

# Biology 347 General Physiology Lab

## Basic Pulmonary Functions: Respirometry and Factors that Effect Respiration

### Objectives

- Students will measure breathing parameters in a resting subject.
- Students will measure breathing parameters immediately following exercise.
- Students will study the effects of gravity on peripheral circulation.
- Students will understand the parameters of breathing associated with sitting, standing and lying down.

### Introduction

Ventilation or breathing is responsible for providing a continual supply of oxygen and the removal of carbon dioxide from the body. From the simple observation of a normal subject it is evident that inspiration and expiration should follow each other rhythmically, without either phase being unduly prolonged. This basic rhythm of ventilation is established within the respiratory centers of the brain. The basic ventilation rate and rhythm are not static, but vary as neuronal and hormonal mechanisms act to alter the frequency and amplitude of ventilation in response to the physiological demands of the organism.

A common misconception is that variation in the O<sub>2</sub> levels within the system cause changes in the ventilation rate. Actually, the O<sub>2</sub> concentration, under normal conditions, has little to do with the determination of respiratory rate. The critical determining factor is the level of CO<sub>2</sub> and/or the level of free protons circulating in the blood. For example, an increase in CO<sub>2</sub> or H<sup>+</sup> levels will induce changes which result in an acceleration of the ventilation rate and volume until these levels return to the normal range. Conversely, conditions associated with alkalosis and lower than normal CO<sub>2</sub> levels depress the ventilation rate.

The receptors that are sensitive to changes in the concentrations of CO<sub>2</sub> and H<sup>+</sup> are located within the arterial system and the medulla of the brain. Excitations of these receptors trigger neural reflexes which alter the respiratory rate and depth. Additionally, other parts of the nervous system influence the basic ventilation pattern established by the respiratory center. For example, the spinal cord has an overall facilitative effect on respiration. If the activity of the ascending sensory pathways within the spinal cord were increased, we would also expect to see an increase in respiration. If the spinal cord were injured or severed there would be a decrease in respiratory activity.

The funny thing about this lab is that it is hard for you to think about how you breathe without you changing how you breathe. In and out normally is tidal breathing, volume about half a liter. There is an expiratory reserve, over a liter, and an inspiratory reserve, maybe three liters. From the top of inspiration to the bottom of expiration is vital capacity. Even after you empty your lungs as much as possible there is a residual volume, over one liter.

Carbon dioxide reflected across the blood brain barrier from the blood stream, increases H<sup>+</sup> (lowers pH) of cerebrospinal fluid (CSF). Chemoreceptors in the medulla detect this to stimulate breathing via medullary centers. Remember this: CO<sub>2</sub> and CSF acidity are stimuli for breathing, and that is why oxygen given to patients has CO<sub>2</sub> in it.

The principle value of these pulmonary measurements is to track changes in the volumes for use as a diagnostic tool. For example, the vital capacity decreases in left heart disease. This is due to the blood congestion in the lung capillaries which in turn leads to pulmonary edema and a decrease in VC. As the person recovers and the heart becomes stronger, the pulmonary congestion and edema decrease followed by an increase in vital capacity. The vital capacity also decreases in paralytic polio due to the partial paralysis of the respiratory muscles.

## Procedure: Equipment Set-up

1. Open the LabScribe program.
2. Click on the **Settings** menu and select the **Breathing-Rest-Exercise-LS2** settings file.
3. After a short time, LabScribe will appear on the computer screen as configured by the **Breathing-Rest-Exercise** settings.
4. The spirometer will monitor breathing from a volunteer. It is important that the volunteer is healthy and has no history of respiratory or cardiovascular problems.
5. The outlets on the flow head should always be in the upright position to avoid problems with condensation developing in the airflow tubes.
6. Turbulence in the flow head will produce a noisy signal. To reduce turbulence, the subject should place his or her lips around the outside of the opening of the flowhead, or its mouthpiece.
7. Use a clip to prevent air from entering or leaving the nose as the subject is breathing. Air that passes through the nose is not included in the volume measurements and causes errors in these values.
8. The settings file, **Breathing-Rest-Exercise**, programmed LabScribe to display the **Airflow** of the spirometer and the **Volume**. The settings file activates an algorithm designed specifically to convert the airflow data of the iWorx SP-304 spirometer to volume data.
9. Click on the words **Spirometry (AirFlow)** that are next to the title of the **Volume** channel, to open the **computed function pull-down menu**. Select **Set-up** from this pull down menu to open the **Spirometer Calibration Dialogue window**. Enter the calibration voltage that is listed on the label of the spirometer unit, into the equation that sets the calibration voltage equal to one liter.
10. Make sure the reset time is set to 60 seconds and the first five seconds of the recording are used to zero the baseline of the **Volume** channel. Click OK.
11. Allow the SP-304 to warm up for 10 minutes before recording for the first time. Do not hold the spirometer amplifier in your hand; the heat of your hand will alter the volumes recorded. Plug the unit into **Channel 4**.
12. Determine which opening of the flowhead should be used to make the deflection on the **Airflow** channel go up as the subject inhales. Inhalation is always displayed as an upward deflection.

## Procedure: Normal Breathing in the Resting Subject

1. The subject should sit quietly and become accustomed to breathing through the spirometer. They should be breathing normally before any recordings are made. Remember to hold the flowhead so that the outlets are on top.
2. Have the subject remove the flowhead from their mouth. They should hold it at the same level as their mouth, but in a position that prevents their breath or any other airflow from moving through the flowhead.

*Note: The LabScribe software will zero the **Volume** channel during the first five seconds of recording. No air should be moving through the flow head during this time.*

3. Click **Record**. Type "Resting" in the comment line to the right of the **Mark** button. After waiting five seconds for the **Volume** channel to zero, have the subject place the flowhead in their mouth and begin breathing. Press the **Enter** key on the keyboard. Click the **AutoScale** buttons for the **Air Flow** and **Volume** channels. Notice the slowly moving wave on the **Volume** channel. Record 5 breaths, which normally takes about 30 seconds. During this time, the **Volume** channel may automatically reset itself to zero; which it will do every 30 seconds after the recording begins.
4. Type "Maximum" on the comment line. Press the **Enter** key on the keyboard as the subject inhales as much as possible. As quickly as possible after reaching their maximum inhalation volume, the subject should exhale as quickly and as completely as possible.
5. The volunteer should return to normal breathing through the spirometer.
6. Click **Stop** to halt recording.
7. Save the file.

## Procedure: Data Analysis Normal Breathing

1. Scroll through the recording and find the section of data recorded when the subject was breathing while **resting**.

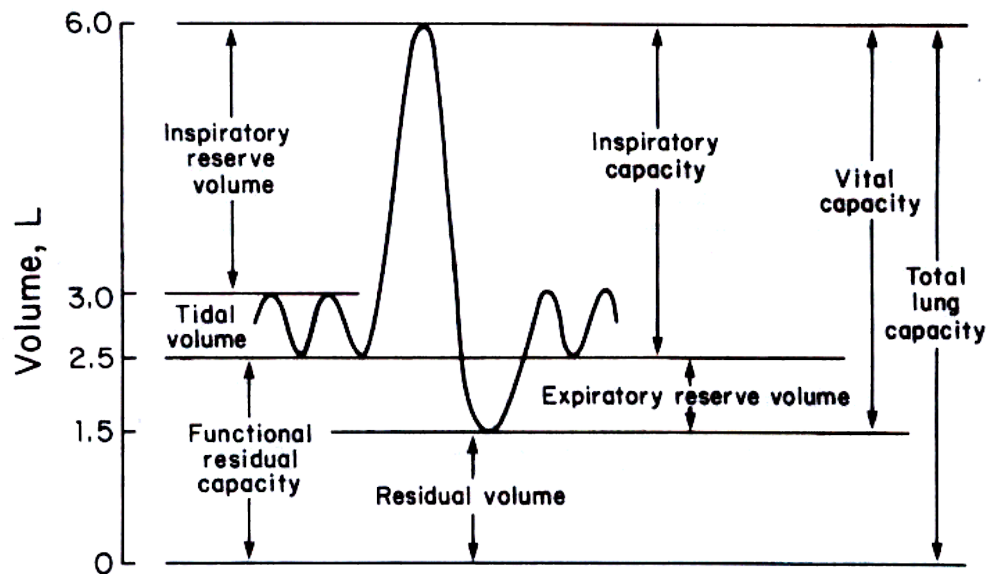
2. Use the **Display Time** icons to adjust the **Display Time** of the **Main window** to show at least four complete breathing cycles on the **Main window**. Four adjacent breathing cycles can also be selected by:
  - Placing the cursors on either side of a group of four complete breathing cycles; and
  - Clicking the **Zoom between Cursors** button on the LabScribe toolbar to expand the four selected breathing cycles to the width of the **Main window**.
3. On the **Volume** channel, use the mouse to click on and drag the cursors to specific points on the recording to measure the following volumes:
  - **Tidal Volume (TV)**, which is the volume of air inhaled or exhaled during a normal breathing cycle. To measure the tidal volume of the subject during breathing at rest, place one cursor in the trough prior to inhalation, and the second cursor on the peak of the cycle. The value for the **V2-V1** function on the **Volume channel** is the tidal volume
  - **Maximum Inspiratory Flow Rate**, which is the maximum rate of air movement during inhalation. To measure the maximum inspiratory flow rate of the subject during breathing at rest, leave the cursors in the same positions used to measure the tidal volume. The value for the **Max\_dv/dt** function on the **Volume channel** is the maximum inspiratory flow rate of that breath cycle.
  - **Maximum Expiratory Flow Rate**, which is the maximum rate of air movement during exhalation. To measure the maximum expiratory flow rate of the subject during breathing at rest, place one cursor on the peak of the breath cycle, and the second cursor in the trough to the right of that peak. The value for the **Min\_dv/dt** function on the **Volume channel** is the maximum expiratory flow rate of that breath cycle. This function is used since the exhalation portion of the breath cycle has a negative slope.
  - **Breath Period**, which is the duration of each breathing cycle. To measure the breath period of the subject during breathing at rest, place one cursor on a peak of a breath cycle, and the second cursor on the peak of an adjacent cycle. The value for **T2-T1** on the **Volume channel** is the period of that breath cycle.
4. Repeat the measurements of tidal volume, maximum inspiratory flow rate, maximum expiratory flow rate, and breath period on at least four normal breath cycles.
5. Average the values obtained for each parameter and enter the means in Table 1.

### **Procedure: Data Analysis Forced Breathing**

1. Use the slider or the arrows on the scroll bar, at the bottom of the **Analysis** window, to position data recorded when the subject exhaled with maximum force in the window.
2. Use the **Display Time** icons to adjust the **Display Time** of the **Analysis window** to show the forced expiration curve and the two normal breaths, that occur before the force expiration curve, on the same window. These breathing cycles can also be selected by:
  - Placing the cursors on either side of the group of appropriate breathing cycles; and
  - Clicking the **Zoom between Cursors** button on the LabScribe toolbar to expand the selected breathing cycles to the width of the **Main window**.
3. Use the same techniques used earlier to record necessary volumes and rates in the Table.
4. Place the cursors on the forced expiration data displayed on the **Volume** channel to measure the following volumes and rates using the **V2-V1**, **T2-T1**, **Max\_dv/dt**, and **Min\_dv/dt** functions.
  - **Tidal Volume (TV)**, by placing one cursor in the trough before the inhalation segment of the resting breath and the second cursor on the peak of that resting breath cycle. The value for the **V2-V1** function on the **Volume channel** is the tidal volume.
  - **Inspiratory Reserve Volume (IRV)**, by placing one cursor on the peak of the normal breath prior to the maximum inhalation and the second cursor on the peak of the forced breath cycle. The value for the **V2-V1** function on the **Volume channel** is the inspiratory reserve volume.
  - **Forced Inspiratory Flow Rate**, by keeping the cursors in the same positions used for measuring **IRV**. The value for the **Max\_dv/dt** function on the **Volume channel** is the forced inspiratory flow rate.
  - **Forced Vital Capacity (FVC)**, by placing one cursor on the peak of the forced breath cycle and the second cursor on the flat line after the subject has expelled all the air from his or her lungs. The value for the **V2-V1** function on the **Volume channel** is the forced vital capacity.
  - **Forced Expiratory Flow Rate**, by keeping the cursors in the same positions used for measuring **VC**. The value for the **Min\_dv/dt** function on the **Volume channel** is the forced expiratory flow rate.
  - **Expiratory Reserve Volume (ERV)**, by placing one cursor in the trough before maximal inhalation and the second cursor on the flat line after subject has expelled all the air from his or her lungs. The value for the **V2-V1** function on the **Volume channel** is the expiratory reserve volume
  - **Forced Expiratory Volume at 1 Second (FEV1)**, by placing one cursor on the peak of the maximum breath cycle and the second cursor on the data point that is one second after the peak. Use the **T2-T1** function to

determine the data point that is one second after the peak. The value for the **V2-V1** function on the **Volume channel** is the forced expiratory volume at one second.

• **Forced Expiratory Volume at 3 Seconds (FEV3)**, by placing one cursor on the peak of the maximum breath cycle and the second cursor on the data point that is three seconds after the peak. Use the **T2-T1** function to determine the data point that is three seconds after the peak. The value for the **V2- V1** function on the **Volume channel** is the forced expiratory volume at three seconds.



**Table 1: Normal Breathing Parameters**

	Tidal Volume (liters)	Period (secs/breath)	Maximum Inspiratory Flow	Maximum Expiratory Flow	Breath Rate (breaths/min)
Cycle 1					
Cycle 2					
Cycle 3					
Cycle 4					
Average					

**Table 2: Maximum Breathing**

	Tidal Volume	Inspiratory Reserve Volume	Expiratory Reserve Volume	Forced Vital Capacity	Forced Inspiratory Flow Rate	Forced Expiratory Flow Rate	FEV1	FEV 3
Maximum Breathing Cycle								

**Calculations for Breathing FEV1/FVC Ratio and FEV3/FVC Ratio**

Use the mean duration of the breathing cycles to measure breathing rate:

$$\text{Breathing Rate (breaths/ min )} = (60 \text{ sec / min } ) / \text{mean duration (sec /breath)}$$

Determine the ratio of forced expiratory volume at one second (FEV1) to the forced vital capacity (FVC) by dividing FEV1 by FVC. Normal values are between .80-0.95.

Determine the ratio of forced expiratory volume at one three seconds (FEV3) to the forced vital capacity (FVC) by dividing FEV3 by FVC. Normal values are between .80-0.95.

### **Procedure: Breathing Immediately After Exercise**

1. For this exercise, use a subject whose resting breathing parameters have been measured. The volunteer should exercise to sufficiently elevate breathing rate, but with minimal class disruption. Running up and down flights of stairs is a good method.
2. The volunteer should immediately sit down after concluding exercise. Click **RECORD**. Type “After Exercise” on the comment line to the right of the **Mark** button.
3. After waiting five seconds for the **Volume** channel to zero, have the subject place the flowhead in their mouth and begin breathing. Press the **Enter** key on the keyboard. Record five complete breathing cycles. Click the **AutoScale** buttons for the **Air Flow** and **Volume** channels.
4. Type “Maximum” on the comment line. Press the **Enter** key on the keyboard as the subject inhales as much as possible. As quickly as possible after reaching their maximum inhalation volume, the subject should exhale as quickly and as completely as possible.
5. The volunteer should return to normal breathing through the spirometer.
6. Click **Stop** to halt recording.
7. Save the recording.

**Procedure: Data Analysis**

1. Follow the procedure for data analysis above and complete Tables 3 and 4.
2. Then complete Table 5, which compares the data between rest and exercise.

**Table 3: Breathing Parameters Following Exercise**

	Tidal Volume (liters)	Period (secs/ breath)	Maximum Inspiratory Flow	Maximum Expiratory Flow	Breath Rate (breaths/min)
Cycle 1					
Cycle 2					
Cycle 3					
Cycle 4					
Average					

**Table 4: Maximum Breathing after Exercise**

	Tidal Volume	Inspiratory Reserve Volume	Expiratory Reserve Volume	Forced Vital Capacity	Forced Inspiratory Flow Rate	Forced Expiratory Flow Rate	FEV1	FEV 3
Maximum Breathing Cycle								

**Calculations for Breathing FEV1/FVC Ratio and FEV3/FVC Ratio**

Use the mean duration of the breathing cycles to measure breathing rate:

$$Breathing\ Rate\ (breaths/\ min) = (60\ sec / \ min) / mean\ duration\ (sec / breath)$$

Determine the ratio of forced expiratory volume at one second (FEV1) to the forced vital capacity (FVC) by dividing FEV1 by FVC. Normal values are between .80-0.95.

Determine the ratio of forced expiratory volume at one three seconds (FEV3) to the forced vital capacity (FVC) by dividing FEV3 by FVC. Normal values are between .80-0.95.

**Table 5: Comparison of Breathing Parameters Before and After Exercise**

	Resting	After Exercise
Period (seconds/breath)		
Rate (breaths/minute)		
Tidal Volume (mLs/breath)		
Minute Air Flow (liters/min)		
Maximum Normal Air Flow (mLs/sec)		
<i>During inhalation</i>		
<i>During exhalation</i>		
Lung Volumes (liters)		
<i>Tidal Volume</i>		
<i>Inspiratory Reserve</i>		
<i>Expiratory Reserve</i>		
<i>Vital Capacity</i>		
<i>Forced Expiratory Volume – 1 sec (FEV1)</i>		
Forced Air Flow (mLs/sec)		
<i>During inhalation</i>		
<i>During exhalation</i>		
<i>FEV1/VC Ratio</i>		
<i>FEV3/VC Ratio</i>		

**Procedure: Breathing When Standing**

1. Unplug the pulse plethysmograph from the iWorx unit.
2. The subject should stand quietly and become accustomed to breathing through the spirometer. They should be breathing normally before any recordings are made. Remember to hold the flowhead so that the outlets are on top.
3. Have the subject remove the flowhead from their mouth. They should hold it at the same level as their mouth, but in a position that prevents their breath or any other airflow from moving through the flowhead.

*Note: The LabScribe software will zero the **Volume** channel during the first five seconds of recording. No air should be moving through the flow head during this time.*

4. Click **Record**. Type “Resting” in the comment line to the right of the **Mark** button. After waiting five seconds for the **Volume** channel to zero, have the subject place the flowhead in their mouth and begin breathing. Press the **Enter** key on the keyboard. Click the **AutoScale** buttons for the **Air Flow** (CH 4) and **Volume** (CH 5) channels. Notice the slowly moving wave on the **Volume** channel. Record 5 breaths, which normally takes about 30 seconds. During this time, the **Volume** channel may automatically reset itself to zero; which it will do every 30 seconds after the recording begins.
5. Type “Maximum” on the comment line. Press the **Enter** key on the keyboard as the subject inhales as much as possible. As quickly as possible after reaching their maximum inhalation volume, the subject should exhale as quickly and as completely as possible.
6. The volunteer should return to normal breathing through the spirometer.
7. Click **Stop** to halt recording.
8. Save the file.





## Calculations for Breathing FEV1/FVC Ratio and FEV3/FVC Ratio

Use the mean duration of the breathing cycles to measure breathing rate:

$$\text{Breathing Rate (breaths/ min )} = (60 \text{ sec / min}) / \text{mean duration (sec /breath)}$$

Determine the ratio of forced expiratory volume at one second (FEV1) to the forced vital capacity (FVC) by dividing FEV1 by FVC. Normal values are between .80-0.95.

Determine the ratio of forced expiratory volume at one three seconds (FEV3) to the forced vital capacity (FVC) by dividing FEV3 by FVC. Normal values are between .80-0.95.

### Procedure: Breathing When Lying Down

1. The subject should lie down (face-up) quietly and become accustomed to breathing through the spirometer. They should be breathing normally before any recordings are made. Remember to hold the flowhead so that the outlets are on top.
2. Have the subject remove the flowhead from their mouth. They should hold it at the same level as their mouth, but in a position that prevents their breath or any other airflow from moving through the flowhead.

*Note: The LabScribe software will zero the **Volume** channel during the first five seconds of recording. No air should be moving through the flow head during this time.*

3. Click **Record**. Type “Resting” in the comment line to the right of the **Mark** button. After waiting five seconds for the **Volume** channel to zero, have the subject place the flowhead in their mouth and begin breathing. Press the **Enter** key on the keyboard. Click the **AutoScale** buttons for the **Air Flow** and **Volume** channels. Notice the slowly moving wave on the **Volume** channel. Record 5 breaths, which normally takes about 30 seconds. During this time, the **Volume** channel may automatically reset itself to zero; which it will do every 30 seconds after the recording begins.
4. Type “Maximum” on the comment line. Press the **Enter** key on the keyboard as the subject inhales as much as possible. As quickly as possible after reaching their maximum inhalation volume, the subject should exhale as quickly and as completely as possible.
5. The volunteer should return to normal breathing through the spirometer.
6. Click **Stop** to halt recording.
7. Save the file.

### Procedure: Data Analysis

1. Follow the data analysis procedures that were used in the before and after exercise experiment. Record your data in Tables 9 and 10.
2. Then use your data from the standing exercise and lying down exercise and complete Table 11.

**Table 8: Breathing Parameters When Lying Down**

	Tidal Volume (liters)	Period (secs/breath)	Maximum Inspiratory Flow	Maximum Expiratory Flow	Breath Rate (breaths/min)
Cycle 1					
Cycle 2					
Cycle 3					
Cycle 4					
Average					

**Table 9: Maximum Breathing When Lying Down**

	Tidal Volume	Inspiratory Reserve Volume	Expiratory Reserve Volume	Forced Vital Capacity	Forced Inspiratory Flow Rate	Forced Expiratory Flow Rate	FEV1	FEV 3
Maximum Breathing Cycle								

**Calculations for Breathing FEV1/FVC Ratio and FEV3/FVC Ratio**

Use the mean duration of the breathing cycles to measure breathing rate:

$$\text{Breathing Rate (breaths/ min )} = (60 \text{ sec / min } ) / \text{mean duration (sec /breath)}$$

Determine the ratio of forced expiratory volume at one second (FEV1) to the forced vital capacity (FVC) by dividing FEV1 by FVC. Normal values are between .80-0.95.

Determine the ratio of forced expiratory volume at one three seconds (FEV3) to the forced vital capacity (FVC) by dividing FEV3 by FVC. Normal values are between .80-0.95.

**Table 10: Comparison of Breathing Parameters in Standing and Lying Down Positions**

	Standing	Lying Down
Period (seconds/breath)		
Rate (breaths/minute)		
Tidal Volume (mLs/breath)		
Minute Air Flow (liters/min)		
Maximum Normal Air Flow (mLs/sec)		
<i>During inhalation</i>		
<i>During exhalation</i>		
Lung Volumes (liters)		
<i>Tidal Volume</i>		
<i>Inspiratory Reserve</i>		
<i>Expiratory Reserve</i>		
<i>Vital Capacity</i>		
<i>Forced Expiratory Volume – 1 sec (FEV1)</i>		
Forced Air Flow (mLs/sec)		
<i>During inhalation</i>		
<i>During exhalation</i>		
<i>FEV1/VC Ratio</i>		
<i>FEV3/VC Ratio</i>		

**Questions and Conclusions for Breathing Before and After Exercise**

1. Did tidal volume change after exercise? Did inspiratory and expiratory reserves change after exercise?
2. Did exercise influence the time taken for each breathing cycle?
3. Did the rate of air flow during the inhalation phase increase with exercise? How can you account for this?
4. Did the rate of air flow during the exhalation phase increase with exercise—how can you account for this?

5. Did the volume of air passing in and out of the resting volunteer's lungs each minute increase due to exercise? If so, was this due to an increase in the rate of breathing, the depth of breathing (tidal volume) or a combination of both factors?
6. Did exercise influence the vital capacity of the individual?
7. If the tidal volume changed due to exercise, can this be accounted for by changes in the TV, IR, the ER or some combination of both?

### **Questions and Conclusions for Breathing and Gravity**

1. What happens to the size of the finger pulse when the hands are raised?
2. If the size of the finger pulse signal indicates blood pressure, what do you think happens to blood pressure in the fingers when the hands are raised over the head?
3. What effect does lying down have on the minute volume (the total amount of air breathed into the lungs in a minute)?
4. What effect does lying down have on the various lung volumes, including tidal volume?
5. From your analysis of the finger pulse data, what is the effect of raising your hand on blood pressure?
6. With the answer to Question 5 in mind, would blood pressure be the same in the pulmonary capillaries at the base of the lung and the apex (top) of the lung in the standing individual? Which would have the lower blood pressure?
7. With the answer to Question 6 in mind, if lower blood pressure allows more pulmonary capillaries to be open, where in the lung would you expect to find more collapsed capillaries, at the base or at the apex?
8. If some capillaries are collapsed, what is the functional effect on the diffusion of O<sub>2</sub> and CO<sub>2</sub>?

9. Would you expect any difference in capillary blood pressures in the apex or in the base of the lungs in a subject who is lying down? If the blood pressure in all pulmonary capillaries is the same, would you expect the capillaries to be open or collapsed?
  
10. If all pulmonary capillaries are open when lying down, what is the functional effect on the diffusion of  $O_2$  and  $CO_2$ ?
  
11. If the demands for  $O_2$  and  $CO_2$  are the same in either body position, should tidal volume and breathing rate be affected by body position? Do you see this?
  
12. Would you expect any difference in the levels of  $O_2$  and  $CO_2$  in the exhaled air of a subject who is standing or lying down?