that may be required by the student to complete the unit. The student may be required to obtain all the basic data or the instructor may supply all or any part of the data. The desirability of supplying basic data is determined by the amount of time and equipment available for the exercise. Although the use of a Spectronic 20 permits a more nearly accurate measurement of Hb concentration than the other methods mentioned, the other methods do provide satisfactory results. It is possible to analyze one student's blood and have all the other students use these same basic data but utilize their own weight to determine TBV. Table 2 provides values that can be utilized for this exercise. Table 3 lists some sample calculations that illustrate how the various values are obtained.

The exercise can be expanded by posing other problems to the students. It is possible to determine the amount of O_2 that must be provided each minute or hour for various types of activity. Assuming that the amount of O_2 used is directly proportional to the alveolar ventilation, one can use a spirometer to measure air volume intake for a given period (measure the volume of air per minute as a result of a specific activity). Use Figure 2 to obtain the amount of oxygen absorbed for a given amount of alveolar ventilation. This is a simplified method, but it gives some idea of the increased use of O_2. It also is possible to determine O_2 requirement on the basis of the amount of calories utilized for a specific type of activity. Assume that one obtains 4.825 calories per liter of O_2 consumed; this is based upon the mean values for the use of the different types of organic molecules consumed by the energy yielding process (glucose = 5.02; starch = 5.05; fat = 9.70; and protein = 4.60). Then, using the values from Table 4, one can determine the number of calories/hr for a specific exercise and using this amount calculate the quantity of O_2 required.