Human Physiology (Biology 4)

Laboratory Exercises

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Laboratory Exercises

At the end of each lab, you should be able to understand the concepts and processes learned and answer any of the questions investigated in the activity. You are expected to identify microscopic tissues/organs and state functions, concisely express concepts learned, and explain outcomes of experiments on lab practical exams.

Lab 1: The Microscope and Overview of Organ Systems

Lab Goals and Guidelines

For Microscope

- you will learn how to properly use and care for the microscope
- follow instructions in lab carefully
- instructor will review care and cleaning of microscopes
- field size activity will be done as a whole class
- do not use oil immersion today, but fill out as much of the chart as you can

For Organ Systems

- know the names of each organ system and the organs in each
- find the following organs on torso models: heart, kidneys, lungs, trachea, brain, esophagus, blood vessels, adrenal glands, liver, stomach, small and large intestine, pancreas, gall bladder, ureters, bladder, spleen
- know basic functions of organ systems

The Microscope

Microscope Basics

The microscope must always be handled properly. You must observe the following rules for its transport, cleaning, use, and storage:

* Transport in an upright position with one hand on the arm and the other supporting the base. Set it down carefully at your work station. Do not drag it across the table.
* Use only special lens paper to clean the lenses. Clean all lenses before and after use. Slides should also be cleaned.
* Always begin the focusing process with the 4x or 10x objective lens in position, changing to the higher-power lenses as necessary.
* The coarse adjustment knob may be used with the 4x or 10x lens, but use only the fine adjustment with 40x or 100x.
* Adjust lighting appropriately. Turn off the light when not in use.
* Always use a cover slip with temporary (wet mount) preparations.
* When you put the microscope away, remove the slide from the stage, and rotate the lowest-power objective lens into position. Wrap the cord around the clips on the back, not around the base.
* Never remove or loosen any parts from the microscope.
* Inform your instructor of any mechanical problems.

Activity 1:
Identifying the Parts of a Microscope

1. Obtain a microscope and bring it to the laboratory bench. (Use the proper transport technique!)

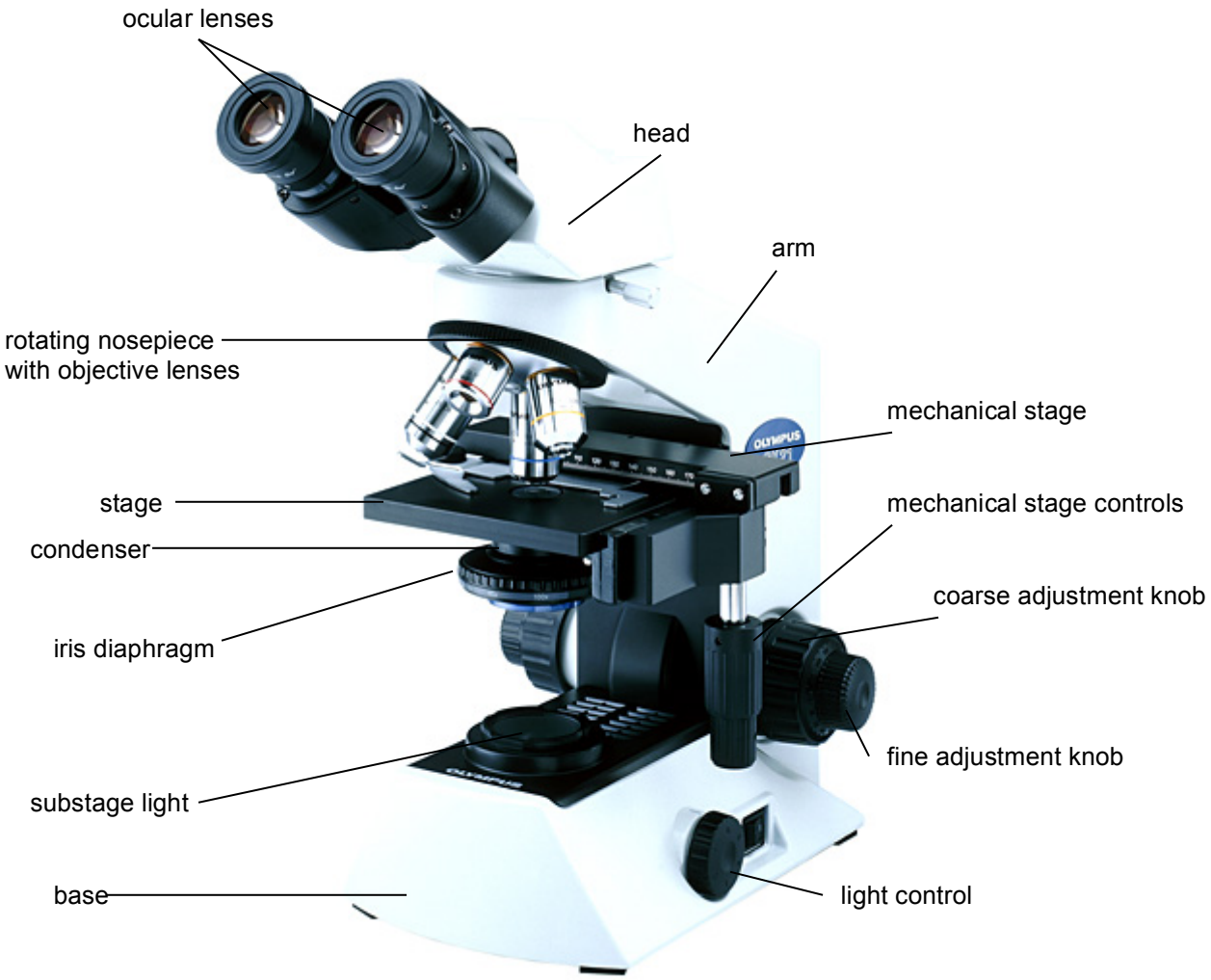
Compare your microscope with the figure on the following page and identify the following microscope parts:

Base: Supports the microscope.

Substage light: Located in the base, the light passes directly upward through the microscope.

Stage: The platform the slide rests on while being viewed. The stage has a hole in it to permit light to pass through both it and the specimen. The mechanical stage permits precise movement of the specimen.

Condenser: Concentrates the light on the specimen. The condenser has a height-adjustment knob that raises and lowers the condenser to vary light delivery. Generally, the best position for the condenser is close to the inferior surface of the stage.



Iris diaphragm dial: Dial attached to the condenser that regulates the amount of light passing through the condenser. The iris diaphragm permits the best possible contrast when viewing the specimen.

Coarse adjustment knob: Used to focus on the specimen when on 4x or 10x.

Fine adjustment knob: Used for precise focusing once coarse focusing has been completed. Use only this knob when on 40x or 100x.

Head or body tube: Supports the objective lens system, and the ocular lenses.

Arm: Vertical portion of the microscope connecting the base and the head.

Ocular (or eyepiece): There are two lenses at the superior end of the head, through which observations are made. An ocular lens has a magnification of 10x. If your

microscope has a pointer, it is attached to the right ocular and can be positioned by rotating the ocular lens

Nose piece: Has four objective lenses and permits sequential positioning of these lenses over the light beam passing through the hole in the stage. Use the nose piece to change the objective lenses.

Objective lenses: Adjustable lens system that permits the use of a scanning lens, a low-power lens, a high-power lens, or an oil immersion lens. The objective lenses have different magnifying and resolving powers.

2. Look at the objective lenses carefully. The shortest lens is the scanning lens, and has magnification of 4x. The low power lens is 10x. The high-power objective lens is 40x. The oil immersion objective lens is usually the longest of the objective lenses and has a magnifying power of 100x. Record the magnification of each objective lens of your microscope in the first row of the summary chart.

3. Rotate the lowest power objective lens until it clicks into position, and turn the coarse adjustment knob about 180 degrees. Notice how far the stage (or objective lens) travels during this adjustment. Move the fine adjustment knob 180 degrees, noting again the distance that the stage (or objective lens) moves.

SUMMARY CHART

	Scanning	Low Power	High Power	Oil Immersion
Magnification of objective lens	x	x	x	x
Total magnification	x	x	x	x
Working distance	mm	mm	mm	mm
Detail observed (draw or describe)				
Field size (diameter)	mm μm	mm μm	mm μm	mm μm

Magnification and Resolution

The microscope is designed to magnify specimens. Your microscope is called a compound microscope because it uses two lenses to magnify the specimen. The objective lens magnifies the specimen to produce a real image that is projected to the ocular. This real image is magnified by the ocular lens to produce the virtual image seen by your eye.

The total magnification of any specimen being viewed is equal to the power of the ocular lens multiplied by the power of the objective lens. If the ocular lens magnifies 10x and the objective lens magnifies 50x, the total magnification is 500x (10 x 50).

* Determine the total magnification with each of the objectives on your microscope and record in the chart.

With a compound light microscope such as the one you are using, the level of magnification is almost limitless, but the resolution (resolving power) is not. Resolution refers to the ability to discriminate two close objects as separate. The human eye can resolve objects about 100 μm apart, but the compound microscope has a resolution of 0.2 μm under ideal conditions. Objects closer than 0.2 μm are seen as a single fused image. Resolving power is determined by the amount and physical properties of the visible light that enters the microscope. In general, the more light delivered to the objective lens, the greater the resolution. The size of the objective lens aperture (opening) decreases with increasing magnification, allowing less light to enter the objective. You will likely need to increase the light intensity at the higher magnifications.

Activity 2:
Viewing Objects Through the Microscope

1. Obtain a millimeter ruler and a letter "e" slide. Adjust the condenser to its highest position and switch on the light source of your microscope.

2. Place the slide on the stage (in the slide holder) so that the letter e is centered over the light beam passing through the stage.

3. With your lowest power objective lens in position over the stage, use the coarse adjustment knob to bring the objective lens and stage as close together as possible.

4. Look through the ocular lens and adjust the light for comfort using the iris diaphragm. Now use the coarse adjustment knob to focus slowly away from the e until it is as clearly focused as possible. Complete the focusing with the fine adjustment knob.

5. Sketch the letter e in the space on the summary chart just as it appears in the field (the area you see through the microscope).

How far is the bottom of the objective lens from the specimen? This is the working distance. Use a millimeter ruler to make this measurement (an estimate is fine).

Record the detail observed and the working distance in the summary chart.

How has the apparent orientation of the e changed (compared to what you see looking at the slide with the naked eye)?

_____

6. Move the slide slowly away from you on the stage as you view it through the ocular lens. In what direction does the image move?

_____

Move the slide to the left. In what direction does the image move?

_____

7. Most laboratory microscopes are parfocal. This means the slide should be in focus (or nearly so) at the higher magnifications once you have properly focused. Without touching the focusing knobs, increase the magnification by rotating the next higher magnification lens into position over the stage. Make sure it clicks into position. Using the fine adjustment only, sharpen the focus. Notice the decrease in working distance. On high power or oil immersion, focusing with the coarse adjustment knob could drive the objective lens through the slide, breaking the slide and possibly damaging the lens. Sketch the letter e in the summary chart. What new details can you observe?

_____

Record the detail observed, and working distance in the summary chart.
Is the image larger or smaller than on the previous magnification?

_____

Approximately how much of the letter e is visible now?

_____

Is the field larger or smaller?

_____

Why is it necessary to center your object (or the portion of the slide you wish to view) before changing to a higher power?

_____

Move the iris diaphragm lever while observing the field. What happens?

_____

Is it more desirable to increase or decrease the light when changing to a higher magnification? Why?

_____

8. Repeat the steps given in direction #7 using the high-power objective lens.

Record the detail observed, and working distance in the summary chart.

9. We will not use the oil immersion lens today, but you will learn to use it later in the semester. Never click an oil immersion lens into place without using oil properly. You should fill in as much of the summary chart as you can right now. What do you think the working distance should be on oil immersion? What do you think you would observe?

Size of the Microscope Field

By this time you should know that the size of the microscope field decreases with increasing magnification. For future microscope work, it will be useful to determine the diameter of each of the microscope fields. This information will allow you to make an estimate of the size of the objects you view in any field. For example, if you have calculated the field diameter to be 4 mm and the object being observed extends across half this diameter, you can estimate the length of the object to be approximately 2 mm.

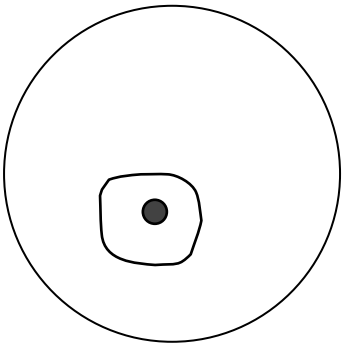
Microscopic specimens are usually measured in micrometers (μm) and millimeters (mm), both units of the metric system.

Activity 3:
Determining the Size of the Microscope Field

1. We will do the first part of this activity as a class. You will learn to measure the size of the field of view on 4x, then calculate it for the other objective lenses. The ultimate goal is to be able to estimate the size of an object in your field of view. If you are waiting for other groups to finish, you may take a quick break (make sure you are back in time to do field size) or move on to one of the other activities scheduled for today.

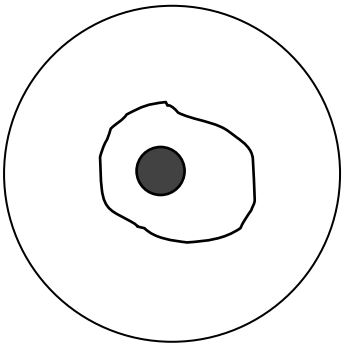
2. Estimate the length (longest dimension) of the following microscopic objects. Base your calculations on the field sizes you have determined for your microscope.

a. Object seen in low-power field:



approximate length: _____ mm

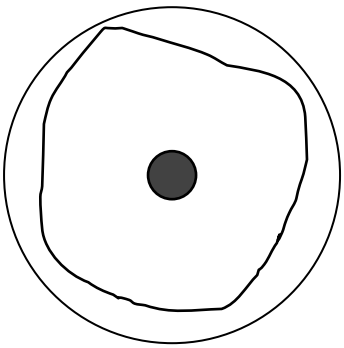
b. Object seen in high-power field:



approximate length: _____ mm

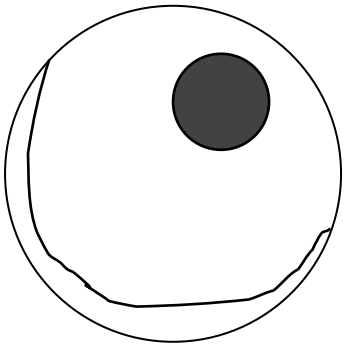
or _____ μm

c. Object seen in oil immersion field:



approximate length: _____ μm

3. If an object viewed with the oil immersion lens looked as it does in the field depicted below, could you determine its approximate size from this view? If not then how could you determine it?

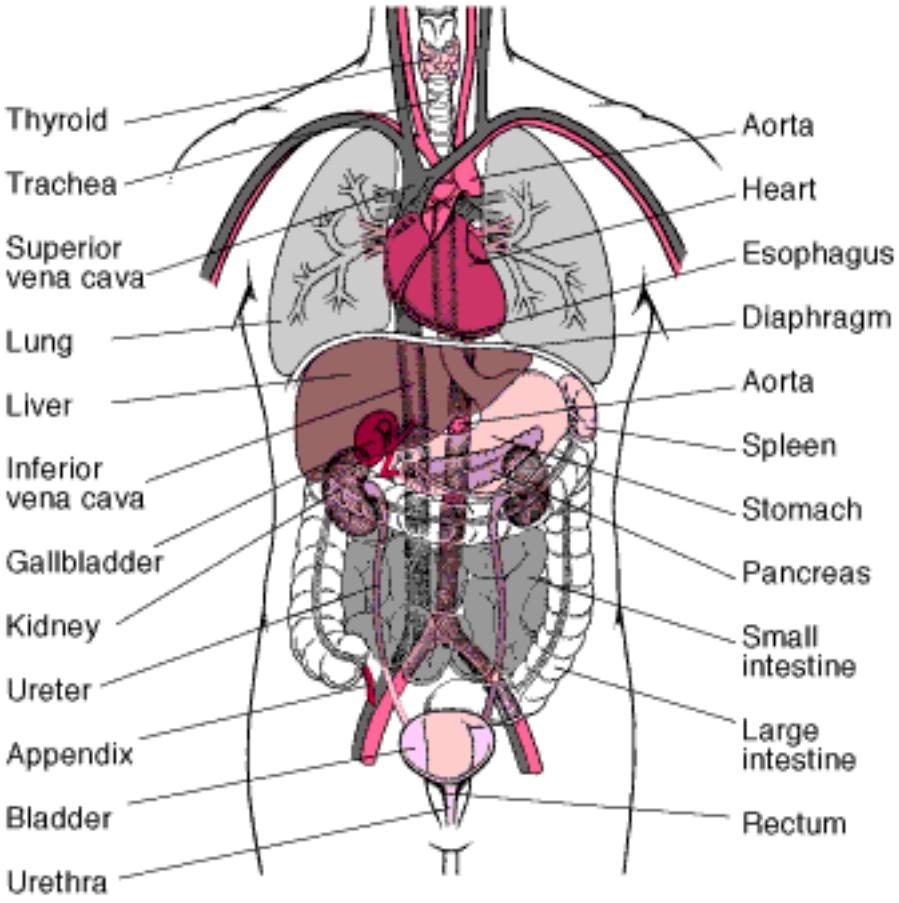


Perceiving Depth

Any microscopic specimen has depth as well as length and width. You will rarely view a tissue slide with just one layer of cells. Normally you can see two or three cell thicknesses. In microscope work the depth of field (the depth of the specimen clearly in focus) is greater at lower magnifications. In other words, you can clearly see more layers of cells on lower magnifications. On high magnifications, you can only focus on one layer at a time, and the other layers may appear blurred. Keep this in mind when working with the microscope.

Overview of Organ Systems

Organ System	Major Organs	Functions
Integumentary	Skin, including epidermis and dermis; glands	Protect deeper organs; excrete wastes such as salt and urea; regulate body temperature; vitamin D production
Skeletal	Bones, cartilages, tendons, ligaments, joints	Support and protect internal organs; provide levers for muscle action; form blood cells in marrow
Muscular	Muscles	Contraction of muscles allows movement such as locomotion and facial expression; generate heat
Nervous	Brain, spinal cord, nerves, sensory receptors	Control system with rapid response, activates muscles and glands
Endocrine	Pituitary, thyroid, parathyroid, adrenal, and pineal glands; ovaries, testes, pancreas	Control system which acts through hormones
Cardiovascular	Heart, blood vessels, blood	Transport of various substances in blood, e.g., blood gases, nutrients, wastes, hormones, ions
Lymphatic	Lymphatic vessels, lymph nodes, spleen, thymus, tonsils, other lymphoid tissues	Return leaked fluids to blood; destroy pathogens and remove debris; house defense cells and provide a location for activating immune responses
Respiratory	Nasal passages, pharynx, larynx, trachea, bronchi, lungs	Obtain oxygen and remove carbon dioxide; pH balance
Digestive	Oral cavity, esophagus, stomach, small and large intestines, teeth, salivary glands, liver, gall bladder, pancreas	Digest and absorb nutrients; eliminate wastes
Urinary	Kidneys, ureters, bladder, urethra	Remove wastes from blood; maintain water, electrolyte and pH balance
Reproductive	Typical male: testes, scrotum, penis, duct system Typical female: ovaries, uterine tubes, uterus, vagina	Make gametes for reproduction; make hormones



Use this diagram to help you find the following organs on the torso models:
heart, kidneys, lungs, trachea, brain, esophagus, blood vessels, adrenal glands, liver, stomach, small and large intestine, pancreas, gall bladder, ureters, bladder, spleen

Lab 2: Cells & Tissues

Lab Goals and Guidelines

- activities can be found in your lab supplement, read ahead for descriptions of four major tissue types; use pictures to guide you; review sheets at the end are optional
- view all tissue slides at appropriate magnifications, typically 10 or 40x
- find a view similar to the pictures for most tissues
- learn to recognize the 4 major tissue types and the various subtypes (there are more than this in the supplement, these are the 20 you need to know):

Major Type	Subtypes
Epithelial	simple squamous, simple cuboidal, simple columnar, pseudostratified columnar, stratified squamous, transitional
Connective	areolar, adipose, reticular, dense regular, dense irregular, hyaline cartilage, elastic cartilage, fibrocartilage, bone, blood
Muscle	skeletal, cardiac, smooth
Nervous	neurons

- always state the full tissue name on an exam, eg., "blood, connective tissue," or "simple squamous epithelium"
- you may use CT to abbreviate connective tissue, and E for epithelium
- know 2 functions and 2 locations for each subtype of tissue
- some slides may be set up on demo, most you will find yourself; you may need to look at more than one slide of each type; a particular slide may have multiple tissue types, you'll have to look for the correct view
- adjust lighting and contrast appropriately, do not use oil immersion
- we will stop in the middle of lab to go over some hints for recognizing tissues
- take only 2-3 slides at a time
- turn off microscopes will they will be unused more than a few minutes
- the slides you see today are a sampling of the major tissues in the body

Lab 3: Transport 1 & 2

Lab Goals and Guidelines

- you will learn various concepts regarding transport
- Transport 1 activities should be set up and performed as directed
- be sure to answer all questions
- Transport 2 activities can be found in the lab supplement, and should be done in groups of 2-3 people/computer
- answer all questions (exception: skip activity 4 on filtration)
- when asked a "what if?" question, think about it, answer, and try it in the simulation
- Chart 1: record rate
- Chart 2: record rate
- Chart 3: record numerical change in osmotic pressure or rate
- extra questions after completing Chart 3: What effect, in general, does solute concentration have on osmotic pressure? Why does 9mM NaCl have greater osmotic pressure than 9mM albumin or 9mM glucose?
- skip activity 4, but do activity 5
- review sheets at end are optional
- we will not print data

Transport 1

Be sure to read all the directions for a topic before you begin

Brownian Motion (do this exercise in pairs or groups of three)

Brownian motion refers to the random movement of particles. All atoms and molecules vibrate, and these vibrations can be observed indirectly. Make a wet mount of carmine dye solution (place a few drops of dye on a clean slide, cover with a cover slip). The slide should look red to the naked eye. Observe the dye particles with the 10x and 40x lenses of your microscope.

What do you observe about the dye particles?

What do you think is causing this?

Are you actually seeing molecules move? (is the microscope powerful enough for you to see something as small as a molecule?)

What do you think would happen if you heated the slide?

Diffusion (6 set-ups available, one for each lab bench)

Diffusion is the movement of molecules from where they are more concentrated to where they are less concentrated. Be extra careful! Try not to get the dye on yourself or anywhere but where it belongs - it will stain. You will use agar plates with holes already cut out of the agar. You should fill the hole with the dye but DO NOT allow the dye to overflow. Using the dropper bottles, place methylene blue dye (molecular weight 320) in the center of one agar plate, and place potassium permanganate dye (molecular weight 158) in the other plate. Depending on our lab supplies, you may use only one plate with two holes, and one dye will go into each hole. Using a millimeter ruler, measure the distance the dye has diffused in 5 minutes, 15 minutes, and 30 minutes (measure the diameter of the circle of dye). Depending on how much dye you used, the experiment may be finished in fewer than 30 minutes.

Which dye moved faster?

What is the relationship between molecular weight and the rate of diffusion?

Osmosis Through a Nonliving Membrane (demonstration)

Osmosis is diffusion of water through a membrane. Each tube originally contained 1.5 M sugar solution. Tube 1 is placed in distilled water, tube 2 in 1.5 M sucrose, and tube 3 in 3 M sucrose. What happened? There are two kinds of molecules involved here, sugar and water.

Which one is moving through the membrane?

Why do you suppose it is this one and not the other? (Remember that this is not a living membrane, but, if it were, how might you explain that one molecule moves through the membrane but the other does not?)

Osmosis Through a Living Membrane (demonstration)

You will observe a figure from your textbook. Red blood cells have been suspended in the following 3 solutions. Observe each picture and note the appearance of the blood cells. What has happened to the cells? (recall that you saw normal red cells in the tissues lab last week)

1. Isotonic solution (concentration of solutes equal to that in cells)
2. Hypertonic solution (concentration of solutes greater than that in cells)
3. Hypotonic solution (concentration of solutes less than that in cells)

Labs 4 & 5: Nervous System (ADAM)

Lab Goals and Guidelines

- this lab should enhance your understanding of the lectures on these topics and give you a visual explanation of events
- work in groups of 2-3/computer
- do not try to write down the information on the disc - it is already in your lecture notes
- ADAM Interactive Physiology is available for purchase if you would like your own copy, just do an internet search
- complete all the parts of the Nervous System activity, including the quizzes at the end of each section:

    Anatomy Review
    Ion Channels
    Membrane Potential (ignore p. 2 of quiz)
    Action Potential

- if the disc gives different information from lecture, give preference to lecture (for example, "active" channels = gated channels; "passive" channels = leakage channels; all cells have Na^+/K^+ pumps and we discussed direct vs. indirect creation of gradient; the part on equilibrium potential can be confusing, ask as necessary)
- there are no written directions to follow, simply do the ADAM activities
- for additional time with these activities, ADAM discs are available at the library or during my office hours (no borrowing from department)

Lab 6: Reflexes & Senses

Lab Goals and Guidelines

- you will learn a variety of concepts regarding these topics, including the names of some reflexes and how they are tested, and basic sensory physiology
- on an exam, you will be expected to know the names of the tests, descriptions of the tests, and results
- work in a group of 4-5 people, and switch subjects occasionally
- for some of the eye reflexes, it may be difficult to precisely determine pupil size, do your best
- for the tests with scented oils, the subject cannot know what is available ahead of time
- in this lab there tends to be differences in results obtained between groups, so we will discuss what the results should be at the end of class (or if there is not time, at the beginning of the next lab)

Reflexes & Senses

The Reflex Arc

Reflexes are rapid, predictable responses to stimuli. The pathway along which the electrical signals travel is called a reflex arc. There are five parts to a reflex arc:

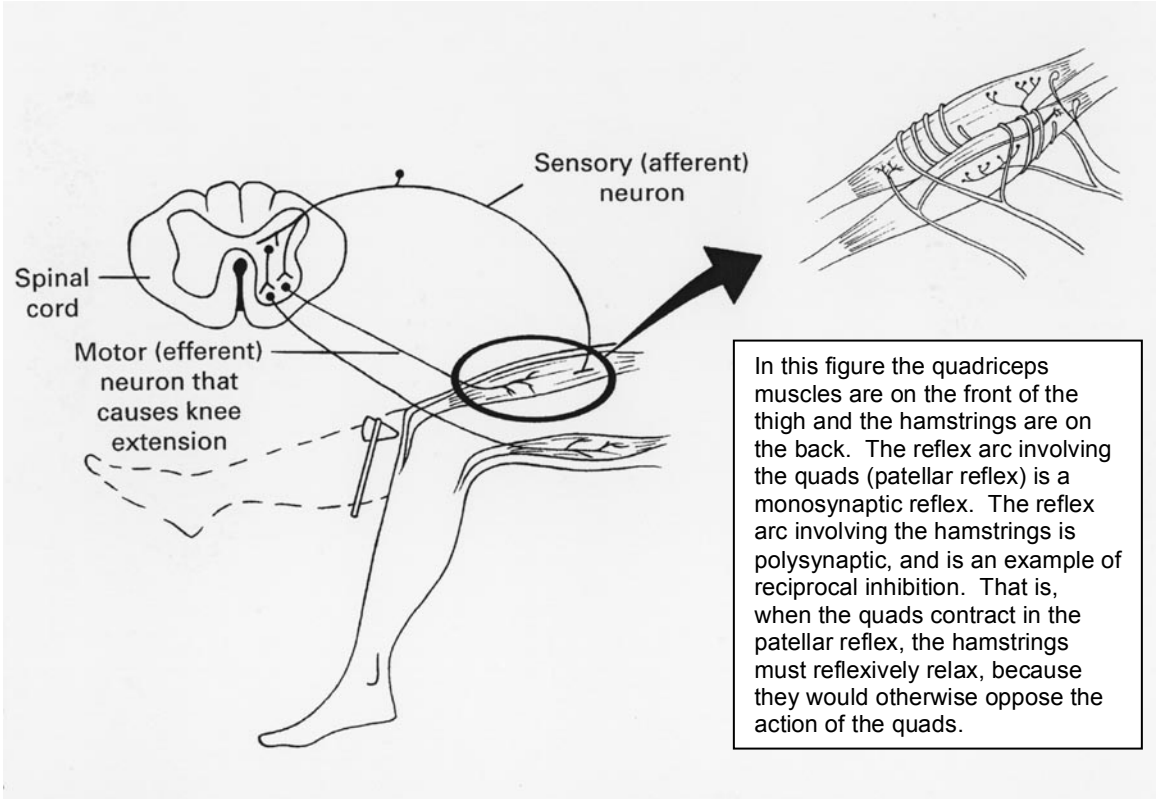
1. The *receptor* detects a stimulus.
2. The *sensory (afferent) neuron* sends an electrical signal to the CNS.
3. The *integration center* consists of one or more synapses in the CNS, and processes the information.
4. The *motor (efferent) neuron* sends an electrical signal from the CNS to the effector.
5. The *effector*, which may be muscle tissue or a gland, responds appropriately.

A monosynaptic reflex has only one synapse. An example is the patellar or knee-jerk reflex, which we will demonstrate today. Most reflexes, however, are polysynaptic, involving more than one synapse. The more synapses involved, the longer the reflex takes. A spinal reflex needs only the spinal cord to function, while other more complex reflexes require brain participation. Somatic reflexes involve skeletal muscle stimulation by the somatic division of the nervous system. Autonomic reflexes are dealt with through the autonomic division and activate smooth muscle, cardiac muscle or glands. Reflex testing is an important diagnostic tool for assessing the general health of the nervous system. Distorted, exaggerated or absent reflexes may indicate pathology. If the spinal cord is damaged, reflex tests can help pinpoint the level of damage.

Spinal Reflexes

Activity 1: The Patellar Reflex

The patellar (or knee-jerk) reflex is called a stretch reflex because it is initiated by tapping a tendon, which stretches the muscle, stimulating the muscle spindle (the proprioceptor inside the muscle) and causing reflex contraction of the quadriceps muscles. Stretch reflexes generally act to maintain posture, balance and locomotion. While this reflex is occurring, the antagonistic muscle group, in this case the hamstrings, reflexively relaxes to prevent interference with the patellar reflex. The brain will also receive information and the subject will be consciously aware of what is happening, although this is not necessary for the reflex to operate. Stretch reflexes tend to be absent or hypoactive with peripheral nerve damage or ventral horn disease, and hyperactive in corticospinal tract lesions. They are absent with deep sedation or coma.



In this figure the quadriceps muscles are on the front of the thigh and the hamstrings are on the back. The reflex arc involving the quads (patellar reflex) is a monosynaptic reflex. The reflex arc involving the hamstrings is polysynaptic, and is an example of reciprocal inhibition. That is, when the quads contract in the patellar reflex, the hamstrings must reflexively relax, because they would otherwise oppose the action of the quads.

1. The subject should sit on the lab bench with legs hanging freely. Tap the patellar ligament (see figure above). This assesses the L2-L4 level of the spinal cord. Test both sides. This will represent the baseline response.

2. Have the subject add several numbers together as you test again. This tests the effect of mental distraction. Is the response greater than or less than the baseline?

3. Test again while the subject pulls up on the lab bench with the arms while relaxing the lower limbs. This tests the effect of other simultaneous muscular activity. Is the response greater than or less than baseline?

4. Which is more likely responsible for the changes you observed - nervous system activity or muscular system activity?

Activity 2: Crossed Extensor Reflex

This is more complex than the patellar reflex. It involves a withdrawal of one limb followed by extension of the other limb. This would work if a stranger suddenly grabbed

your arm as you walked down the street - you would pull away with the grabbed arm and push with the other. It rarely works under laboratory conditions because people typically do not feel threatened in lab, but try it.

1. The subject should sit with eyes closed and one hand resting, palm up, on the lab bench. With a sharp pencil prick the subject's index finger. What happens?

2. Even if the extensor part of the reflex did not work, do you think it should be slow compared to the reflexes you have observed so far? Why?

Autonomic Reflexes

Activity 3: Pupillary Reflexes

We will test the pupillary light reflex and the consensual reflex. In both, the retina of the eye is the receptor, the optic nerve holds the afferent fibers, the oculomotor nerve contains the efferent fibers, and the smooth muscle of the iris is the effector organ. Many CNS areas are involved. Absence of these reflexes indicates severe trauma or damage to the brain stem from metabolic imbalance.

1. For the pupillary light reflex, have the subject in a relatively dim area (turn off lights in lab if helpful). The subject should shield the right eye. Shine a penlight into the subject's left eye. What happens to the pupil?

2. Also observe the right pupil. Does the same change (called a consensual response) occur?

When a reflex is observed on the same side of the body that was stimulated, that is called an ipsilateral response. When a reflex occurs on the opposite side of the body that was stimulated, that is a contralateral response.

3. If there is a contralateral response in a reflex, what does that indicate about the pathways involved in the reflex?

4. What is the purpose of the pupillary reflex you just tested?

5. Do you think these reflexes involve sympathetic or parasympathetic pathways? (you may want to try the next reflex before you answer)

Activity 4: Ciliospinal Reflex

This response is somewhat unusual, but interesting.

1. Have the subject stare straight ahead. Look into the subject's eyes as you gently stroke the skin, or just the hairs, on the left side of the back of the neck, near the hairline. What is the reaction of the left pupil? Is there any reaction on the right? If you see no reaction, try a gentle pinch instead of stroking.

2. Do you note a contralateral response?

The dilation of the pupil you should have noted is a sympathetic response. This can happen when one pupil receives more sympathetic stimulation than the other for any reason. Try to explain why dilation is sympathetic while constriction is a parasympathetic response.

Activity 5: Reaction Time of Unlearned Responses

The body's reaction time to a stimulus depends on many things, including sensitivity of receptors, speed of nerve conduction, number of synapses involved, etc. The type of response is also key. If the response involves an established reflex arc, response time will be short. If the response is an unlearned response, as we will demonstrate, then more pathways and higher level processing will be needed, and response time will be greater. It is critical that you follow the instructions precisely, or results will not be valid. You must use the same person as the subject for all three parts.

1. The subject should sit with hand out, thumb and index finger extended. Hold a ruler vertically so it is one inch above the subject's hand, numbers read from the bottom up. Drop the ruler, and let the subject grasp it with index finger and thumb. The relative speed of reaction time is determined by reading the number at the subject's fingertips. Record five successful trials. If the subject cannot catch the ruler, hold it a bit higher above the hand before dropping.

trial 1_____ trial 2_____ trial 3_____ trial 4_____ trial 5____

2. Test again, this time saying a simple word before dropping the ruler. Designate a certain word that will be the signal for the subject to catch the ruler. If any other word is said, the subject must let the ruler pass through the fingers. If the subject catches the

ruler on the wrong word, disregard that trial. Does this increase or decrease the reaction time?

trial 1_____ trial 2_____ trial 3_____ trial 4_____ trial 5____

3. Do the test again, now with word association. Say a simple word just before you drop the ruler. The subject must say a response word he/she associates with the stimulus word, before catching the ruler. If the subject cannot think of a word, the ruler must be allowed to pass through the fingers. Does this increase or decrease response time? How many times did the subject miss the ruler?

trial 1_____ trial 2_____ trial 3_____ trial 4_____ trial 5____

There was probably a great deal of variation in this particular set of trials. Why?

General Sensation and Sensory Receptor Physiology

The general sensory receptors of the body react to touch, pressure, temperature, pain and changes in body position. Cutaneous receptors are found in the skin. There is probably a great deal of overlap in the kinds of stimuli that the receptors respond to. Unencapsulated receptors include free dendritic endings, which sense mainly pain and temperature, Merkel discs, which sense light pressure and root hair plexuses, which sense touch via movement of hairs. The encapsulated receptors are enclosed in a capsule of connective tissue, and include Meissner's corpuscles, Pacinian corpuscles, and Ruffini's corpuscles. They are all mechanoreceptors, sensing stimuli such as touch, light and deep pressure, stretch, and vibration. Density of skin receptors is greater in areas that are designed to sense our environment.

Activity 6: Two-point Discrimination Test

1. Using calipers, test the ability of the subject to differentiate two distinct sensations when the skin is touched simultaneously at two points. If calipers are not available, use two blunt probes (or forceps) and a metric ruler. Start with the points right together, then gradually increase the distance apart. Record the distance at which the subject first reports feeling two distinct points of contact with the skin (the two-point threshold). Test the areas of the body as listed in the chart below. Of the tested areas of the body,

which ones seem to have the greatest density of receptors (smallest two-point threshold)?

Body area	Two-point threshold (mm)
Face	
Back of hand	
Palm of hand	
Fingertips	
Lips	
Back of neck	
Back of calf	

Activity 7: Tactile Localization

Tactile localization is the ability to determine exactly which portion of the skin has been touched. Some areas of the body have a high density of touch receptors, and a strong ability to localize a stimulus. Other areas have a lower density of receptors and less ability to localize a stimulus.

1. The subject should sit with eyes closed. Touch the palm of the subject's hand with a colored marker or pen. The subject then tries to touch that exact point with a different colored marker or pen. Measure the error in millimeters. Test all the areas three times, recording the results in the table below.

Body area	Trial 1	Trial 2	Trial 3	Average
Upper back				
Fingertip				
Anterior forearm				
Anterior arm				
Palm of hand				

Which areas seem to have a greater density of receptors? Does this agree with your findings in the two-point discrimination test?

Does the ability to localize the stimulus consistently improve over all three trials? Explain.

Activity 8: Adaptation of Touch Receptors

The number of signals sent by the sensory receptors may change with the intensity of the stimulus and the length of time the stimulus is applied. When the awareness of a stimulus decreases, it is called adaptation. Some receptors adapt rapidly, such as certain types of touch receptors, and others, such a pain receptors, may not adapt at all.

1. The subject should sit with eyes closed, arm resting on the lab bench. Place a coin on the anterior surface of the subject's forearm. Time (in seconds) how long it takes for the sensation to disappear.

2. Now stack three more coins on top of the first one. Does the sensation return? How long does it take for the sensation to disappear?

Do you think the same receptors are being stimulated by the four coins as with the one coin?

3. Using the tip of a pen or pencil, slowly bend back one of the tiny hairs on the subject's forearm. This is being sensed by the root hair plexus. If this type of receptor did not adapt, what would be the consequences to a person wearing their hair in a ponytail?

Special Senses

The special senses are vision, hearing, smell, taste, and equilibrium.

Activity 9: Demonstration of the Blind Spot

A portion of the retina (the sensory part of the eye) does not have any photoreceptors, because this is where the neurons head out of the eye via the optic nerve. Use the procedure below to demonstrate the existence of the blind spot.

1. Hold the figure of the ✖ and the ● about 18 inches from your eyes, straight out in front of your face. Close your left eye, and keep your right eye focused on the ✖, which should be directly in line with your right eye. Move the figure slowly toward your face. The spot should disappear at some point, then reappear as the figure is moved closer. You can try it with the left eye as well, but you'll look at the ●.

✖ ●

Activity 10: Afterimages

When light bouncing off an object strikes the rhodopsin pigment in the rods of the retina, the rhodopsin is split into its colorless precursor molecules. This is called bleaching of the pigment, and it ultimately results in a signal being sent along the optic nerve. When bleaching occurs, the pigment must then be remade before the rod can be stimulated again. This takes a bit of time. Both the stimulation of the rods and the following inactive period can be demonstrated.

1. Stare at a bright light bulb for a few seconds, then close your eyes.

First, you should have seen a positive afterimage caused by the continued firing of the rods after you first closed your eyes. Then, a negative afterimage (a dark image of the light bulb on a lighter background) is seen. This is because the pigment in the rods had been bleached.

Activity 11: More Eye Reflexes

1. Test the accommodation pupillary reflex by having the subject stare at a distant object (not a light source). Observe the subject's pupils. Then hold up printed material several inches in front of the subject and have him/her focus on it. What happens to the pupils? Why is this change useful?

2. Test the convergence reflex by having the subject stare at a distant object (not a light source). Observe the subject's pupils. Then hold up a pen or pencil and have the subject focus on it. How does the position of the eyeballs change? Why is this important?

Activity 12: Sound Localization

The tests should be performed in a relatively quiet area. If the room is too noisy, you may step outside.

1. The subject should close the eyes. Hold a watch with an audible tick (if one is not available, click together two blunt probes to make a noise) and move it to various locations around the subject's head (front, back, sides, above). Have the subject locate the position of the noise by pointing toward it. Is the sound localized equally well at all positions?

The ability to localize a source of sound depends on the difference in loudness of the sound reaching each ear and the time difference in the arrival of the sound at each ear. How does this help explain your results?

Activity 13: Tests of Balance and Equilibrium

The equilibrium apparatus, sometimes known as the vestibular apparatus, is part of your inner ear, but separate from the structures of hearing. The following tests demonstrate proper functioning of these structures.

1. Have the subject walk in a straight line, placing one foot directly in front of the other. Does the subject experience wobbling or dizziness? If not, this indicates a properly functioning equilibrium apparatus.

2. Have the subject stand with his/her back to the blackboard. Draw parallel lines on each side of the subject's body. Subject should stand straight, eyes open, for about two minutes while you observe. Do you note any gross swaying movements?

3. Now repeat the test, this time with the subject standing with one side of the body toward the blackboard.

4. Repeat steps 2 and 3, this time with the subject's eyes closed. What difference do you note with eyes closed?

5. Have the subject stand on one foot for about one minute, eyes open. Then try it with eyes closed. What is the difference?

Do you think that the subject's equilibrium apparatus was working equally well in all the tests? Were the subject's proprioceptors working? What conclusions can you draw about which factors are necessary for maintaining balance and equilibrium?

Activity 14: Effects of Smell on Taste

1. Obtain fresh cotton swabs dipped in three different scented oils. Place the swabs on a paper plate or paper towel to keep them clean and do not dip a swab back in the bottle of oil after it has touched any surface. The subject should not know what oils are available beforehand. Have the subject sit with eyes closed and nostrils pinched shut. Apply one of the oils to the subject's tongue. Can the subject identify the oil (or at least describe the taste)?

2. Now have the subject open the nostrils. Can the subject identify the oil?

3. Use the other two oils for this step. Simultaneously place one swab near the subject's nostrils and the other on the tongue. Which oil is identified first?

Do you think smell is important in what we generally call flavor?

Activity 15: Olfactory Adaptation

You will need two swabs dipped in scented oils for this test. They may be different from the previous ones used if you wish.

1. Place one swab near the subject's nostrils while the subject breathes through the nose, and record the time it takes for the scent to disappear (if it does not completely disappear, record the time it takes for the sensation to significantly decrease). Once the sensation has disappeared or decreased, immediately place the new oil at the subject's nostrils. Is the new scent detected?

What can you conclude about olfactory adaptation?

Lab 7: Muscular System (ADAM)

Lab Goals and Guidelines

- this lab should enhance your understanding of the lectures on these topics and give you a visual explanation of events
- do not try to write down the information on the disc - it is already in your lecture notes
- complete the following parts of the Muscular System activity, including the quizzes at the end of each section:

    Anatomy Review

    Neuromuscular Junction

    Sliding Filament Theory

    Contraction of Motor Units

    Contraction of Whole Muscle do only p. 16, length-tension relationship, then quiz p. 6-8

- there are no written directions to follow, simply do the ADAM activities

Lab 7a: Whole Muscle Function (Simulation)

this lab is not done every semester - check your schedule

Lab Goals and Guidelines

- you will learn how a whole muscle works, as opposed to the cellular level that you explored last week
- this activity can be found in your lab supplement
- do this activity in groups of 2-3/computer
- the graph you see is NOT a graph of an AP, it is force generated
- review sheets are optional

Lab 8: ECG & Cardiovascular System

Lab Goals and Guidelines

- read the appropriate section in your text before beginning (look up ECG in the index to find the right pages in your edition of the text)
- learn what an ECG can be used for, the basics of reading the tracing, and be able to calculate heart rate from the tracing
- learn what causes heart sounds, types of murmurs, and how to measure blood pressure
- do the ECG portion in groups of 4-5, your instructor or lab aide will help each group get started
- at the end of the ECG activity, everyone in the group should have a portion of the resting and the exercise readings
- after completing ECG reading, calculations should be done at your usual lab station
- we will stop during class to discuss heart sounds
- when an activity calls for the subject to exercise, the subject must really make an effort in order to obtain appropriate results

Electrocardiography and Cardiovascular System

Read the appropriate section in your text before beginning

Preparing the Subject

Use an alcohol swab to clean the skin of the subject at the electrode attachment sites on the inner wrists and inner ankles. Peel the electrodes off the packaging and apply to attachment sites. Clip the appropriate line to each electrode (RA = right arm, LA = left arm, RL = right leg, LL = left leg). The subject should sit in a comfortable position and not make any unnecessary movements.

Baseline Readings

Run a baseline (at rest) recording for leads I-III. Be sure to record until you have a strip of stable readings long enough for each group member to have a segment for calculations.

Exercise Readings

After the baseline recording is finished, stop recording, and have the subject run in place for 2-3 minutes. As soon as the subject stops, have him/her sit down and begin recording. When you have enough readings for each group member, stop recording. Clean up by throwing away the disposable electrodes. The subject may clean any residue off the skin with an alcohol pad. Return to your seat to perform calculations.

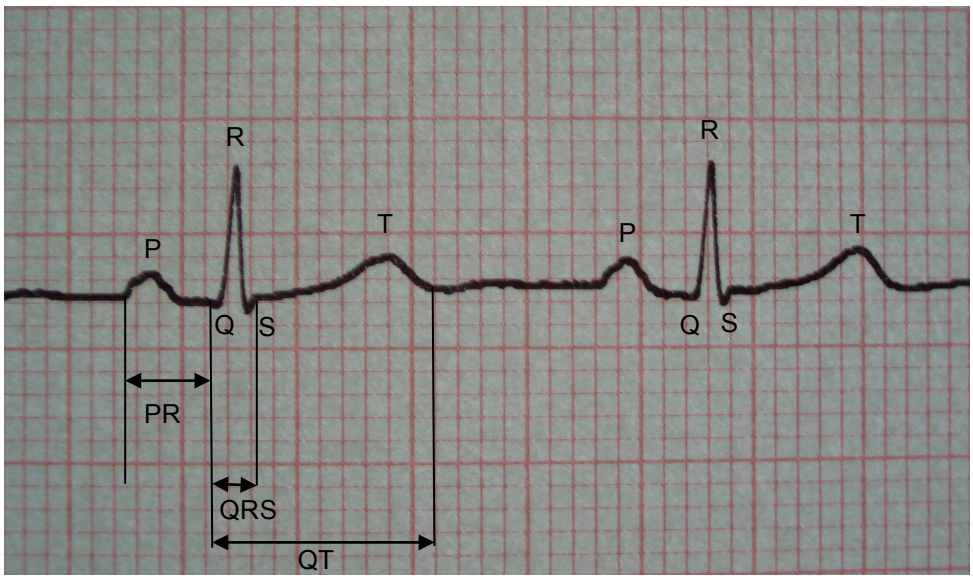
Understanding the Recording

P wave: atrial depolarization

QRS complex: ventricular depolarization (obscured atrial repolarization)

T wave: ventricular repolarization, may be inverted, elevated, or depressed depending on the lead sampled, or pathology

see your text for examples of abnormal ECG recordings



PR Interval
This is the time from the initiation of SA nodal depolarization to the initiation of ventricular depolarization. It encompasses the time it takes for the action potential to pass through the AV node. A typical value, from 120-200 msec, indicates that the electrical impulses are originating from the atria and following the proper conduction pathways. The PR interval may shorten slightly during tachycardia, and lengthen during bradycardia within the stated limits. A significantly longer than normal interval may suggest a partial AV heart block caused by damage to the AV node.

QRS Duration
The normal QRS duration is 60-100 msec. A longer QRS indicates conduction problems, often caused by bundle branch blocks that cause one ventricle to contract later than the other.

QT Interval
This interval represents the time from onset of depolarization to the completion of repolarization of the ventricles. Normal values are around 300-400 msec at a heart rate of 70 beats/min. As heart rate increases, the interval becomes shorter. As heart rate decreases, the interval becomes longer. An exceptionally long QT interval may indicate slowed ventricular repolarization, possibly due to hypokalemia, or other electrolyte imbalances. Shortened QTs are seen with hypercalcemia and digitalis toxin.

Baseline Calculations

Look at the figure on the previous page and use the following equations. You do not need to memorize equations, but you do need to know how to use them.

Computing Heart Rate

Use the lead which gave the most ideal recording. Measure the distance in millimeters (the strip of paper is divided into millimeters) from the beginning of one QRS complex to the beginning of the next QRS complex (that is, measure from Q to the next Q).

_____ mm x 0.04 sec/mm = _____ sec/beat

heart rate (beats/min) = $\frac{60 \text{ sec/min}}{\_\_\_\_\_ \text{ sec/beat}}$

Computing Intervals

Compute the duration of the following intervals in msec by multiplying mm x 40

QRS interval _____

Q-T interval _____

P-R interval _____

Are the computed values for the intervals and heart rate within normal limits (see above)? As the T-P interval increases, how might this affect cardiac output?

Exercise Calculations

Which calculations do you expect to be different from baseline? Repeat the baseline calculations for the exercise readings to verify your predictions.

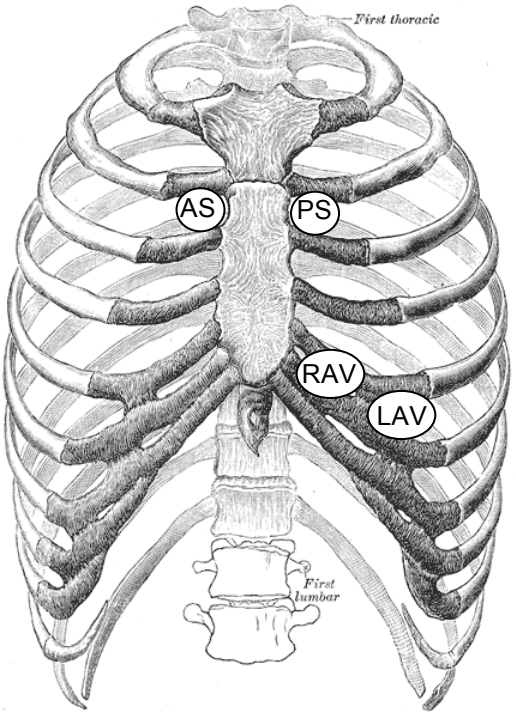
Diagnosing Abnormalities Using the ECG

What three types of problems can be diagnosed using an electrocardiogram? Also, define the terms tachycardia, bradycardia, arrhythmia, fibrillation, heart block, and myocardial infarction.

Heart Sounds

Closing of the heart valves produces vibrations in the walls of the ventricles and major arteries that are termed heart sounds. These sounds are commonly described as "lub-dup." The first sound, "lub," is due to the closing of the AV valves at the beginning of systole. The second sound, "dup," is due to the closing of the semilunar valves at the beginning of diastole. Listen to your own or your partner's heart sounds using a stethoscope. Use the alcohol pads to clean the ear pieces.

Individual valve sounds can be heard by placing the stethoscope in the appropriate thoracic region. Try this, referring to the figure that follows. Note that the semilunar valves are heard best at the second intercostal space, while the AV valves are heard at the fifth intercostal space.



AS = aortic semilunar valve
PS = pulmonary semilunar valve
RAV = right atrioventricular (tricuspid) valve
LAV = left atrioventricular (bicuspid or mitral) valve

Heart Murmurs

Abnormal heart sounds are usually associated with cardiac disease and are called murmurs. Sometimes a non-pathological murmur (called a functional murmur) can be heard, probably due to relatively thin heart walls that vibrate with rushing blood. Functional murmurs are more common in very young or elderly individuals.

Normally, blood flows smoothly through the structures of the heart. Pathological murmurs are heard due to turbulent blood flow that causes vibrations in the heart. A stenotic valve is a stiff, narrowed valve that does not open completely. Blood is forced

through, and a characteristic whistling or screeching sound is heard. An insufficient or incompetent valve does not close completely, and backward flow of blood creates a swishing or gurgling sound. This is sometimes referred to as regurgitation. Both stenotic and insufficient valves are most often caused by infection.

An experienced health care practitioner can tell by the location and timing of the murmur which valve is involved. A murmur occurring between the first and second heart sounds is a systolic murmur (lub-murmur-dup). If the murmur occurs between the second and first heart sounds it is a diastolic murmur (lub-dup-murmur).

Pulse

A pulse is felt due to the alternating expansion and recoil in an artery. It can be felt where arteries pass close to the surface of the body. Palpate (feel) as many of the pulse points as you can on yourself and/or your partner as appropriate. Use the fingertips of your first two fingers to feel for the pulse. A healthy pulse is strong and regular. What do you think a weak pulse could indicate?

Pulse Point	Where to find it
Temporal	At the “temples,” over the temporal bone
Facial	Approximately the middle of the mandible (jaw)
Carotid	Neck
Brachial	Arm just above the inner elbow
Radial	Inner wrist on thumb side
Femoral	In the groin, where the thigh meets the trunk
Popliteal	Back of knee
Posterior Tibial	Medial side of leg just above ankle
Dorsalis Pedis	Top of foot

Blood Pressure

Blood pressure is the pressure the blood exerts against the blood vessel walls, and is generally measured in the arteries. First measure your partner's blood pressure using the automatic equipment. This will give you an idea of what you will get using the manual method. Now for the rest of the procedures measure your partner's blood pressure with a manual sphygmomanometer. Understand the instructions before proceeding - don't keep the cuff inflated longer than necessary. The partner should sit comfortably with one arm resting on the lab bench. Make sure the cuff is deflated and wrap it around your partner's arm just above the elbow. Place the diaphragm of a stethoscope over the brachial artery (under the cuff). Inflate the cuff slightly above the pressure you expect based on the automatic reading and slowly release the pressure valve. Watch the pressure gauge as you feel for a radial pulse and listen for soft thudding sounds. When you first feel a pulse that should be the systolic pressure. The first sound you hear occurs at systolic pressure. The sound disappears at diastolic pressure. Record your partner's blood pressure, and calculate the mean arterial pressure.

MAP = diastolic pressure + (*pulse pressure/3)

*pulse pressure = systolic - diastolic

Once each student has a baseline value for blood pressure, get in a group of four and select one member for further testing. Have the subject stand still for a few minutes and measure pulse rate and blood pressure. Then have the subject exercise for several minutes and measure pulse rate and blood pressure. Calculate MAP for all blood pressure measurements. What changes do you notice?

Lab 9: Cardiovascular System (Simulation, ADAM)

Lab Goals and Guidelines

- work in groups of 2-3/computer
- do the simulation activity first (see lab supplement), to learn the effects of various electrical conditions and chemical substances on the heart
- do optional, highly recommended ADAM Cardiovascular System, focusing on the heart (no written instructions, begin with heart anatomy and do as many sections as you can including quizzes)
- activity 5: be sure to note changes in strength of contraction (amplitude of force) as well as rhythm

Lab 10: Blood

Lab Goals and Guidelines

- learn universal precautions (make sure to read this section before coming to lab)
- learn the concept of blood typing, how to read a blood type, and why it is important to determine blood type in the case of transfusions

Blood

Universal Precautions

In a health care setting, all body fluids must be treated as though they harbor infectious agents. This is to protect all individuals in the clinical setting. The precautions include techniques designed to prevent contact with pathogens and contamination, and if this is not possible, to take purposeful measures to decontaminate potentially infectious materials.

1. Barrier precautions, including masks and gloves, should be taken to prevent contact of skin and mucous membranes with patients' blood or other body fluids. Because gloves may have small invisible tears, double gloving decreases risk further. For protection during surgery, venipuncture, or emergency procedures, gowns, aprons, and other body coverings should be worn. Dental workers should wear eyewear and face shields to protect against spattered blood and saliva.

2. More than 10% of health care personnel are pierced each year by sharp (and usually contaminated) instruments. These accidents carry risks not only for HIV but also for hepatitis B and C, and other diseases. All disposable needles, scalpels, or sharp devices from invasive procedures must immediately be placed in puncture-proof containers for sterilization and discard. Under no circumstances should a worker attempt to recap a syringe, remove a needle from a syringe, or leave unprotected used syringes where they pose a risk to others. Reusable needles or other sharp devices must be heat-sterilized in a puncture-proof holder before they are handled. If a needlestick or other injury occurs, immediate attention to the wound such as thorough degermination and application of strong antiseptics can prevent infection.

3. Dental instruments should be sterilized between patients.

4. Hands and other skin surfaces that have been accidentally contaminated with blood or other fluids should be scrubbed immediately with germicidal soap. Hands should likewise be washed after removing rubber gloves, masks, or other barrier devices.

5. Because saliva may be a source of some types of infections, barriers should be used in all mouth-to-mouth resuscitations.

6. Health care workers with active, draining skin or mucous membrane lesions must refrain from handling patients or equipment that will come in contact with patients. Pregnant health care workers risk infecting their fetuses and must pay special attention to these guidelines. Personnel should be protected by vaccination whenever possible.

These are the basics. More information can be obtained at: http://www.cdc.gov

Blood Typing

In this activity, you will use simulated blood to practice typing.

Red blood cells have genetically determined proteins on their plasma membranes called antigens. The body recognizes its own antigens, but will fight against antigens it does not recognize as self. The worst transfusion reactions occur with the ABO and Rh blood groups. There are other antigen systems, but these usually do not cause as severe a reaction as with the ABO/Rh groups. If someone receives blood of the wrong type, the worst problem is the reaction of the recipient's antibodies on the donor's RBCs. When someone must receive blood, typically one would perform a type and cross match, where the donor cells and the recipient's plasma are mixed to test for agglutination. When the body encounters a foreign antigen, agglutination occurs. Agglutination is the clumping of RBCs due to binding of antibodies (part of the immune system) to antigen, and causes blockage of blood vessels and eventually death. In your blood, you have antibodies for the antigens you don't have (see below).

ABO blood groups	Rh factor
type O: no antigens, antibodies for A and B	Rh+: antigen present
type A: A antigen, B antibodies	Rh-: no antigen (antibodies form only if body exposed to Rh+ blood)
type B: B antigen, A antibodies	
type AB: both A and B antigens, no antibodies	

Simulated Blood Typing

You will use 4 unknown samples to explore the concept of blood typing. The "agglutination" of the simulated blood will not look exactly like real agglutination, but it will demonstrate the concept. Use a plastic typing slide for each of the 4 unknowns. Wash and dry the plastic slides when you are finished. Note that “anti-A” means you are adding antibodies for the A antigen, after which you check for agglutination. The same concept applies to the other antigens.

1. Put a few drops of Patient 1’s simulated blood in each well of slide #1.
2. Put a few drops of Patient 2’s simulated blood in each well of slide #2.
3. Put a few drops of Patient 3’s simulated blood in each well of slide #3.
4. Put a few drops of Patient 4’s simulated blood in each well of slide #4.
5. Add a few drops of simulated anti-A serum to the A well on each slide.
6. Add a few drops of simulated anti-B serum to the B well on each slide.
7. Add a few drops of simulated anti-Rh serum to the Rh well on each slide.
8. Stir each well with a different toothpick. Avoid cross contamination. Read and record results. Put a + where agglutination occurred, a - where it did not.

		anti-A	anti-B	anti-Rh	Blood Type
1	Patient 1				
2	Patient 2				
3	Patient 3				
4	Patient 4				

Lab 11: Blood Slides and Respiratory System (ADAM)

Lab Goals and Guidelines

For Blood Slides:

- learn to identify the components of blood
- learn causes of diseases involving the blood
- you should be able to find everything you need on one blood slide, but you may look at multiple slides if you wish
- focus on 10x, then 40x, then ask for help with oil immersion
- slide may be moved around while on oil immersion but may not be moved back to 40x unless cleaned first
- when finished, clean slides and lens with dry paper first, then lens cleaner

For Respiratory System (ADAM):

- this lab should enhance your understanding of the lectures on these topics and give you a visual explanation of events
- work in groups of 2-3/computer
- do not try to write down the information on the disc - it is already in your lecture notes
- there are no written directions to follow, simply do the ADAM activities
- complete the following sections and quizzes:

    Anatomy Review
    Pulmonary Ventilation
    Gas Exchange
    Gas Transport especially quiz p. 9 & 10

Blood Cells

Microscopic Examination of Blood

Locate the following structures on blood slides, using display materials for assistance and note the information below. Draw the structures and record definitions. Refer to your text for definitions.

Erythrocytes: (red blood cells) range from 4.5-5 million cells/mm^3. (note: 1 mm^3 = 1 μl) The cells are small, reddish, and have no nucleus. Define anemia and explain at least three potential causes. Define polycythemia and explain two causes.

Platelets: these small, purple-stained cell fragments range from 250,000-500,000/mm^3. They are important in the clotting process.

Leukocytes: (white blood cells) range from 4000-11,000 cells/mm^3. The basic function of these cells is protective, and they can move in and out of blood vessels (diapedesis) and wander through body tissues by amoeboid motion. Find a neutrophil, lymphocyte and monocyte. If possible, also find an eosinophil and a basophil. There are several characteristics that can be used to tell WBCs apart, but the best ones are size of the cell (relative to a RBC) and color of the cytoplasm.

If the size of the cell is:

- similar to RBC or just barely larger than RBC = lymphocyte
- much larger than RBC = **not** a lymphocyte

If the color of the cytoplasm is:

- Pink or lavender = neutrophil
- Blue or grey = lymphocyte **OR** monocyte

Granulocytes are larger than RBCs and have lobed nuclei and granules in their cytoplasm.

> Neutrophils: 40-70% of WBCs, 3-7 lobed nucleus, pale lilac or pink cytoplasm contains very fine granules which are difficult to see. They are active phagocytes and fight bacterial invasions (important in inflammatory response) as well as cleaning up debris.
>
> Eosinophils: 1-4% of WBCs, figure 8 or bilobed nucleus, large red/orange cytoplasmic granules. Important in ending allergic reactions (phagocytize antibody-bound allergens) and fighting parasitic worms.
>
> Basophils: less than 1% of WBCs, nucleus often U or S shaped with indentations, large dark purple cytoplasmic granules. Mediate inflammatory response (release histamine and other molecules) during allergic responses and parasitic infections.

Agranulocytes have no observable granules and nuclei are usually roughly spherical.

> Lymphocytes: 20-40% of WBCs, about the size of a RBC, dark blue or purple nucleus, sparse gray/blue cytoplasm. Important role in immune system.
>
> Monocytes: 4-8% of WBCs, largest of the WBCs, dark blue nucleus, abundant gray/blue cytoplasm. They are active phagocytes and considered important in long-term clean up.

Lab 12: Respiratory System

Lab Goals and Guidelines

- learn to define and measure respiratory capacities and volumes
- determine the most efficient way of breathing
- slides: identify the organs and functions, use 10x

    trachea - identify epithelial, submucosal, and cartilage layers

    lung - identify alveoli and bronchiole (note that bronchioles have various epithelial linings, ranging from pseudostratified ciliated columnar to simple columnar or cuboidal)

Respiratory System

Pulmonary ventilation (breathing) has two phases: inspiration, when air is taken into the lungs, and expiration, when air leaves the lungs. The lungs normally remain partially inflated throughout the phases of ventilation. The lungs cannot be completely deflated because the smallest airways collapse during forced expirations, trapping some air in the alveoli. This constant partial inflation of most alveoli means that gas exchange can continue to occur throughout the stages of respiration. Also, less effort is expended fully inflating the partially inflated alveoli than would be needed if they were completely collapsed.

Model Lung

Use the demonstration model to show how pulmonary ventilation works. Keep in mind the relationships between volume, pressure and airflow. Which part of the body does each part of the model represent?

Spirometry - Measuring Respiratory Volumes and Capacities

Measurement of various respiratory volumes and capacities is a useful way of determining general respiratory health. The type of spirometer we will use can only measure exhaled air, so some volumes will be measured indirectly by calculating them based on other values. A person's size, sex, age, and physical condition produce variations in respiratory volumes. You will need to memorize the following definitions, and the "typical" values. There will be considerable variation in actual measured values.

Tidal Volume (TV): amount of air inhaled or exhaled with each breath under resting conditions (500 ml)

Inspiratory Reserve Volume (IRV): amount of air that can be forcibly inhaled after a normal tidal volume inhalation (1900 - 3100 ml)

Expiratory Reserve Volume (ERV): amount of air that can be forcibly exhaled after a normal tidal volume exhalation (700 - 1200 ml)

Vital Capacity (VC): maximum amount of air that can be exhaled after a maximal inspiration (3100 - 4800 ml), this is the total amount of exchangeable air

Residual Volume (RV): amount of air left in the lungs after a maximal expiration (1200 ml), cannot be measured with a spirometer (this is the volume that makes it possible for gases to be exchanged continuously)

Total Lung Capacity (TLC): vital capacity plus residual volume (4200 - 6000 ml), this is the total amount of air that can fit in the lungs

Each student should follow the procedures below and obtain values for their respiratory volumes and capacities. Work in small groups or with a partner and follow directions carefully. Be aware that students tend to breathe faster and more deeply when they are paying attention to their breathing, so make an effort to breath as normally as possible for the experiments. Also, you must breath out only through your mouth, so plug your nose if necessary. Perform 3 trials for each test and average the values (be sure to reset the spirometer to 0 for each new trial).

1. Without using the spirometer, count and record the subject's normal respiratory rate (respirations/minute). The subject should sit and read quietly while the observer counts breaths.

2. Identify the parts of the spirometer you will be using. Some students will use wet spirometers, some dry. Make sure you know how to read the scale. The subject should stand up straight during testing. Obtain a disposable mouthpiece and attach it to the spirometer (each student will use one for all their experiments, and throw it away when finished).

a. Tidal Volume - Inhale and exhale normally a few times, then exhale into the spirometer. Do not force the expiration. Some of the dry spirometers may need to be set to 1000 instead of 0 to measure small volumes. Make sure you account for this when recording your volumes. If you have trouble measuring tidal volume on your spirometer, do the other measurements first, then switch to another spirometer for tidal volume.

b. Pulmonary ventilation is often described in units of ml/min and can be called the minute respiratory volume (MRV). Compute your MRV (MRV = TV x respirations/min). This is the total amount of air exchanged in one minute at rest.

c. Expiratory Reserve Volume - Inhale and exhale normally a few times, then exhale forcibly as much of the additional air as you can into the spirometer.

d. Vital Capacity - Inhale and exhale normally a few times, then bend over as you exhale all the air you can. Then, as you raise yourself into an upright position, inhale as fully as possible. Exhale as forcibly as you can into the spirometer.

e. Inspiratory Reserve Volume - Plug your average values for VC, TV and ERV into the following equation and calculate IRV

$$IRV = VC - (TV + ERV)$$

f. Residual Volume - Estimate your residual volume using the following equation

$$RV = VC \times *factor$$

ages 16-34	factor = 0.250
ages 35-49	factor = 0.305
ages 50-69	factor = 0.445

g. Total Lung Capacity - Calculate your total lung capacity

$$TLC = VC + RV$$

3. Compare your values to the typical values given in this lab exercise. Keep in mind those values are for comparison only, and may not match your values. The values you have obtained are useful for assessing overall health of the lungs. They can be combined with information from tests like x-rays and blood gas determinations to diagnose disease. Typically, low values for VC are seen in patients with lung disease, with varying changes in other respiratory volumes depending on the specific type of disease (for example, in emphysema and other obstructive lung disease VC is typically low, while RV is higher than normal).

Dead Space and Alveolar Ventilation

Anatomical dead space is the air in the conducting airways (trachea, bronchi and bronchioles). This air is not exchangeable. At a tidal volume of 500 ml, about 150 ml is in the airways. This means about 350 ml of air is actually in the alveoli and can potentially be exchanged with the blood. The dead space influences alveolar ventilation such that as requirements for air increase, a particular way of breathing is more efficient. Use the following information to determine whether it is more beneficial to breath slowly and deeply, or fast with shallow breaths.

Recall that pulmonary ventilation can be measured in ml/min and is calculated as TV x respirations/min. Alveolar ventilation (the amount of air that can actually be exchanged) is (TV - dead space volume) x respirations/min. In other words:

$$PV = TV \times resp/min$$

$$AV = (TV - 150) \times resp/min$$

Calculate PV and AV for slow, deep breathing (assume TV = 1200 ml, dead space = 150 ml, and 5 resp/min) and for fast, shallow breathing (assume TV = 200 ml, dead space = 150 ml, and 40 resp/min). Which way of breathing is a more efficient way to increase the amount of air exchanged at the lungs during periods of activity?

Lab 13: Urinary System

Lab Goals and Guidelines

- learn normal and abnormal urine constituents, and possible reasons for abnormal values
- be sure to use labels on test tubes (do not write directly on tubes)
- be very careful with hot plates
- do not waste dip sticks (use only 3/group)
- slides: identify the organs and functions, use 10 and 40x

    kidney - identify glomeruli vs. tubule (tubule will have simple cuboidal epithelium)

    bladder - identify epithelium and smooth muscle layers

Urinary System

The kidneys filter 150-180 liters of plasma each day. This means the entire plasma volume is filtered up to 65 times a day. The amount of urine produced per day is generally between 1.0 and 1.8 liters. Even with wide variations in diet and metabolic activity, healthy kidneys maintain a relatively constant plasma composition. The kidneys can produce urine of varying concentrations of solutes and water to maintain plasma composition. Urine produced by a healthy person has some general characteristics that we will investigate in this lab exercise. We will also explore some abnormal constituents of urine and potential causes of the abnormalities.

Characteristics of Urine

Freshly voided urine is generally clear and pale yellow (straw) to amber in color. The color is from urobilins, pigments that come from the breakdown of hemoglobin. Generally speaking, paler urine has a lower concentration of solutes. Abnormal color of the urine can come from certain foods, such as beets, drugs, bile or blood. The odor of freshly voided urine is characteristic, but when left standing bacterial action gives a strong odor of ammonia. Some drugs, vegetables, and disease conditions such as diabetes mellitus alter the characteristic odor. The pH of urine averages 6.0 but may range from 4.5 to 8.0. Diets high in protein typically increase the acidity of urine, while a vegetarian diet can result in a more alkaline urine. Bacterial infections may also cause urine to be more alkaline. The major normal urine constituents include water, urea, sodium, potassium, phosphates, sulfates, creatinine, and uric acid. Calcium, magnesium and bicarbonate ions can also be found in small amounts. Abnormally high concentrations of any of these urine constituents may indicate pathology.

Analyzing Urine Samples

We will be using artificial urine, so no special precautions need to be taken. However, keep in mind that with real urine samples you would use precautions as when dealing with any body fluid (gloves, proper disposal of samples and contaminated materials, cleaning up spills with bleach solution). There is one set-up for each lab table, and students at that table should work together. Each group will analyze one "normal" sample and 2 "unknowns". For each of the activities below, perform the observations or tests for each sample and record your results in the appropriate place in the table that follows. Before beginning, you should obtain about 50 ml of each urine sample. Be sure to label each one carefully.

Physical Characteristics of Urine

Determine the color, transparency, and odor of your samples. Color should be described as pale, medium or dark yellow, or "other" with description if applicable. Transparency should be indicated as clear, slightly cloudy or cloudy. Odor should be listed as "characteristic" or give a description if unusual. Note that since these are artificial samples you may not notice a typical odor. The pH can be determined by dipping a strip of pH paper in each sample two or three times and then comparing to the test chart (pH can also be determined using a dip stick, which you will do later).

Inorganic Urine Constituents

Sulfates - Add about 2.5 ml of urine to a test tube (measure the normal sample with the graduated cylinder to get an idea of how much you need, then estimate for all other samples and for the rest of the tests). Add a few drops of dilute hydrochloric acid and 1 ml of 10% barium chloride solution. A white precipitate (barium sulfate) indicates the presence of sulfates in the sample. Record as present or absent.

Phosphates - Prepare a hot water bath using a beaker half filled with water and your hot plate. Add about 2.5 ml of urine to a test tube, then add 3 or 4 drops of dilute nitric acid and 1.5 ml ammonium molybdate. Mix well with a glass stirring rod (be sure to rinse rod with distilled water and dry between samples) and heat gently in the hot water bath. A yellow precipitate indicates the presence of phosphates. Record as present or absent.

Chlorides - Place 2.5 ml of urine in a test tube and add several drops of silver nitrate. A white precipitate (silver chloride) indicates the presence of chlorides in the sample. Record as present or absent.

Rinse all glassware thoroughly, remove labels, and place in tub in sink

Organic Urine Constituents

Urea - Place two drops of urine on a clean microscope slide and carefully add one drop of concentrated nitric acid. Slowly warm the mixture on a hot plate until it begins to dry at the edges. Hold the slide with your fingers, and if it gets too hot pull it away from the heat. Once the slide has cooled, examine the edges of your preparation under low power to identify crystals of urea nitrate. You may be able to see the crystals without a microscope. Record as present or absent.

Glucose, Albumin (protein), Ketone bodies, RBCs/hemoglobin, Leukocytes - Use the dip sticks, following the directions on the package carefully and reading at the appropriate time. Record as positive or negative.

Unknowns

Determine what, if anything, is abnormal about each unknown sample. Speculate on the cause of the abnormality (see the section on abnormal urinary constituents for help). When everyone is done we will discuss results as a class.

Observation/Test	Normal Values	Normal Sample	Unknown 1	Unknown 2
Physical Characteristics				
Color	Pale yellow			
Transparency	transparent			
Odor	characteristic			
pH	4.5-8.0			
Inorganic components				
Sulfates	present			
Phosphates	present			
Chlorides	present			
Organic components				
Urea	present			
Glucose	negative			
Albumin (protein)	negative			
Ketones	negative			
RBCs/hemoglobin	negative			
leukocytes	negative			

Abnormal Urinary Constituents

Glucose
Presence of glucose in the urine is called glycosuria. It indicates abnormally high blood sugar levels. At normal blood sugar levels of 80-100mg/100ml, all the glucose in the filtrate is reabsorbed. The capacity of the renal tubules to reabsorb glucose may be temporarily exceeded with an excessive carbohydrate intake. Pathological glycosuria occurs in uncontrolled diabetes mellitus, in which body cells are unable to absorb glucose from the blood because the pancreatic islet cells do not make enough insulin, or there is an abnormality of insulin receptors.

Albumin
Presence of albumin in the urine is called albuminuria. Albumin, the most abundant plasma protein, is too large to pass through the filtration membrane. Thus, albuminuria is generally indicative of an abnormally increased permeability of the filtration membrane. Nonpathologic conditions like excessive exertion, pregnancy, or high protein intake can temporarily increase membrane permeability. Pathologic conditions include things that damage the membrane, such as kidney trauma due to blows, ingestion of heavy metals, bacterial toxins, glomerulonephritis, and hypertension.

Ketones
Presence of ketones in the urine is called ketonuria. These intermediates of fat metabolism are normally present in only trace amounts. If present in excessive amounts, this usually indicates abnormal metabolic processes. The results may be acidosis and associated complications. Ketonuria is expected during starvation, as the body uses up its fat stores. When coupled with glycosuria, it is generally diagnostic for diabetes mellitus.

RBCs
Presence of RBCs in the urine is called hematuria, and nearly always indicates pathology. Causes include kidney stones, infections, or physical trauma to the urinary organs. Accidental contamination with menstrual blood is possible.

Hemoglobin
Presence of hemoglobin in the urine is called hemoglobinuria. It is a result of hemolysis of red blood cells. It may be caused by hemolytic anemias, transfusion reactions, burns, or renal disease.

Bile Pigments
Presence of bilirubin (bile pigments) in the urine is called bilirubinuria. It is detected by a yellow foam that forms when the sample is shaken, and generally indicates liver pathology such as hepatitis or cirrhosis.

WBCs
Presence of WBCs or other pus constituents in the urine is called pyuria. It indicates inflammation of the urinary tract.

Casts

Casts are hardened cell fragments flushed out of the urinary tract. There are many types, and they always indicate pathology. White blood cell casts are typical of pyelonephritis, red blood cell casts are common in glomerulonephritis, and fatty casts indicate severe renal damage.

Lab 14: Digestive System and Acid/Base Demo

Lab Goals and Guidelines

For Digestive System:

- learn which enzymes digest which nutrients and where they act
- understand appropriate conditions for digestion
- understand the use of experimental controls
- your lab bench will be assigned one part of the experiment, and the class will share results
- be sure to use labels on test tubes (do not write directly on tubes)
- be very careful with hot plates
- after mixing, tubes must go immediately to incubation conditions
- when adding bile salts, add only a small amount
- for the boiling water bath, use the larger beaker; use the smaller beaker for the ice bath (ice bath will not be quite 0 degrees)
- assay results can be recorded as negative, strong positive, weak positive, etc.
- slides: identify the organs and functions, use mainly 10x

    esophagus - identify epithelium and muscle layers

    stomach - identify epithelium and smooth muscle layers

    duodenum - identify epithelium and smooth muscle layers

    colon - identify epithelium and smooth muscle layers

    liver - find lobules w/central vein, 4x and 10x

    pancreas - find islets (endocrine) and acinar (exocrine) cells, 10 x and 40x

For Acid/Base Demo:

- learn what a buffer is and what body fluid acts as a buffer

Digestive System

Enzymes are large protein molecules made by the body that act as catalysts. The digestive enzymes can be called hydrolases, because they break down food molecules by adding water to the molecular bonds, breaking them apart. Each enzyme is specific to one or a small group of substrates, and requires specific conditions to function properly.

Starch Digestion by Salivary Amylase

1. Obtain 6 test tubes.

2. Label each tube and load the tubes as in the table titled Salivary Amylase Digestion of Starch. Use 3 drops of each substance.

3. Place all tubes in their incubation condition for about 1.5 hours.

Protein Digestion by Trypsin

1. Obtain 5 test tubes.

2. Label each tube and load as indicated in the table titled Trypsin Digestion of Protein. Use 3 drops of each substance.

3. Place all tubes in their incubation condition for about 1.5 hours.

Pancreatin (Pancreatic Lipase) Digestion of Fats with Bile

1. Obtain 9 test tubes.

2. Prepare 2 tubes, label them 1V and 2V. To tube 1V add 10 drops water and 2 drops vegetable oil. To tube 2V add 10 drops of water, 2 drops of vegetable oil, and a pinch of bile salts. Cover each tube with Parafilm, shake, and let stand for 10-15 minutes. Observe for emulsification.

3. Label the rest of the tubes and load as indicated in the table titled Pancreatic Lipase Digestion of Fat. Use 5 drops of each substance. Add bile where appropriate. The bile is in powder form and you should take a *tiny* amount on the tip of the scoop for each tube that requires it. Note that pancreatic lipase is also known as pancreatin. Cover tubes with Parafilm, shake, and place in incubation condition for about 1.5 hours.

Enzyme Assays

Amylase Assay

1. Mark the spot plate with labels. Pour about a drop from each tube into the appropriate spot. Place a drop of Lugol's solution (Iodine) in each spot. A blue-black color indicates the presence of starch. Record results in the appropriate table.

2. Prepare a boiling water bath. Into the remaining mixture from each tube, place three drops Benedict's solution. Put each tube in the boiling water bath for about 5 minutes. A green-to-orange precipitate indicates a positive result for sugar (maltose, the product of starch digestion). Record results in the appropriate table.

Trypsin Assay

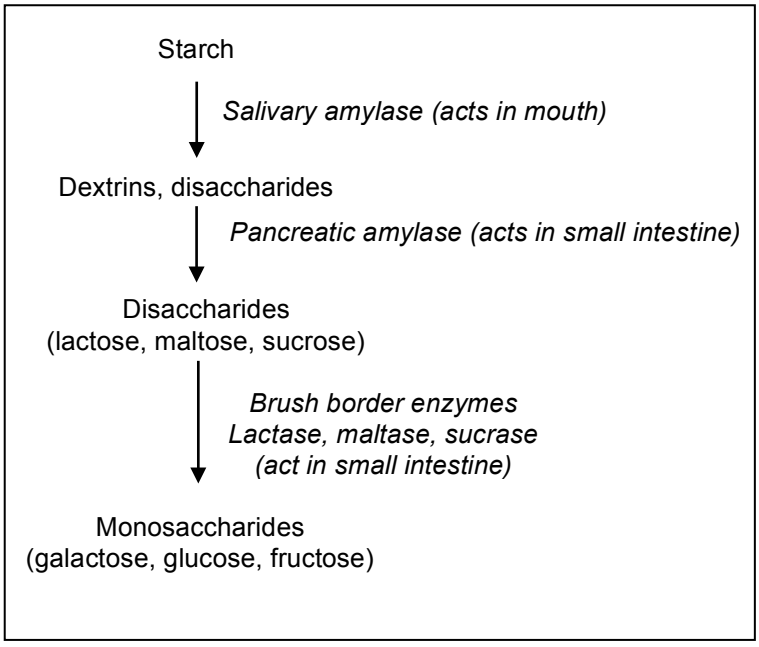
1. A deep pink color indicates a positive test for amino the products of protein digestion. Record results in the appropriate table.

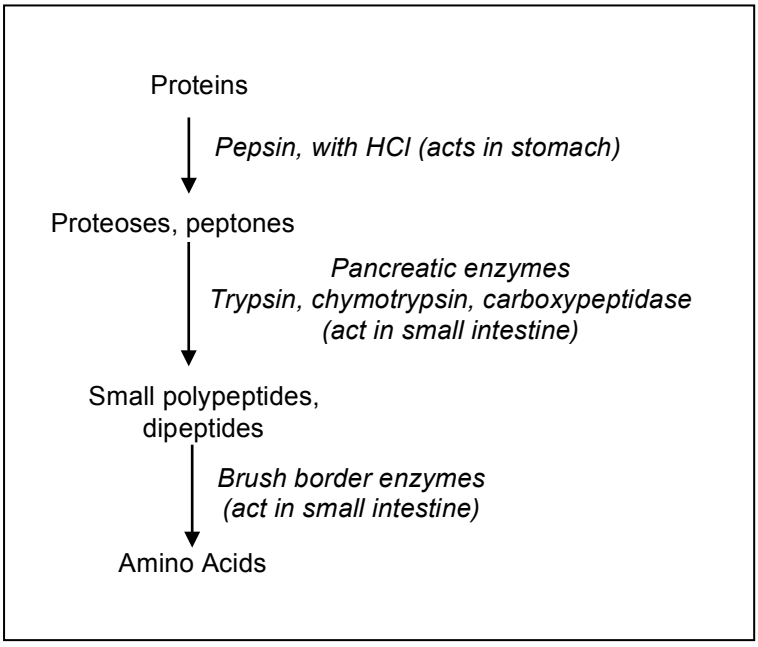
Pancreatin Assay

1. Prepare a color control by adding 0.1 M HCl drop by drop to tubes 1F and 2F until one turns pink. A pink color in the experimental tubes indicates the presence of fatty acids (one of the products of fat digestion). Record results in the appropriate table.

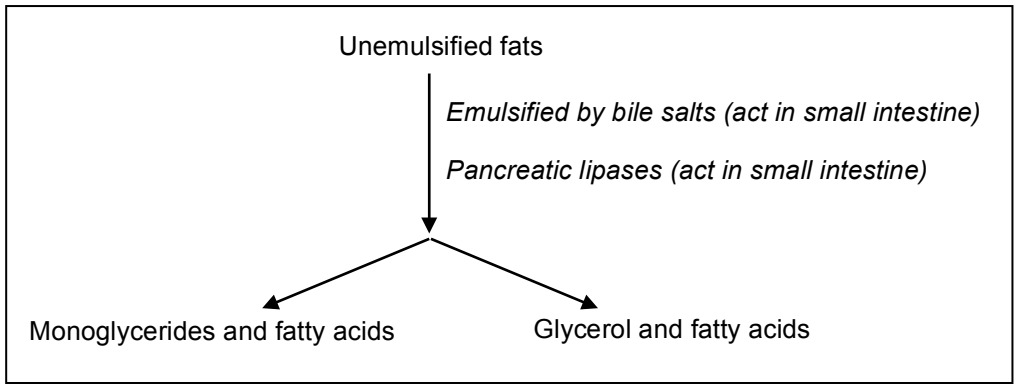
Questions to Think About

What is the purpose of a control? How do you explain the results of your experiments? What is the point of using various incubation temperatures? When everyone has completed these experiments we will go over the results as a class.





Monosaccharides and amino acids are absorbed into the blood in the capillaries of the villi in the small intestine, then go to the liver via the hepatic portal system.



Some glycerol and fatty acids are absorbed into the blood in the capillaries of the villi in the small intestine. Most of the products of fat digestion are absorbed into the lacteals of the villi and travel in the lymph until they enter the circulation via the thoracic duct.

Salivary Amylase Digestion of Starch						
	1S (control)	2S (control)	3S (control)	4S	5S	6S
Add 3 drops each	Amylase dH_2O	Starch dH_2O	Maltose dH_2O	Starch Amylase	Starch Amylase	Starch Amylase
Incubation condition	37°C	37°C	37°C	Boil 4 minutes, then 37°C	37°C	0°C
Lugol's test (Iodine)						
Benedict's test						

Trypsin Digestion of Protein					
	1P (control)	2P (control)	3P	4P	5P
Add 3 drops each	Trypsin dH_2O	Biuret Albumin dH_2O	Biuret Albumin Trypsin	Biuret Albumin Trypsin	Biuret Albumin Trypsin
Incubation condition	37°C	37°C	Boil 4 minutes, then 37°C	37°C	0°C
Color change					

Pancreatic Lipase Digestion of Fat							
	1F (control)	2F (control)	3F	4F	5F	4FB	5FB
Add 5 drops each	Pancreatin dH_2O	Litmus cream dH_2O	Litmus cream Pancreatin	Litmus cream Pancreatin	Litmus cream Pancreatin	Litmus cream Pancreatin Bile salts	Litmus cream Pancreatin Bile salts
Incubation condition	37°C	37°C	Boil 4 minutes, then 37°C	37°C	0°C	37°C	0°C
Color change							

Acid/Base Demo

Measure the pH of the solutions and answer the questions.

Beaker #	Contents	pH
1	150 ml water	
2	150 ml water + 1 drop concentrated HCl	
3	150 ml water + 1 drop concentrated NaOH	
4	150 ml buffer + 1 drop concentrated HCl	
4a	150 ml buffer + 4 drops concentrated HCl	
5	150 ml buffer + 1 drop concentrated NaOH	
5a	150 ml buffer + 4 drops concentrated NaOH	

What is a buffer? Did your results show that the buffer did its job?

Beaker #	Contents	pH
1	10 ml water	
2	10 ml water + 2 drops dilute HCl	
3	10 ml plasma	
4	10 ml plasma + 2 drops dilute HCl	

Is plasma a good buffer?