Lab Manual
for
Biology for Science Majors I
(BIOL 1406)

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Richland College
Dallas County Community College District
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Lab 1: Safety in the Biology Laboratory

Objectives

- Learn the safety rules for working with chemicals and participating in a safe manner when performing laboratory procedures
- Know the proper disposal of hazardous materials
- Know how to read the NFPA (National Fire Protection Association) label
- Understand what Safety Data Sheets (MSDS's) are
- Sign the "Safety Roster", which testifies your commitment to follow the safety rules and procedures presented in the lab
- Complete the safety questions

Pre-Lab Reminder: *Before arriving to this scheduled lab, read the entire lab (background, procedure, and questions), and use that information to complete the pre-lab in eCampus.*

Background Information

Experiments in laboratories involve equipment and materials that can pose safety considerations if used incorrectly. In order to avoid dangerous accidents, or minimize their damage, precautions must be taken by every student to ensure the safety of everyone working in the laboratory. Your lab instructor will demonstrate the proper handling of the tools of scientific investigation. Be sure to follow all suggestions and cautions as indicated by the instructor for safe behaviors in the lab. As to the chemicals that we will encounter, normal handling and adequate ventilation will minimize exposure.

We need to be informed in writing with medical verification in cases of pregnancy or other medical conditions that may be affected or aggravated by chemicals used in the laboratory. In such cases, the student is required to meet with the instructor to discuss ways to safely use or to avoid certain chemicals.

Preparing for Lab Work

Pre-read Before you come to the laboratory, read the background information and procedures for the experiment you will be doing. Since we want to make sure that you know what the experiment is about before you start the actual work, we have prepared a "Pre-Lab" activity for each experiment, which you must complete before starting the lab.

Prepare your work area Before you begin a lab, make sure that the lab bench was left clean by the previous group. If not, obtain a wet paper towel and clean your area. Also, clear the work area of all your personal items, such as backpacks, books, sweaters and coats. Find a storage place for them in the lab. All you will need is your laboratory

manual, calculator, pencil, and equipment provided to you on lab trays.

Chemicals used in the lab

The following shows you a list of chemicals that you may be using in the lab. Many of them are found in products that you use on a daily basis. In addition, we generally use concentrations that are relatively low, posing minimal risk to you.

Acetic Acid
Acetone
Albumin
Alconox (detergent)
Alanine (Demo)
Agar
Arginine (Demo)
Bleach (Sodium Hypochlorite)
Caffeine
Congo Red
Copper (II) Sulfate
Coverage Plus (Ammonia)
Ethyl Alcohol (Ethanol)
Levulose (Fructose)
Galactose
Gelatin
Glucose
Glycerol
Guaiacol
Hydrochloric Acid
Hydrogen peroxide
Hydroxylamine Hydrochloride
Iodine (IKI):Lugol's
-Iodine
-Potassium Iodide
Immersion Oil
Isopropyl Alcohol (Isopropanol)
Lactose
Linolenic fatty acid (Demo)
Lysine (Demo)
Malachite Green
Maltose
Methylene Blue
Monostearin (monoglyceride) (Demo)
Neutral Red
Petroleum ether
pH Buffers: 3.0, 5.0, 7.0, 9.0
Phenolphthalein
Phenol Red
Potassium Permanganate
Saline Solution
Sodium Hydroxide
Starch
Stearic (fatty) Acid (Demo)
Sucrose
Sudan IV
Tistearin (triglyceride) (Demo)

Last revised 7/24/2015

Material Safety Data Sheets (MSDS's)

Each chemical has an "MSDS" which contains information about hazards and safety precautions. If you want to know more about a particular chemical, you should feel free to ask your instructor for the MSDS of that chemical. They can be found in a single, designated place in the lab room and the prep area. In the MSDS you will find physical (molecular weight, melting point, boiling point) as well as chemical (reactivity) information of the chemical.

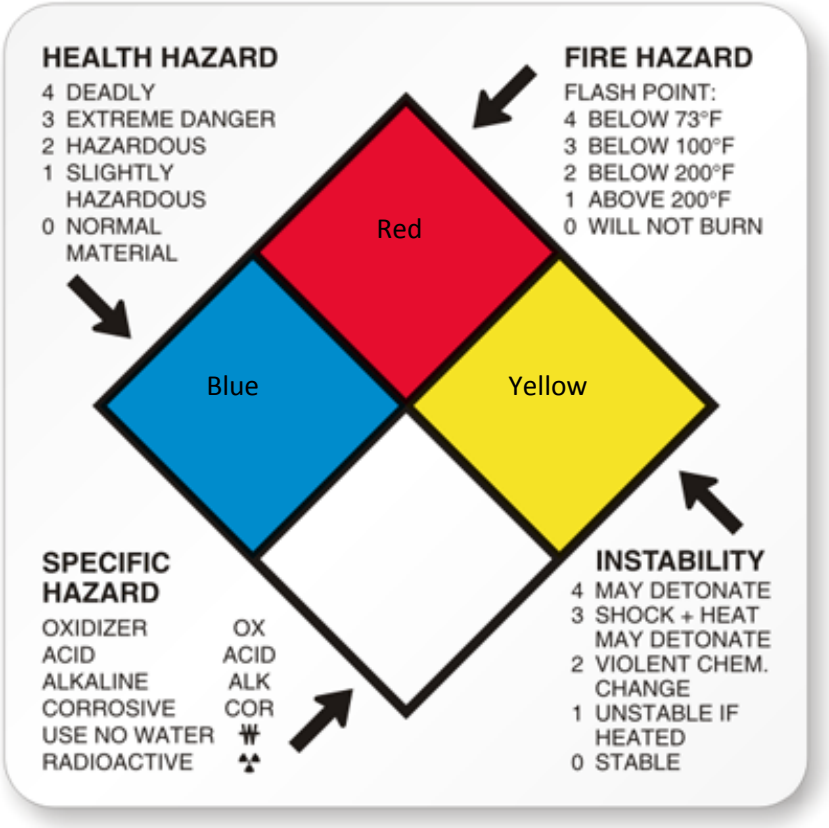
National Fire Protection Association (NFPA) Label

The NFPA label is characterized by a multicolored diamond shape that identifies the hazards of a material and the severity of the health, flammability, and reactivity hazards. A numerical rating ranging from (0) to (4) indicates hazard severity. A rating of (0) indicates minimal hazard, and (4) indicates a severe hazard.

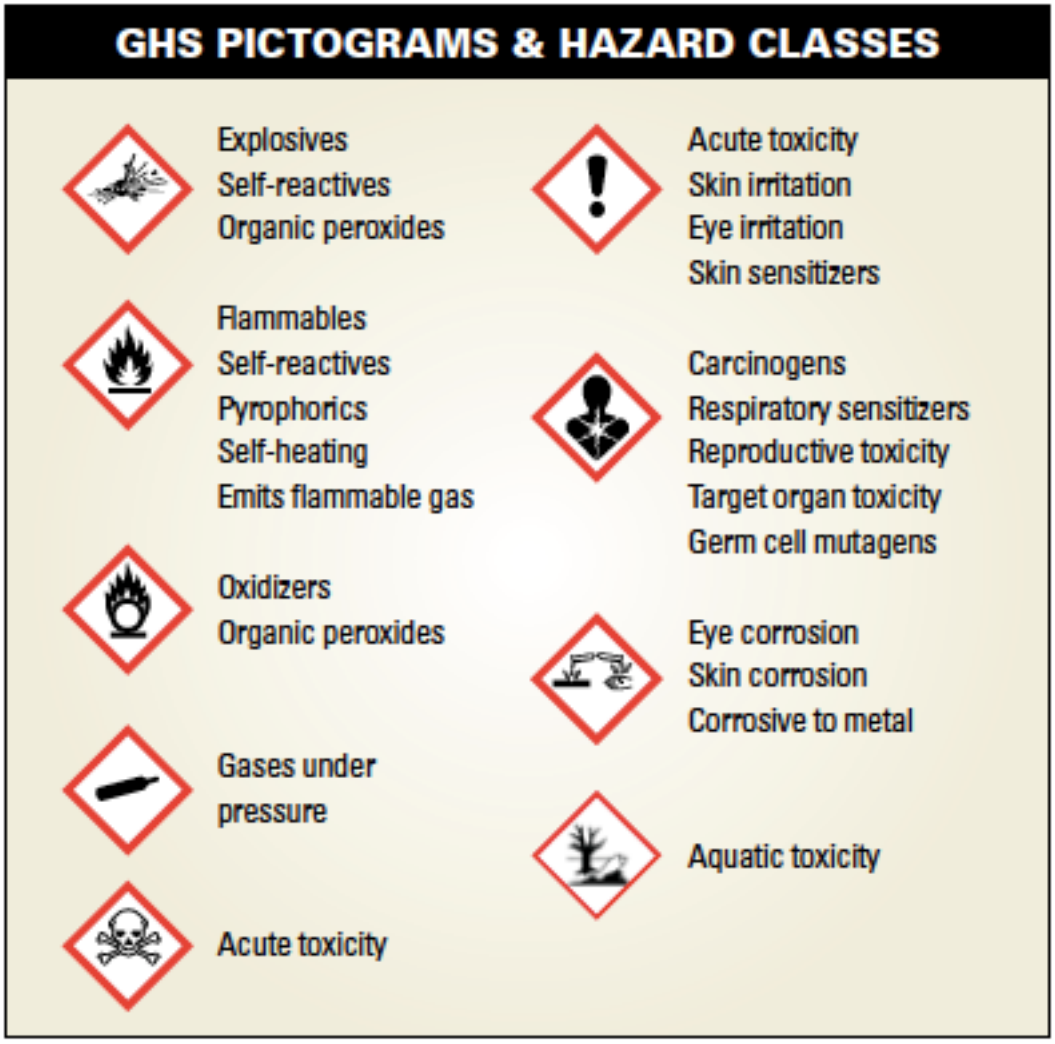
The hazards are arranged spatially as follows: health at nine o'clock position, flammability at twelve o'clock, and reactivity at the three o'clock position. In addition to their positions, they are also color-coded as follows: blue for health, red for flammability and yellow for reactivity.

The six o'clock position represents special hazards, and has a white background. Examples of special hazards include ALK (for alkali), ACID, OX (for oxidizer), and others.

ALL CHEMICALS IN THE LAB MUST HAVE THE NFPA LABEL.
IF YOU SEE ONE WITHOUT IT, NOTIFY YOUR INSTRUCTOR IMMEDIATELY.



The **Globally Harmonized System of Classification and Labeling of Chemicals (GHS)** is being implemented in our campus. This system includes criteria for the classification of health, physical and environmental hazards, and specifies what information should be included on labels of hazardous chemicals as well as safety data sheets. You should become acquainted with these **pictograms** and their meanings.



BIOLOGY LABORATORY PROCEDURES AND SAFETY RULES

It is extremely important that you follow the safety rules and lab procedures carefully.

Important Laboratory Procedures

1. Read labels on chemical bottles for safety precautions. If chemicals come in contact with the skin, **wash immediately with water** and inform your lab instructor.

2. Make sure you know how to properly dispose of any chemicals used in lab. Do **not** return chemicals to the original containers.

3. Make sure all chemical containers are closed tightly after use.

4. Keep the laboratory clean and organized.

5. **Clean** your work area carefully before leaving the lab. Glassware (including microscope slides) must be washed with soap and water, rinsed, and returned to the tray at your workstation. Test tubes should be inverted to dry, but **do not** invert Erlenmeyer flasks.

6. Handle hot glassware with appropriate clamps or tongs. Small beakers or flasks may be removed from heat using paper towels. Test tube holders are for lifting test tubes only.

7. Be familiar with experiments you will be doing **before** coming to the laboratory.

Safety Rules

1. **DO NOT EAT OR DRINK IN THE LAB!** Smoking is prohibited in all of the Richland College buildings.

2. Broken glassware must be disposed of in the glass disposal box located in the lab. This box is for **glass only**. A brush and dustpan are available to use in cleaning up broken glassware. Ask your instructor for assistance.

3. If you are cut or injured in any way during the lab, you must inform the instructor **immediately** (regardless of how small you think the injury is). In addition to administering first aid if needed, the lab personnel must take special precautions when cleaning areas and objects contaminated with human blood. **Do not attempt to clean blood-contaminated spills!**

4. If a mercury thermometer is broken, **do not** try to retrieve the mercury. **It is very poisonous!** Inform the instructor immediately so that lab personnel can retrieve the mercury safely.

5. Use extreme caution around an open flame. Keep long hair and loose clothing well away from the flame. Make sure that gas jets are off completely when burner is not in use.

6. Use extra care when working with scalpels, razor blades, and glass pipets. A glass pipet should **never** be forced into a pipump. Wrap the pipet in a paper towel when inserting it into a pipump and when removing it.

7. Bare feet are **not** permitted in lab. Wear shoes as protection against broken glass or spillage.

8. Know where to find emergency equipment such as the **eyewash, fire extinguisher, fire blanket, electricity and gas shut off switch, and telephone (dial 911 for campus police).** Report all accidents to the instructor immediately!!

9. Report any conditions that appear hazardous to the instructor.

10. **Do not** bring children to the lab. Unaccompanied children are not permitted on the campus.

You should have a copy of these safety rules, a chemical list for this course, and an example of an NFPA label. Make sure you understand all of these pages and keep them with your lab manual or in a folder or notebook, which you will bring to every lab.

Signing the Safety Roster

You must sign a laboratory Safety Roster. By doing so, you are saying that you understand the safety rules and that you agree to follow them and all other instructions stated in the handouts and given by the instructor.

If you do not sign the Safety Roster,
you will NOT be allowed to participate in subsequent lab exercises!!

End of Lab Questions

What is an “MSDS” ? What information does it provide? Where are they located?

Draw a mini-NFPA label and indicate the correct position of the colors and their meanings.

How is the degree of hazard indicated in the NFPA label?

What do you do if you break glassware in the lab?

What should be done with blood-contaminated materials?

Can you bring water bottles to drink during the lab?

What should you do about the lab work area at the beginning and at the end of every experiment?

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(to ensure each lab starts on a front side in 2-sided printing)

Lab 2: Microscopy

Using the Compound Light Microscope

Objectives

- Learn the differences between a light microscope, an electron microscope and a stereomicroscope
- Define and differentiate magnification, resolution, and contrast
- Name the parts of the compound microscope and explain their function
- Explain the terms field of view and depth of field, and know how they are related to the magnification power
- Learn how to focus a prepared microscope slide with the scanner, low power and high power objective lens
- Measure the field of view diameter and area with all four objective lenses
- Learn how to estimate the size of a specimen with the microscope
- Prepare a wet mount slide and use it to locate living organisms in the sample taken
- Learn the basic operation of the stereomicroscope

Pre-Lab Reminder: *Before arriving to this scheduled lab, read the entire lab (background, procedure, and questions), and use that information to complete the pre-lab in eCampus.*

Background Information

Biology involves the study of living organisms of a diverse array of sizes. Some organisms – such as giraffes, ants, mushrooms, corn plants and most seeds-can be studied to some extent with the naked eye. At the cellular level, however, important biological structures cannot be observed without a microscope.

There are two main types of microscopes based on the kind of energy used to view specimens. **Light microscopes** use visible light going through the specimen and through glass lenses, resulting in an enlarged image seen by the eye. They are called "compound" microscopes because they consists of two or more lenses that collect and focus the light that passes through a transparent specimen (i.e. it works by transmitted light passing through the specimen). Since light has to go through the specimen, the specimen must be thin or transparent. The light microscope provides magnification power as well as resolution.

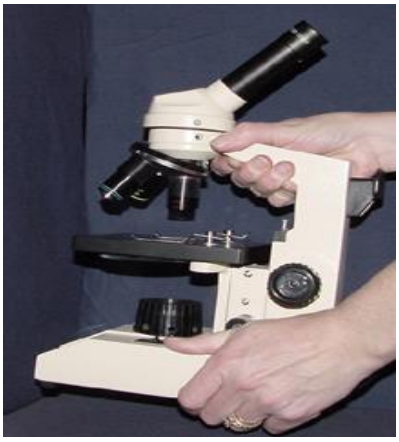
Magnification means that the image appears many times its natural size. **Resolution** refers to the capacity to distinguish two points in space as separate points. Magnification without resolution is not very useful, but both together provide an enlarged and sharp image. The resolving power of the naked eye is about 0.1 mm, and that of the light microscope can be 0.1 μm (that is, a 1000x increase!).

Electron microscopes use a beam of electrons originating from an electron source within the instrument. This is a much more expensive instrument, requiring a trained technician for its

operation. The beam of electrons is focused by magnetic lenses resulting in a magnified image appearing on a fluorescent screen (similar to a television tube) or on photographic film. Even though the images produced by an electron microscope are always in black and white, the electron microscope provides much more resolution and images that are amazingly clear and detailed. The resolution power of an electron microscope is about 0.2 nm, about a thousand fold increase over the light microscope!

The capacity to discern detail depends also on **contrast,** or the degree to which the details on the specimen stand out against their background. That is why many of the specimens examined with the light microscope are stained with chemical dyes that increase the contrast, making the specimen more visible. In addition, certain dyes are capable of differentially staining certain parts of the cells. Thus, the nucleus and the mitochondria, for example, can be observed with the use of different dyes. Moreover, in many cases, adjusting the light going through the specimen can allow you to get better contrast.

There are times when the specimen cannot be observed with the compound light microscope, because it is too large and/or opaque (e.g. a small flower or insect). These specimens can be observed using the **stereomicroscope** (or dissecting scope). The stereomicroscope has two eyepieces that provide a three-dimensional view of the specimen. It allows the viewing of opaque specimens by using <u>reflected light</u>: the magnified image is formed as the lenses gather and focus light that strikes and bounces off the specimen.



Parts of the Compound Light Microscope

Activity 2-1: Familiarize yourself with the microscope

1. Each individual will work on their own microscope.
2. Your microscope is heavy, and expensive! Please use BOTH hands when carrying it.
3. Using Fig. 2-1 a) and b) on the next page as a reference, find all of the following components in or on your microscope:

   a. **ocular lenses** (eyepiece): the lenses that you look through. In your microscope they provide a fixed magnification power of **10x**. ***Note:** One of the oculars in your microscope has a line that serves as a **pointer**. If you don't see it when using both eyes, try closing one eye and looking through each ocular lens separately.**
   b. **arm**: used to hold the microscope when carrying it in the lab
   c. **revolving nosepiece**: holds four objective lenses and rotates to allow you to change the objective lens
   d. **objective lenses**: provide various magnification powers
      scanner: magnifies **4x** by itself. Used to scan the microscope slide to locate the specimen. <u>**ALWAYS**</u> the first objective you use to start looking for something on a slide.
      low power: magnifies **10x** by itself
      high power: magnifies **40x** by itself

oil immersion: magnifies **100x** by itself. To be used with a special microscopy oil **ONLY**.

e. **stage**: where the microscope slide goes

f. **slide holder**: keeps the slide in its proper, secured place

g. **coarse adjustment knob**: larger knob on the lower part of the arm; typically only on one side, not both. Moves the stage up and down; use for finding the sample only under the 4x/scanning or 10x/low power objectives. Do NOT use with 40x or 100x objectives.

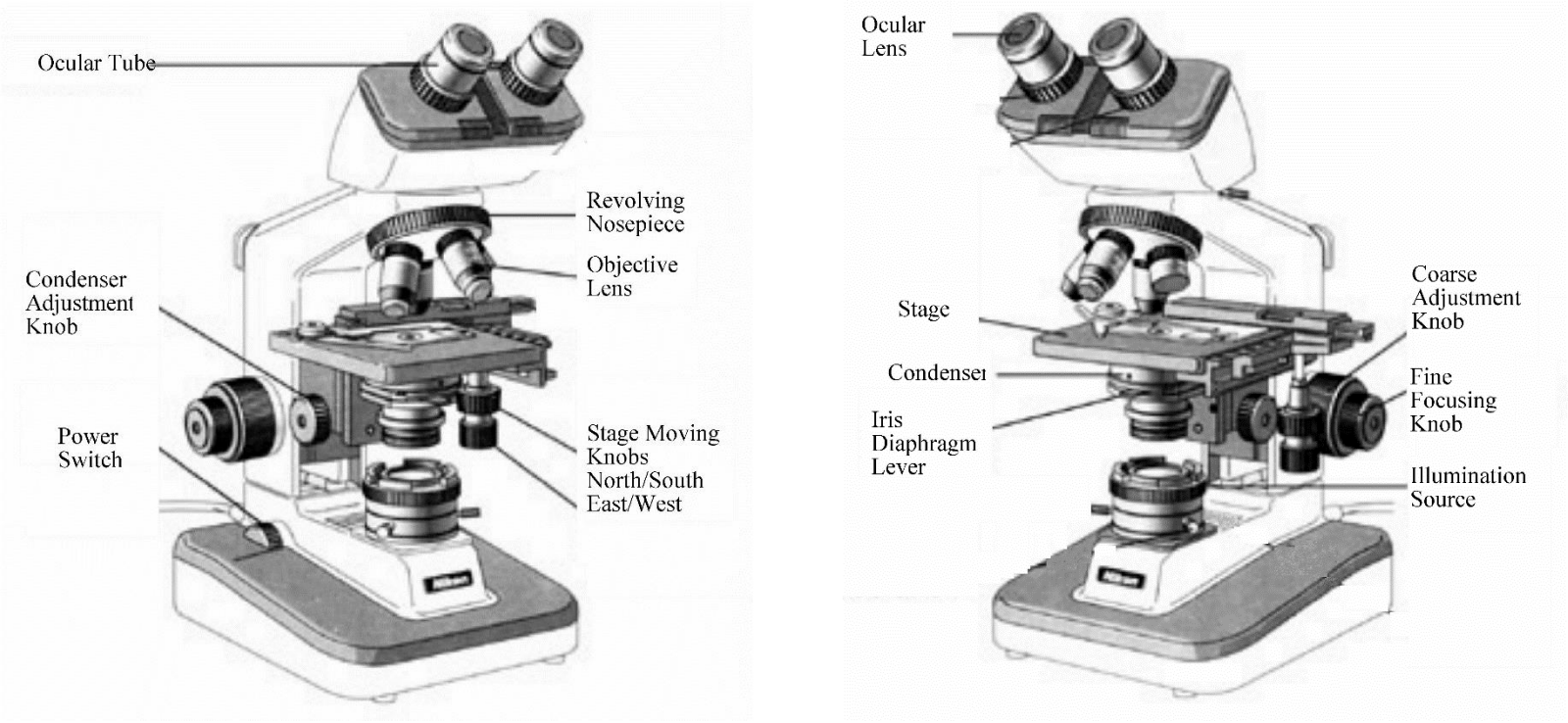
h. **fine adjustment knob**: inside the coarse adjustment knob on one side; typically a smaller knob by itself on the other side. Use for fine focusing AFTER you can already see the sample.

i. **stage moving knobs**: located underneath the stage, they move the stage north/south or east/west

j. **iris diaphragm**: lever underneath the stage that regulates the amount of light going through the specimen (serving as a "window" to let more or less light)

k. **condenser**: a lens that concentrates the beam of light going through the specimen

l. **condenser adjustment knob**: a knob underneath the stage that raises or lowers the condenser. *For your microscopes, keep the condenser raised as high as it will go during use.*



a) **b)**

Figure 2-1 a) and b) Side views of the microscope.

Activity 2-2: **Setting up the Compound Light Microscope**

1. Clean all the microscope's lenses with lens paper, **NEVER** rub the glass surfaces with a paper towel or Kimwipe – they contain fibers that could scratch the glass. When magnified by the glass lens's curve, even a small scratch can ruin your view.
2. Turn on the light – flip switch, but may also need to increase light level by turning knob.
3. Use the coarse adjustment knob to lower the stage all the way.
4. Rotate the nosepiece until the scanner lens (4x) is in position. You should hear a "click" sound when it is in proper position.
5. Looking through the oculars, with both eyes open and relaxed, make sure that you see a circle of light. This circle is called the **field of view (abbreviated "FOV")**.
6. If you do not see a full circle, you may need to adjust the "interpupillary distance": move the eyepiece laterally to bring the ocular lenses closer to or farther from your nose as needed until you see a **single, round circle of light**.
7. Look for the **pointer** – it is only inside one of the ocular lenses, so if you don't see it, try closing one eye at a time and looking through the other ocular. If you have a "strong" eye, you might not be using the weak one when both eyes are open. *When using a microscope, adjusting the stage to move the cell or structure of interest to the tip of the pointer is necessary so your instructor (and your classmates) can see what you have been observing and ensure it is the correct object.*

Activity 2-3: Focusing a prepared microscope slide

1. Check that the stage is a low as possible and the scanning objective (4x) is in place.
2. Obtain a slide of three crossed threads available in the laboratory. Check that the slide looks clean. If not, wipe it gently with lens paper moistened with the cleaning solution.
3. Open up the clip that holds the slide on stage. Position the slide securely on the holder. Let go of the clip to secure the slide in the proper position.
4. Looking at the stage (NOT through the oculars), move the stage north/south/east/west as needed, to bring the central part of the slide (containing the threads) to the center of the stage over where the light comes through.
5. Now look through the oculars and start moving the stage up slowly with the coarse adjustment knob. Stop when you see the threads. Try to get the best focusing you can with the coarse adjustment knob (get the crispest image).

   Note: If the background appears "grainy and/or grayish", that means that the condenser is not positioned properly. Move the condenser adjustment knob until you obtain a background that is white and clear (usually the highest condenser position on these microscopes).
6. While looking through the oculars, move the stage so the point where the three threads cross each other is in the **center** of the field of view.
7. Now, keep the stage at the SAME HEIGHT and rotate the revolving nosepiece to bring the low power lens (10x) in position. Make sure it clicks into place. It will be closer to the slide than the 4x lens, but not too close (if you focused correctly with the 4x objective).

   DO NOT TOUCH THE COARSE ADJUSTMENT KNOB FROM NOW ON!! *Your microscope is* ***parfocal****, meaning the image will remain nearly focused as the objective lenses are changed.*

8. While looking through the oculars, fine focus the specimen by slowly turning ONLY the fine-focusing knob. If will probably start out fuzzy, so fine focus adjustments are needed to focus sharply. *If you can't find the threads after a minute or two, go back to the 4x objective and confirm that you centered your threads before changing objectives.*
9. While looking through the oculars, move the stage until the point where the three threads cross is again in the center of the field (only a slight adjustment may be needed).
10. Repeat steps 7-9, to bring the high power objective (40x) in position. **Do not move the stage up or down!** If you did this correctly, the 40x objective will be about two millimeters above the cover slip on the slide when you click it into place.
11. After fine-focusing at 40x, you may have noticed that the intensity of light decreased. To let more light go through the specimen, **open the iris diaphragm**. Adjust the iris diaphragm and light level (next to the power switch) until you get good contrast.
12. Choose one thread color, and adjust the fine focus until that color thread is as sharp and clear as possible. **Keep your microscope slide focused at 40x on the stage.** You will need it for the checkpoint AND for the next activity.

CHECK POINT: BEFORE PROCEEDING TO THE NEXT ACTIVITY, raise your hand and show the instructor your thread slide in focus at 40x.

_____Instructor's initials

You have learned how to focus a prepared microscope slide.

<u>Remember, always begin with these 8 steps for EVERY new slide you use:</u>

1. Clean lenses with lens paper only → **2.** lower stage → **3.** position scanner (4x) lens → **4.** insert slide → **5.** center slide over light on stage → **6.** look through oculars and turn coarse adjustment knob until you locate specimen → **7.** center specimen before changing to higher power objectives. → **8.** If you can't find the specimen after changing objectives, go back to the lower objective to re-focus and re-center. Do not use the course adjustment know for any higher powers.

Always begin with the scanning (4x) objective and work your way up to higher magnification only AFTER you have found, focused, and centered the specimen at this magnification first.

Activity 2-4: Magnification

While using the microscope, you are always using two types of lenses, an ocular and an objective lens, each one providing a particular magnification. Although we primarily speak of the magnification in terms of the objective lens because that is the one that we can change, the <u>total magnification</u> is the product both magnifications (the ocular x the objective). To

calculate total magnification, multiply the magnification provided by each of the lenses being used in sequence.

1. Determine and record in Table 2-1 the total magnification of each objective lens, using this formula:

   Total magnification = (Magnification $_{objective}$) x (Magnification $_{ocular}$)

   For example, if using a microscope with ocular lenses labeled "10x" and a 10x objective clicked into place, the total magnification obtained is 10x times 10x = 100x, which means the object will appear 100 times larger through the microscope than it really is.

Table 2-1: Magnification Powers of your Microscope

Objective Lens	*Magnification Objective*	*X*	*Magnification Ocular*	*=*	*Total Magnification*
Scanner	_____	X	_____	=	_____
Low Power	_____	X	_____	=	_____
High Power	_____	X	_____	=	_____
Oil immersion	_____	X	_____	=	_____

Activity 2-5: Depth of Field

The depth of field refers to the number of focal planes that can be obtained with a particular magnification. In other words, it is the thickness of the object that can be in focus at a given time. The depth of field varies with the magnification power.

1. Look at the microscope slide and rotate the scanner (4x) lens back into place (remember not to move the stage) and observe the three threads.

   Can you have all three threads focused at the same time? _____

2. Now rotate the nosepiece to bring the low power (10x) lens in position and observe.

   Can you have all three threads focused at the same time at this magnification? _____

The 4x lens allowed you to focus all threads at the same time. We say that the scanner lens has the highest **depth of field**. This is important because cells are 3-dimensional. If you use a higher magnification to obtain more detail, you lose depth of field and might have to focus on only one position in the cell at a time, such as either the top or bottom part of your specimen. *You are finished with the threads slide. Please return it to the tray.*

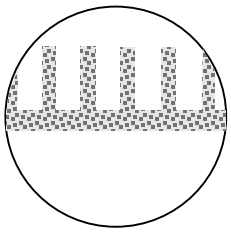
NOTICE: As the magnification power increases, the depth of field decreases.

Activity 2-6: Field of View (FOV) and Estimating the Size of a Magnified Object

The field of view (FOV) is the circle that you see through the oculars and the objective. Knowing the diameter of the field of view is important because it allows you to estimate the size (length) of the specimen you are observing.

We will measure the diameter of the FOV using a transparent ruler ONLY while using the scanner lens. The other field of views will be determined mathematically (no ruler needed).

1. Obtain a clear plastic ruler with a metric scale.
2. Put the scanner lens in position and lower the stage as low as it goes.
3. Place the ruler on the stage, with the metric scale positioned along the central opening on the stage. Make sure the smallest markings are over the circle where light goes through the stage.
4. Locate the ruler while looking through the oculars by moving the stage upward.
5. Once in focus, slide and rotate the ruler as needed until the horizontal line that forms the metric scale goes through the center of the field of view.
6. Then, slide the line left or right until one of the vertical millimeter markings is positioned along on the LEFT edge of the FOV circle. The lines are thick, so center it vertically at the edge as shown below.



Note: *This figure shows you HOW to align the ruler, but is NOT accurate at showing how many millimeters go across the diameter. This ruler shows an FOV diameter of ~5.3 mm, but yours is less than 5 millimeters.*

7. Measure the diameter of the circle in millimeters. **Use one decimal place to estimate**. Do NOT round to the nearest whole number – that is too inaccurate.
8. **Record the FOV diameter of the scanner (4x) lens on the next page (see Step 11).**
9. We will NOT be able to measure accurately the FOV with the other objective lenses using the ruler, so **we will NOT use the ruler anymore**. Remove the ruler from the stage.
10. **Do NOT use the microscope for any of the remaining steps in this activity.**

To mathematically calculate the size of the FOV diameter for the other objective lenses, we will use this relationship obtained from the field of optics:

$$\textbf{(FOV}_{\textbf{scanner}}\textbf{) (Magnification}_{\textbf{scanner}}\textbf{) = (FOV}_{\textbf{other objective}}\textbf{) (Magnification}_{\textbf{other objective}}\textbf{)}$$

where: **FOV** $_{\textbf{scanner}}$ = diameter of the field of view (FOV) using the scanner lens, the one you measured
Magnification $_{\textbf{scanner}}$ = magnification of the scanner lens
FOV $_{\textbf{other objective}}$ = diameter of the FOV that you are trying to calculate (NOT measured directly)
Magnification $_{\textbf{other objective}}$ = magnification of the objective lens for which you are trying to calculate the diameter of the FOV

We can rearrange the equation to allow us to find each FOV diameter we want to know by dividing the left side by the magnification of the objective we are trying to evaluate:

$$\textbf{The diameter of the FOV of any objective} = \frac{\textbf{(FOV}_{\textbf{scanner}}\textbf{) (Magnification}_{\textbf{scanner}}\textbf{)}}{\textbf{(Magnification}_{\textbf{power of that objective}}\textbf{)}}$$

11. The FOV diameter under the scanner lens was measured to be:

$FOV_{scanner}$ = _____ millimeters.

12. Use the measured $FOV_{scanner}$ diameter to CALCUALTE the size of the FOV diameter under the higher power objectives.

a. To find the FOV diameter of the **Low Power (10x) Objective** (no ruler involved!)

$$\textbf{(FOV}_{\textbf{low power}}\textbf{)} = \frac{\text{(FOV scanner) (4x)}}{\text{(10x)}} = \frac{\text{(\_\_\_\_\_) (4x)}}{\text{(10x)}} = \text{\_\_\_\_\_ millimeters}$$

b. To find the FOV diameter of the **High Power (40x) Objective** (no ruler involved!)

$$\textbf{(FOV}_{\textbf{high power}}\textbf{)} = \frac{\text{(FOV scanner) (4x)}}{\text{(40x)}} = \frac{\text{(\_\_\_\_\_) (4x)}}{\text{(40x)}} = \text{\_\_\_\_\_ millimeters}$$

c. To find the FOV diameter of the **Oil Immersion (100x) Objective** (no ruler involved!)

$$\textbf{(FOV}_{\textbf{oil immersion}}\textbf{)} = \frac{\text{(FOV scanner) (4x)}}{\text{(100x)}} = \frac{\text{(\_\_\_\_\_) (4x)}}{\text{(100x)}} = \text{\_\_\_\_\_ millimeters}$$

13. You now know the size of the full diameter of the FOV for every objective you will use in this class. Record all 4 FOV diameters from steps 11 and 12 in Table 2-2 below.

14. Most cells are measured in micrometers (a smaller unit of measure that is more appropriate for microscopic objects like cells and organelles). So, **convert all the FOV diameter values in your table into micrometers (μm) by multiplying the millimeter size by 1000**. *For example, if an ant is 3.1 millimeters long, I can convert 3.1 mm into micrometers and describe that same ant accurately as 3100 μm long.*

Table 2-2: Sizes of the Field of View Under Each Microscope Objective

Objective Lens	FOV diameter (mm)	FOV diameter (μm)
Scanner (4x)	_____ (only one measured)	_____
Low Power (10x)	_____ (calculated above)	_____
High Power (40x)	_____ (calculated above)	_____
Oil immersion (100x)	_____ (calculated above)	_____

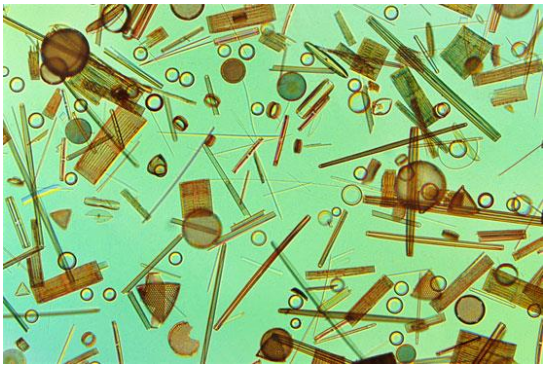
NOTICE: As the magnification power increases, the field of view decreases.

CHECK POINT: BEFORE PROCEEDING TO THE NEXT ACTIVITY, raise your hand and show the instructor your FOV diameter calculations and micrometer conversions.

_____Instructor's initials

Activity 2-7: Estimating the size of a cell

Now that we have the diameters of the FOV with all the objective lenses, we are going to use this information to estimate the size of various cells.



1. Obtain a slide labeled "Diatoms". Diatoms are marine and freshwater algae with unique glass-like walls made of silica embedded in an organic matrix.
2. Focus your slide as you learned before (beginning with the scanning objective) and work your way up to be focused on a field of diatoms under the 10x objective.
3. You will see diatoms of various shapes, some circular, some triangular. Choose any of the **triangular** diatoms or, if you can't find a triangular one, choose one of the medium **rectangular** diatoms (NOT a very small or very large one, please).
4. Center the diatom you chose and then switch to the 40x (high power) objective and adjust focus as needed to sharpen your diatom cell.
5. Now, adjust the stage position until the leftmost side of your selected diatom is aligned with the leftmost side of the FOV. If your diatom is too large to see all of it at the same time, lower the magnification by changing objectives. Look at Fig. 2-3 for reference:

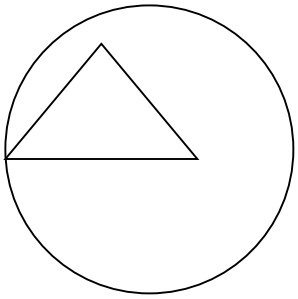


Fig. 2-3 An idealized view of a large, triangular diatom aligned for estimating its size.

6. Now that you can see how your diatom cell lines up with the full field of view, estimate what percent (or fraction) of the FOV diameter is occupied by YOUR diatom along its longest side. *NOTE: We are NOT estimating the cell's volume, we are estimating its <u>length</u> on the <u>longest</u> side. (It is similar to determining a person's height. We don't want to know their width or how much space/volume they take up, so we look only at their longest side and compare that to a known standard – the FOV diameter.)*

Example: If I saw the diatom in Fig. 2-3 under my microscope, I could estimate the percent or fraction of that diatom by **imagining** a line down the middle of the FOV

(halfway mark) and 2 additional lines at the ¼ and ¾ marks (see Fig. 2-4). ***Do NOT try to place a rule on the top of the slide or draw any lines – use your imagination.***

If you like fractions, you might estimate this diatom occupies 2/3 of the FOV because it is between the ½ and the ¾, but is closer to the ¾. If you like percentages, you might estimate this diatom as 65% of the FOV diameter.

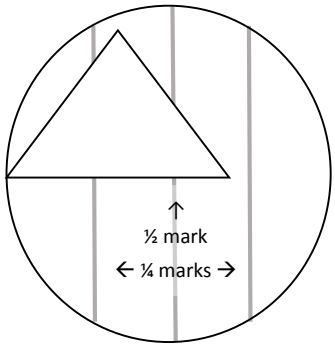


Fig. 2-4 In this example, I've shown the lines I would imagine as I look through the microscope and see the FOV and my cell. Notice that for this to work, my diatom must be lined up so its longest length is across the center of the diameter. If your diatom is taller than it is wide, or it is angled differently, you need to line it up to estimate vertically or at another angle.

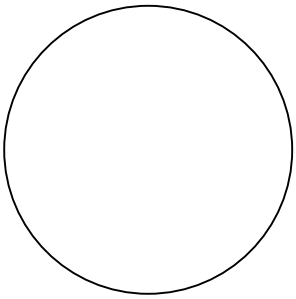
Using either of these numbers, we can estimate the size of our diatom because we know the actual size of the full diameter of this FOV (depending on which objective we used). In sentence format, if the diatom is 2/3 of the FOV, and the full FOV is ____ micrometers, then our diatom is 2/3 of ____ micrometers. The FOV diameter will come from Table Table 2-2, so if I used the 40x objective, I would write in the FOV diameter from the 3rd row of my table.

In equation format:
Note: If using percentages, remember that you need to account for the percent part, so 65% is entered as either 65/100 or 0.65 in your equation, not as "65.")

Cell Size = (FOV $_{\text{high power}}$ in µm) x (fraction of FOV occupied by diatom) = _____ µm

7. Use your fraction or percentage estimate of your diatom to calculate the approximate size of your diatom in micrometers, using the FOV diameter as a reference. Make sure to check which objective you used to get the correct FOV diameter!

   Use the provided FOV circle below to sketch what your diatom looks like after you lined it up (draw anything else you see in the FOV for reference). Show your calculations here and **circle your final answer with units shown**. **Keep your diatom in focus under the microscope –** I will need to compare your drawing and estimate to the real thing!



CHECK POINT: BEFORE PROCEEDING TO THE NEXT ACTIVITY, raise your hand and show the instructor your diatom size estimate. **You must still have your slide in focus at the magnification you used for size calculations** so I can compare the actual diatom to the size estimation.

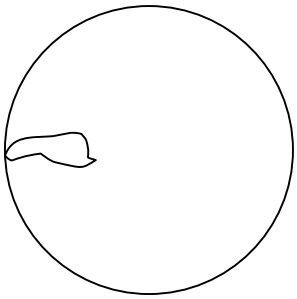
_____Instructor's initials

Activity 2-8: Estimating the size of cells from pictorial representations

Estimate the size of the cells shown. Information about the objective lens being used for each is given. SHOW CALCULATIONS FOR EACH and circle your final answer, including UNITS.

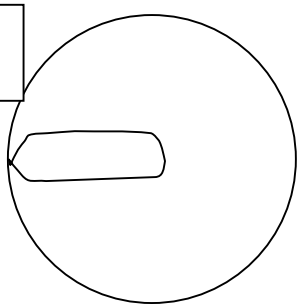
Case 1:

Objective lens used: scanner



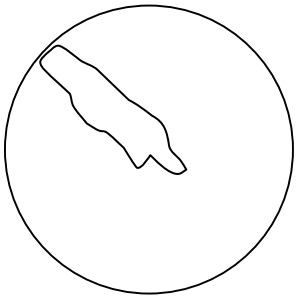
Case 2:

Objective lens used: low power



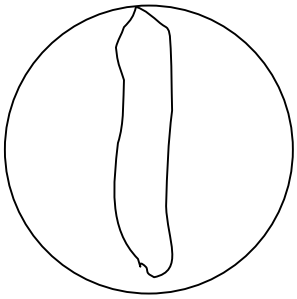
Case 3:

Objective lens used: high power



Case 4:

Objective lens used: oil immersion



CHECK POINT: BEFORE PROCEEDING TO THE NEXT ACTIVITY, have your instructor check your answers.

_____Instructor's initials

Activity 2-9: Using the oil immersion lens (optional, time permitting)

The oil immersion lens is to be used when a lot of magnification is desired as, for example, when observing bacteria or other microorganisms.

1. Obtain a microscope slide label "Bacteria Types". Check that the slide is clean – it is likely to have oil residue on it from previous classes, so use lens paper and cleaner to gently rub until it is clear.
2. Follow the steps learned earlier to focus the slide all the way to the high power lens. You are looking for tiny shapes that are usually stained a bright pink and purple/blue.
3. Once you have something pink and/or purple in focus as well as you can get it with the 40x objective and at least some of the pink/purple is CENTERED in the middle of the 40x FOV, rotate the nosepiece partway towards the oil immersion lens, leaving it halfway between these 2 lenses (the high power and the oil immersion objectives).
4. Obtain the bottle of microscopy oil from your lab tray.
5. Carefully place one or two drops of the oil on top of the cover slip of the microscope slide. – aim for where the light is coming through.
6. **Do NOT move the stage up, down, or sideways!!!** Carefully rotate the nosepiece to bring the **OIL IMMERSION LENS** in position. The oil WILL touch the bottom of the objective lens – this is normal.

Note: *Do NOT turn back towards the 40x lens once the oil is on the slide – the 40x will get into it. If you accidentally allow oil to get on the 40x objective lens, you must clean the lens* ***immediately*** *to avoid damaging this expensive objective! Wet a piece of lens paper with the cleaning solution and wipe off the bottom of the contaminated lens for several seconds.*

7. Once the oil immersion objective is touching the lens, look through the oculars. You may have to adjust the light with the iris diaphragm, but if you centered the bacterial cells and were in focus with the 40x objective, just be patient and you'll find them.
    a. Use the fine focusing knob to bring the specimen into sharper focus. Turn the fine focus slowly in one direction while looking through the oculars. If you do not see anything after 2 full turns, stop turning in that direction and slowly turn the fine focus know the opposite direction (remember it will take 2 full turns to get back to where you started, so be patient and turn slowly).
    b. If neither direction finds the bacteria's bright pink/purple color, call the instructor over for assistance
8. Once you are finished, clean the oil immersion lens and the microscope slide with the cleaning solvent on lens paper. Then, clean all the other objectives with a **fresh** piece of lens paper and cleaning solvent.

Proper storage of the compound microscope in the cabinet

When you are finished using the compound microscope return it to the cabinet with the stage all the way down, the scanner lens in place and with the plastic cover on it. Put the microscope in the cabinet with the arm facing out (to make it easier for the next person to grab it).

Activity 2-10: Using the stereomicroscope (optional, time permitting)

1. Locate a stereomicroscope set up on one of the side lab benches.
2. Turn it on by rotating the power switch knobs at the base of the arm.
3. Adjust the interpupillary distance by moving the ocular lenses sideways as needed.
4. Obtain a watch glass and choose a specimen to observe (these should be labeled appropriately). Good specimens might be insects or flower parts.
1. Adjust the focus and magnification (a switch know on the side) to view the provided specimen.
2. As you observe, notice that you can:
    a. use light that shines from above the specimen (reflected light),
    b. use light from below (transmitted light), or
    c. use both light sources at the same time.
3. Adjust the different types of light and notice the details you see on the surface of the specimen even at these low magnifications.

References for photos and information included in this lab:

1. Perry, J.W., Morton, D., Perry, J.B.. *Laboratory Manual for Starr and Taggart's Biology the Unity and Diversity of Life*, 2002, Brooks/Cole Thomson Learning
2. Vodopich, D., Moore, R., *Biology Laboratory Manual*, 6th edition, 2002, McGraw Hill
3. http://www.slic2.wsu.edu:82/hurlbert/micro101/pages/101lab1.html
4. http://pdnpulse.com/2011/08/frans-lanting-and-art-wolfe-using-photography%E2%80%99s-power-for-planet-earth.html

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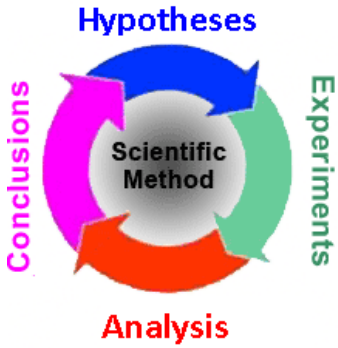
(to ensure each lab starts on a front side in 2-sided printing)

Lab 3: The Scientific Method

Evaluating the Effect of Drugs on an Aquatic Organism



Objectives

- Understand the steps involved in the scientific method
- Define and identify: independent, dependent and control variables
- Calculate the heart rate of *Daphnia* in various experimental conditions
- Analyze the data obtained
- Make conclusions regarding the various variables tested and *Daphnia's* heart rate

Pre-Lab Reminder: *Before arriving to this scheduled lab, read the entire lab (background, procedure, and questions), and use that information to complete the pre-lab in eCampus.*

Background Information

All fields of science have one unifying principle that is a common tie among these diverse scientific disciplines. That unifying theme is the ***scientific method***.

The scientific method is simply an organized, methodical, and structured way of observing and/or investigating a situation in an effort to find information about what is being observed. There are six steps to the scientific method.

1. **Identification of the situation to be investigated.**
   This is vital because no progress can be made towards understanding the situation unless one knows exactly what is being investigated. Let's consider an example. Suppose that you notice (observe) a list of essential nutrients on the label of a box of plant fertilizer. You wonder how plant growth might be affected if plants are deprived of just one of those essential nutrients. You decide to investigate the effect of the lack of potassium on pepper plants.

2. **Obtain information about the situation being investigated.**
   One of the biggest advantages in problem solving is knowing the background information about what is being investigated. This is why researchers do searches of the scientific literature when writing a paper or conducting research. Accordingly, you would go to the library and read as much as you can about plant nutrition and how potassium affects plant growth.

3. **Formulation of a hypothesis.**
   A hypothesis is a possible explanation of the problem or situation based only on what it is known about it so far. The hypothesis must be **testable**: an experiment must be designed to test its validity. Another important characteristic of a hypothesis is that it must be **falsifiable.** This means that the hypothesis must lead to specific predictions that could be proven false if the experimental results are different from the prediction. Finally, a hypothesis must actually be an explanation, not just a prediction. Based on your observations and research, your first hypothesis for the pepper plant situation might be, "Plants use potassium ions to regulate transport of water to the leaves."

4. **Predict the results.**
   If your hypothesis is correct, you ought to be able to predict the outcome of a situation where your hypothesis was actually applied to the problem. For example, if you grow a pepper plant in a potassium-free medium, you would predict (based on your hypothesis), that the leaves would show signs of wilting due to the lack of water transport. Further, you could also predict that a

lack of water transport to the leaves would slow growth, resulting in obvious changes in the development of leaves and/or the stem height.

5. Design and conduct an experiment to test the hypothesis.

An experiment is an investigation conducted under very specific conditions in which all variables are controlled except the one being studied. A variable is any event, condition, or factor subject to change (something that is not always constant). In the potassium study, the lack of potassium is the variable being investigated, but it is not the only variable to consider.

The design of experiments to test hypotheses requires considerable thought. The variables must be identified, appropriate measures developed, and influences outside of the experimental variables must be controlled.

- The **independent variable** is what will be varied intentionally in the experiment. It is the predicted cause of a change in another variable. It is *independent* because you do not expect it to be affected by the experiment; you expect it to have an EFFECT on something else.
- The **dependent variable** is what will be observed or measured in the experiment. It the factor predicted to show an effect because it should change as a result of differences in the independent variable; it is *dependent* on the independent variable.
- Many **controlled variables** must be identified and kept constant throughout the experiment. These are factors that could accidentally vary between tests, so they must be identified in advance so they can be kept constant. If not kept constant, they might influence the dependent variable's response/change, confusing the interpretation of the results.

For example, suppose you were growing plants with the intention of studying how the amount of potassium affects their ability to transport water. In that case, the independent variable would be the amount of potassium provided (none vs. plenty). The dependent variable could be the level of bend to the leaves as a measure of "wilting" (you'd measure this), and some of the controlled variables would include the amount and quality of water provided (because less water in one plant would definitely affect leaf wilting!), the amount and quality of light, the temperature, other minerals provided, and so on.

If, at the end of the experiment, the results do NOT match the prediction, the hypothesis should be found to be wrong. Then it can be modified, further tested or completely discarded. The scientific method commonly results in a long series of repeated testing and hypothesis modification**. A hypothesis can never be proven right unequivocally**. With more and more experimental evidence to support it, a hypothesis gradually evolves into becoming more and more valid for the situation or problem. The evolution of a hypothesis is based on conducting experiments, making observations, gathering data, etc., all of which are done to investigate the validity and to challenge the hypothesis under consideration.

Going back to the initial experiment about the role of potassium in pepper plants, you could conduct your experiment based on a technique discovered in the literature search. You could grow your pepper plants hydroponically (in water with plant nutrients and no soil). In your experiment, you would have two groups of plants, each group consisting of six pepper plants of the same variety and all are the same age, size, and general state of health. In addition, both groups of plants would be grown under exactly the same environmental conditions of heat, light, container size, etc. The only difference between the two groups is that one will be grown with complete nutrients, the other with all nutrients except potassium.

When your experiment is run, the plants should be allowed to grow for a week (to allow time for any previously stored potassium to be used up). At the end of the week, the plants would be compared.

In this design, the plants growing in the complete nutrient solution serve as the **control group**, which is the group forming the basis for judging any differences that may appear in the **experimental group**, the group grown without potassium. A control group is essential in an experiment because it provides a comparison to reveal any differences in the experimental situations.

6. Form a conclusion based on the results.

The validity of a hypothesis may or may not be determined by your experiment. Either the results of the experiment support the hypothesis, the results are confusing or inconclusive, or the results show that the hypothesis is wrong or needs modification. If you found the control plants to have lush, green, springy leaves, while the experimental plants had sagging, yellowish leaves with brown spots, you would have supported your hypothesis. If you instead found both plants to have springy, non-wilted leaves, and the only difference was the color (the experimental group had yellowish leaves with brown spots, but they were not wilted), you would probably need to modify your hypothesis or do further studies to determine if it should be rejected.

The experiment will NEVER PROVE your hypothesis to be correct beyond all shadow of doubt. What the experiment does show is that under the conditions of the experiment you performed, the hypothesis is **supported**. Each time a new and different experiment supports the same hypothesis, you become more confident it will continue to be supported by new data in the future.

The scientific method is neither complicated nor intimidating – nor is it unique to science. It is a powerful tool of logic that can be employed any time a problem or question about the fundamental nature of something. In fact, we all use elements of the scientific method to solve little problems every day, but we do it so quickly and automatically that we are not conscious of the methodology. In brief, the scientific method consists of observing, predicting, testing, and interpreting.

Investigating the Effect of Drugs on *Daphnia magna*

You will base today's experiment on observations of twentieth-century American lifestyles. You have probably observed that when people drink alcohol, their behavior changes. Some people become more active, while others fall asleep. These behaviors are likely due to the interactions between this legal drug and your normal body functions. In this lab, you will be investigating the effect of alcohol on ***Daphnia magna***, a small water crustacean. You will evaluate the effects of alcohol by measuring the heart rate of *Daphnia* when exposed to several concentrations of alcohol.

*NOTE: All organisms are classified by Latin names that specifically identify them. You must always identify an organism by its proper scientific name so that other scientists know what you are talking about. You must also remember to **ALWAYS** italicize or underline the Latin names (genus and species) of organisms EVERY TIME YOU USE THEM!*

The advantage of studying *Daphnia* is that they are almost transparent. You can see the heart beating, the squeezing action of the intestine, muscular movements, and occasionally, babies in the brood pouch. Also, because *Daphnia* is a small, aquatic organism, it makes an excellent subject for studying the effects of drugs on circulation.

Even if you perform your experiments carefully, you cannot be certain that any effect you see is due to the drugs. Perhaps the change in heart rate was caused by heat from the microscope light, or the *Daphnia* was affected by the removal or addition of solutions. To reduce influence of these other variables, we will use a control experiment. A "control experiment" is similar to a control group, except using the same *Daphnia* because heart rates vary significantly between individuals. The control experiment will be to obtain the average heart rate of the *Daphnia* in 0% drug (distilled

water) over 3 trials. The same *Daphnia* will then be exposed to different drug concentrations using the exact same procedure for the "experimental procedure."

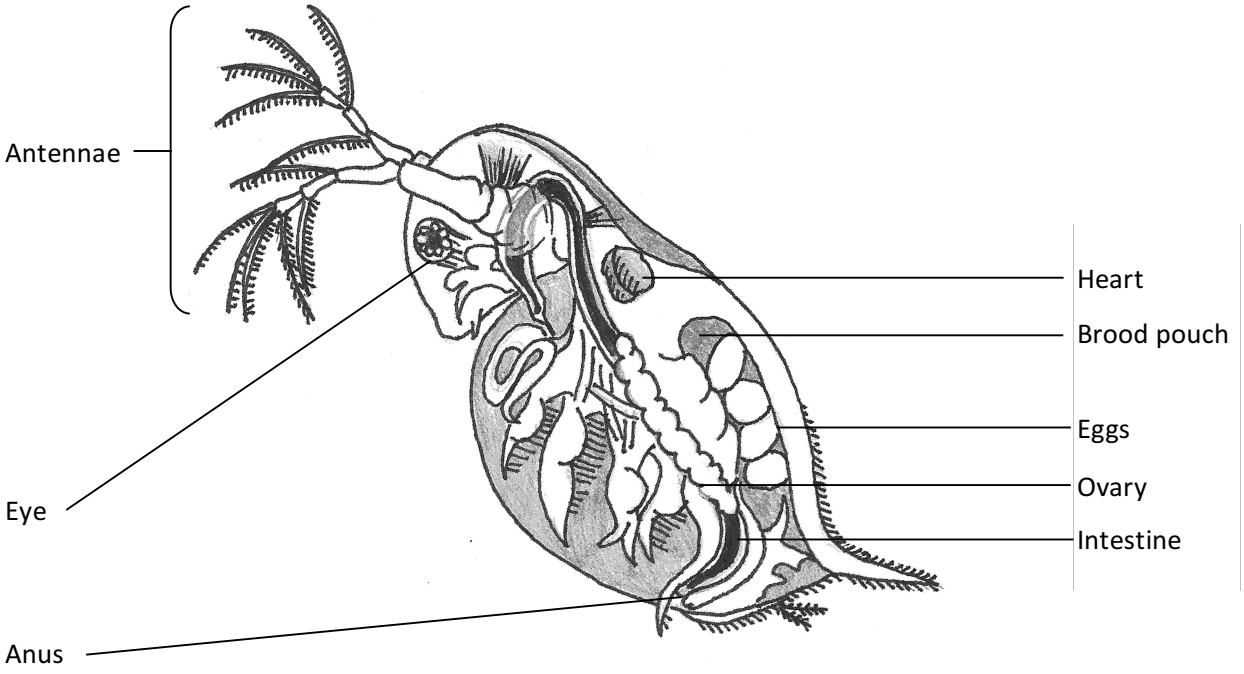


Figure 3-1. Use this image as a reference for locating the indicated structures and organs in *Daphnia*.

Activity 3-1: Experimental Set-Up

Before you begin this procedure, **read through the entire lab one more time** to make sure you know exactly what you'll be doing. Once the *Daphnia* is under the microscope light, it begins to be affected by the heat and its stressful surrounding environment. To control for these factors as much as possible, work safely, but quickly from this procedure forward, recording all observations and errors or deviations from the printed procedure.

1. In a group or 3 or 4 students, discuss your expectations – share some of your own observations about the effect of alcohol in humans. As a group, devise a hypothesis about WHY or HOW chemicals like this affect the cardiovascular system of humans, and then predict whether you think they'll affect an aquatic organism like *Daphnia* the same way.

   Write your group's **hypothesis** (remember, it must be a statement that suggests a HOW or WHY):

   Write your **predictions** here (logical and based on the HOW or WHY in your hypothesis):

2. Now, obtain a depression slide and place it on your microscope stage. Lower the stage as far down as it goes and click the scanning (4x) objective in place.

3. Add 2 drops of water to the center of the slide and place 3 threads into the water to create a triangular corral (see Fig. 3-2). Make sure the threads do not extend off of the slide (you may need to cut them).

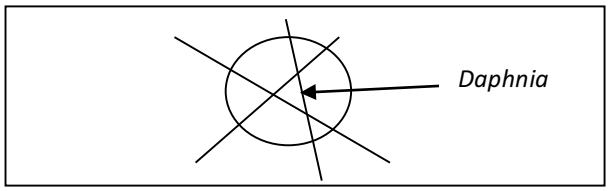


Fig. 3-2 Set up the depression slide with threads as indicated in the figure. The *Daphnia* is placed in the center where indicated. This arrangement will confine the *Daphnia* to a small space and make it easier for you to identify and count the heart beats.

4. Turn the microscope on and look at the light coming through the center of the stage. Adjust the stage to center the threads over the light.
5. Looking through the oculars, slowly raise the stage with the coarse focus knob until you see the threads. Focus them as sharply as possible and then adjust the light level and the iris diaphragm to get the light as low as possible while still allowing you to see details in the threads.
6. Call the instructor over to check your focus and deliver a living *Daphnia* to the center of the corral in the water on your depression slide.
7. Adjust the fine focus while looking through the oculars and observe the *Daphnia*. Make sure all students in your group take a turn observing the *Daphnia* and trying to see the heart beating, but don't look for too long (to avoid overheating the *Daphnia* with the light). **Keep track of the time spent on this:** for every 1 minute of looking, turn the light off and allow the *Daphnia* to rest without the heat of the lamp for 1 minute. Make sure the *Daphnia* is always in plenty of water.
8. Using Fig. 3-1 (previous page) as a reference, locate the following structures:
   - ✓ The most obvious structure is an eye, with a light-colored brain just above the eye.
   - ✓ The pairs of antennae protrude from the head. These are used for locomotion and to sense the environment.
   - ✓ Inside the exoskeleton are five pairs of legs. Comb-like gills are attached to some of the legs. When the legs kick forward, they bring a stream of water across the gills and wash bits of food up to the mouth, which lies just beneath the beak. *The rhythmic kicking of the legs can sometimes look like a heart beating, so be careful to avoid counting this!*
   - ✓ The heart lies in the upper part of the *Daphnia* (above the digestive tract), it is a clear (see-through and colorless) structure and should be contracting rapidly, at a regular rate.
   - ✓ In females, a large brood chamber is located behind the heart. Usually it will contain eggs, but occasionally it may be filled with baby *Daphnia*. The brood pouch will probably not be present in most *Daphnia*.
9. After everyone in your group has had a chance to observe the *Daphnia* and find the heart, turn off the light, check that your *Daphnia* has enough water, and then leave it to rest while you get ready for the experiment. The lamp is HOT, so do not leave the light on when you aren't looking!
10. In your group, assign the following 3 jobs:
    - **A.** For the student who felt most comfortable finding and counting the heart beating, assign them data collector role. For the ENTIRE lab, this same person will count the heart beats.
    - **B.** The second job is the health monitor who will be in charge of keeping the *Daphnia* alive and changing out solutions. This person must watch the water level at all times to avoid letting the *Daphnia* dry out and die, and to change the solutions correctly and quickly.
    - **C.** The third job is the timekeeper and data recorder. This person needs to keep track of the procedure steps to make sure you don't miss anything and keep track of all the times using a digital clock (such as on a smartphone). If you have a 4th person, this job can be split.

Activity 3-2: Control Procedure

This is the exact same procedure you will do with each of the different concentrations of drugs. Make sure that you do not alter the procedure – It is repetitive (changing water out with more water repeatedly) so that we can keep this variable (how often we change solutions) constant between the 0% drug control procedure and the experimental drug procedures.

1. Begin the procedure with the *Daphnia* in distilled water with the light OFF.
2. When ready, the health monitor will do the first solution replacement:
    a. Absorb away the water from the slide using a KimWipe. To do this, gently place the KimWipe at one edge of the water and most of the water will be absorbed.
    b. Immediately add a new drop of the test solution (which is water, or 0% drug, in this control procedure) to the depression slide, making sure to NOT drop the water directly on the *Daphnia's* body, but close enough that it spreads quickly over the organism.
3. Now, the timekeeper will monitor a **1.5 minute (90 seconds) wait period with the light OFF** to let the *Daphnia* acclimate to the new solution (this is water in the control, but later it will be a drug).
4. At the end of 90 seconds, turn the light ON (at the same level previously identified) and the data recorder should find the heart as quickly as possible.
5. When ready, the timekeeper tells the data collector to start counting heart beats and tells them to stop after 15 seconds. *This is the number of heartbeats in 15 seconds, which is NOT the heart rate yet because we need to convert it to BPM (beats per minute).*
6. Immediately after you finish counting, turn the light OFF.
7. Immediately after you turn the light OFF, the health monitor needs to RINSE the test solution away.
    a. Absorb away the test solution from the slide using a KimWipe.
    b. Immediately add a new drop of WATER to RINSE (always water, even when testing the drug solutions) to the depression slide, making sure to NOT drop the water directly on the *Daphnia's* body, but close enough that it spreads quickly over the organism.
    c. Repeat the WATER RINSE a 2nd time to remove any remnants of the drug by absorbing the water away again and adding another new drop of water.
8. Leave the light OFF and allow the *Daphnia* to recover **in the water** for at least 2 full minutes.
9. Calculate the heart rate (in BPM) using the following conversion:

    # of beats in 15 seconds X 4 = _____ BPM

    The heart rate in a healthy *Daphnia* will be very rapid, but varies between organisms. A typical rate is 3-5 beats per second, which is a count of ~45-75 beats in the 15 second time you're counting. **It should calculate out to a heart rate of 180-300 BPM**. If your heart rate is outside this range, call me over to discuss it while your *Daphnia* is resting.
10. Repeat **steps 1 – 9 two more times** so you have a total of 3 heart rate measurements in the water (0% drug) control solution. Record the data in Table 3-1 on the next page. Record the <u>average</u> heart rate in 0% drug in Table 3-2.

CHECK POINT: BEFORE PROCEEDING TO THE NEXT STEP, raise your hand and show the instructor your *Daphnia's* 3 water heart rate measurements in Table 3-1.

_____ Instructor's initials

Activity 3-3: Experimental Procedure to Test the Effect of Alcohol

11. Follow steps 1-9 in the control procedure above to test the effect of 1% alcohol with the following change: use 1% alcohol instead of water as the test solution. Before pipetting the drug solution, swirl the bottle to ensure the drug is evenly mixed. Record your data in Table 3-1.
12. Repeat steps 1-9 with 1% alcohol a second time. Make sure your *Daphnia* rested for at least 2 minutes in water (after the 2 rinses) before you start this second 1% alcohol test. Record your data in Table 3-1 and calculate and record the <u>average</u> heart rate in 1% alcohol in Table 3-2.
13. <u>After</u> the 2 minute recovery in water after the second 1% alcohol test:
    a. If the heart rate in 1% alcohol was ≤ 8 BPM away from (above or below) your average 0% drug (water only) heart rate (≤ 2 counts in 15 seconds different from average count), you may move on to the next drug solution as directed in step 14.
    b. If the heart rate in 1% alcohol was > 8 BPM different from your average 0% drug heart rate, repeat steps 1-9 using WATER as the test solution to determine if the heart rate has returned to normal before moving on to step 14. Record the heart rate check (in water) where indicated in Table 3-1 **and call me over to discuss the result**. *Make sure your* Daphnia *is back in fresh water and resting in the dark before calling me over!*
14. Next, follow steps 1-9 again to test the effect of 3% alcohol, adding the 3% alcohol as the test solution instead of water, again mixing the solution before pipetting it.
15. As with the 3% alcohol solution, repeat the test a second time, calculate the average, and then check that the *Daphnia* recovered and remained healthy after the 3% alcohol test by **following step 13 above** after the second test's 2 minute recovery in water.
16. Repeat **all of this one more time to test the effect of 5% alcohol**, including testing 5% alcohol two times (with appropriate waits, rinses and repeats)**.**
17. Now, no matter what the heart rate was in 5% alcohol, rinse the *Daphnia* one extra time and test the heart rate in water one more time. Record that heart rate in Table 3-1 and have your instructor check your results.

Table 3-1. Results from the alcohol test series.

Test Solution		# of heart beats in 15 sec.	Heart Rate in beats per minute (BPM)	
Water / 0% Drug	*(1st test)*			
Water / 0% Drug	*(2nd test)*			
Water / 0% Drug	*(3rd test)*			
Water / 0% Drug Average				← *write this heart rate into the 0% alcohol box below*
				Rate in WATER after the 2 min. rest period (if needed to check):
1% alcohol	*1st test*			
	2nd test			
3% alcohol	*1st test*			
	2nd test			
5% alcohol	*1st test*			
	2nd test			

18. Obtain data from at least 2 other groups/tables and record the information in Table 3-2 below.
19. Finally, discuss and record any **behavioral changes** that your data collector noticed while the *Daphnia* was in the **alcohol** solutions here:

Table 3-2. Overall results from the alcohol test series. *Record all the **average** heart rates in beats per minute (BPM) and make sure to include the table # in the top row. You must collect data from at least 3 Daphnia total (your own, plus 2 more).*

	***Daphnia* A (YOUR DATA)**	***Daphnia* B (Table # ___)**	***Daphnia* C (Table # ___)**
0% alcohol (= water)			
1% alcohol			
3% alcohol			
5% alcohol			

CHECK POINT: BEFORE PROCEEDING TO THE NEXT STEP, raise your hand and show the instructor your *Daphnia's* complete set of average heart rates in Tables 3-1 and 3-2.

_____ Instructor's initials

Optional Activity 3-4: Experimental Procedure to Test the Effect of Caffeine

Like alcohol, caffeine is often frequently consumed by humans, with varying effects on consumers. You may have seen or experienced yourself that when people drink too much coffee, they sometimes become jittery, nervous, and complain about being unable to relax. Because your previous *Daphnia* has already been exposed to several concentrations of alcohol, it is best to use a fresh, untested *Daphnia* for the caffeine experiment. Because all *Daphnia* have a unique basal heart rate (in water / 0% drug), you'll need to measure the average heart rate in water first, before testing any effects of caffeine.

20. Obtain a NEW *Daphnia* and follow **steps 1-9 using WATER** to test the control heart rate in 0% drug of this new *Daphnia* three times. Record your data in Table 3-3 below.
21. Follow steps 1-9 in the control procedure above to test the effect of 1% caffeine with the following change: use 1% caffeine instead of water as the test solution. Before pipetting the drug solution, swirl the bottle to ensure the drug is evenly mixed. Record your data in Table 3-3.
22. Repeat steps 1-9 with 1% caffeine a second time. Make sure your *Daphnia* rested for at least 2 minutes in water (after the 2 rinses) before you start this second 1% caffeine test. Record your data in Table 3-3 and calculate and record the average heart rate in 1% caffeine in Table 3-4.
23. After the 2 minute recovery in water after the second 1% caffeine test:

a. If the heart rate in 1% caffeine was ≤ 8 BPM away from (above or below) your average 0% drug (water only) heart rate (≤ 2 counts in 15 seconds different from average count), you may move on to the next drug solution as directed in step 23.
b. If the heart rate in 1% caffeine was > 8 BPM different from your average 0% drug heart rate, repeat steps 1-9 using WATER as the test solution to determine if the heart rate has returned to normal before moving on to step 23. Record the heart rate check (in water) where indicated in Table 3-3 **and call me over to discuss the result**. *Make sure your* Daphnia *is back in fresh water and resting in the dark before calling me over!*

24. Next, follow steps 1-9 again to test the effect of 2% caffeine, adding the 2% caffeine as the test solution instead of water, again mixing the solution before pipetting it.
25. As with the 2% caffeine solution, repeat the test a second time, calculate the average, and then check that the *Daphnia* recovered and remained healthy after the 2% caffeine test by rinsing the *Daphnia* one extra time and testing the heart rate in water one more time. Record that heart rate in Table 3-3 and have your instructor check your results.
26. Finally, collect data from 2 other groups for Table 3-4 and record any **behavioral changes** your data collector noticed while the *Daphnia* was in the **caffeine** solutions here:

Table 3-3. Results from the caffeine test series.

Test Solution		**# of heart beats in 15 sec.**	**Heart Rate in beats per minute (BPM)**	
Water / 0% Drug	*(1st test)*			
Water / 0% Drug	*(2nd test)*			
Water / 0% Drug	*(3rd test)*			
Water / 0% Drug Average				*← write this heart rate into the 0% caffeine box below*
				Rate in WATER after the 2 min. rest period (if needed to check):
1% caffeine	*1st test*			
	2nd test			
2% caffeine	*1st test*			
	2nd test			

Table 3-4. Overall results from the caffeine test series. *Record all the **average** heart rates in beats per minute (BPM) and make sure to include the table # in the top row. You must collect data from at least 3 Daphnia total (your own, plus 2 more).*

	***Daphnia* D (YOUR DATA)**	***Daphnia* E (Table # ___)**	***Daphnia F* (Table # ___)**
0% Caffeine (= water)			
1% Caffeine			
2% Caffeine			

DATA ANALYSIS and DRAWING CONCLUSIONS

Once you have finished testing the different solutions, analyze your data. Are the effects of the alcohol similar to the effects of caffeine? What do you think your results today indicate about the effects these beverages if you were to consume them?

During these biology labs, you will occasionally have an experiment that "does not work". This does not necessarily mean that you have disproved the hypothesis. It does mean that the experiment must be repeated so that variations in technique or in an individual organism's response are put in perspective. For example, if your group had *Daphnia* with a very high heart rate in water, how do you think your results might have differed if you had done the experiment with a *Daphnia* whose control heart rate in water was much slower? The answer is to repeat the experiment many times, which is referred to as increasing your **sample size**.

To increase our sample size, collect the results of at least 3 other groups in class using **Table 3-2** below. Compare your group's results to these others. How much variation did we encounter in starting heart rates? What about in the response to alcohol and caffeine? Are you ready to draw conclusions? How much more confident would you be if we had tested 10 *Daphnia*? 20?

Analyzing Data

Results from experiments must be presented in a clear, scientific way. If tables and graphs are well constructed, they provide a concise summary and allow the reader to see at a glance the pertinent results of the experiment. First graph your data on paper, then use the lab computers to practice how to plot your results and create XY scatter plots of the data through Microsoft Excel.

Follow these steps to plot your data on graph paper:

1. Identify which variable is the dependent variable and which is the independent variable.

   Independent variable: _____

   Dependent variable: _____

2. Always place the independent variable on the x-axis (the horizontal axis), and the dependent variable on the y-axis (the vertical axis). *This is because you want to see the RESPONSE (the effect) vertically on the graph – did the heart rate go up or down with increasing drug?*

3. LABEL the axes with a few words describing the variable, and ALWAYS INCLUDE THE UNITS of the variable in parentheses after the variable description. For example, if I were graphing students grades on Lab 3, I would label the x-axis with: "Student Name" and the y-axis with: "Lab 3 Score (percentage)."

4. Choose an appropriate **scale** for the dependent and independent variable so that the highest value of each will fit on the graph paper.

5. Plot the data set (the values of y for the particular values of x). Draw separate graphs for the alcohol data and the caffeine data, but in each graph you should have 4 sets of plotted data (a separate data line for each of the 4 *Daphnia* whose results you collected). Use a different symbol or type of line for each different *Daphnia* – we want to see the trend (did it go up or down); the actual value of each number is not as important.

6. Show the trend for each set of data by drawing a trendline (on paper, this is a straight line using a ruler that gets as close to all the dots for that line as possible – in a software program, this is generated automatically when you choose a "linear trendline"). When creating the graph, do NOT choose the option that connects your dots from point to point – we want to see the trendline only.

7. Every graph should have a legend (showing which line is which) and a **TITLE** (must be in the format of **Figure #: _____** where the # is replaced with 1 for the first figure, 2 for the 2nd, etc. and the _____ is a short sentence explaining what the figure is about. You can include a separate title or use the Figure #: _____ as the title by making it a larger font.

Interpretation and Conclusions

One of the most important features of scientific inquiry is the exchange of information. Scientists publish experimental results to make them known to others, and they include their interpretations of what those results mean. Health professionals use the same format for their publications, so anyone interested in a health field will also benefit from learning to write and read information in the traditional scientific format.

The practice version of a scientific journal article is called a "lab report" by colleges and universities. To practice this method of information exchange **you will write short analysis of this experiment and create graphs of the data from the experiments you did today**. Instructions will be explained fully during lab. Later this semester, you'll be asked to write a complete, formal lab report, so use this practice to help prepare you for the full report writing experience.

References for the lab procedure and images included here:

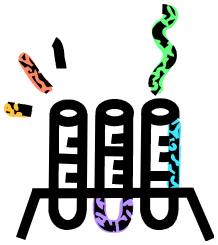
1. Dolphin, W.D., *Biological Investigations: Form, Function, Diversity and Process*, 6th edition, 2002, McGraw Hill
2. Jacklet, A., *Laboratory Manual to Accompany Life*, 1998, McGraw Hill Companies
3. Skavaril, R., Finnen, M., Lawton, S., *General Biology Lab Manual, Investigations into Life's Phenomena*, 1993, Harcourt College Publishers

Lab 4: Chemistry and Life

Investigating Biomolecule Structure

Objectives

- Understand basic concepts of chemistry as they relate to molecules found in living organisms.
- Learn the various functional groups found in biomolecules.
- Learn the basic components of the four main groups of biomolecules: carbohydrates, fats, proteins and nucleic acids.
- Understand how glycosidic linkages, ester linkages and peptide bonds are formed
- Explain the need for positive and negative controls in chemical tests
- Recognize positive and negative results in the Iodine, Biuret's and Sudan's tests.



Pre-Lab Reminder: *Before arriving to this scheduled lab, read the entire lab (background, procedure, and questions), and use that information to complete the pre-lab in eCampus.*

Background Information

From atoms to molecules to cells....

A revolution occurred in the field of biology in the early 1950's. By means of X-ray diffraction data, Watson and Crick elucidated the molecular structure of DNA. At that point, biology became a discipline in which the processes of life were viewed as understandable at the molecular level.

Many molecules are found in living things and many share something in common: they are made of carbon atoms bonded to other atoms such as hydrogen, oxygen, nitrogen, phosphorus and sulfur. How these molecules are put together can be understood if we examine the chemical properties of these atoms. Atoms such as C, H, O, N, P, and S, make bonds among themselves by sharing electrons in their outermost shell: these are called the ***valence*** electrons. When sharing of valence electrons occurs, a ***covalent*** bond is formed. How many bonds are typically made by an atom can be predicted by the number of valence electrons in each atom (and this is related to the position of each atom in the Periodic Chart!!) This is summarized in Table 4-1. Make sure to remember this, because it will help you later in understanding how molecules are formed.

Table 4-1. Generalized information regarding number of bonds made by various atoms.

Atom	Family #	Valence electrons	Number of bonds
C	IV A	4	4
N,P*	V A	5	3
O,S*	VI A	6	2
H	I A	1	1

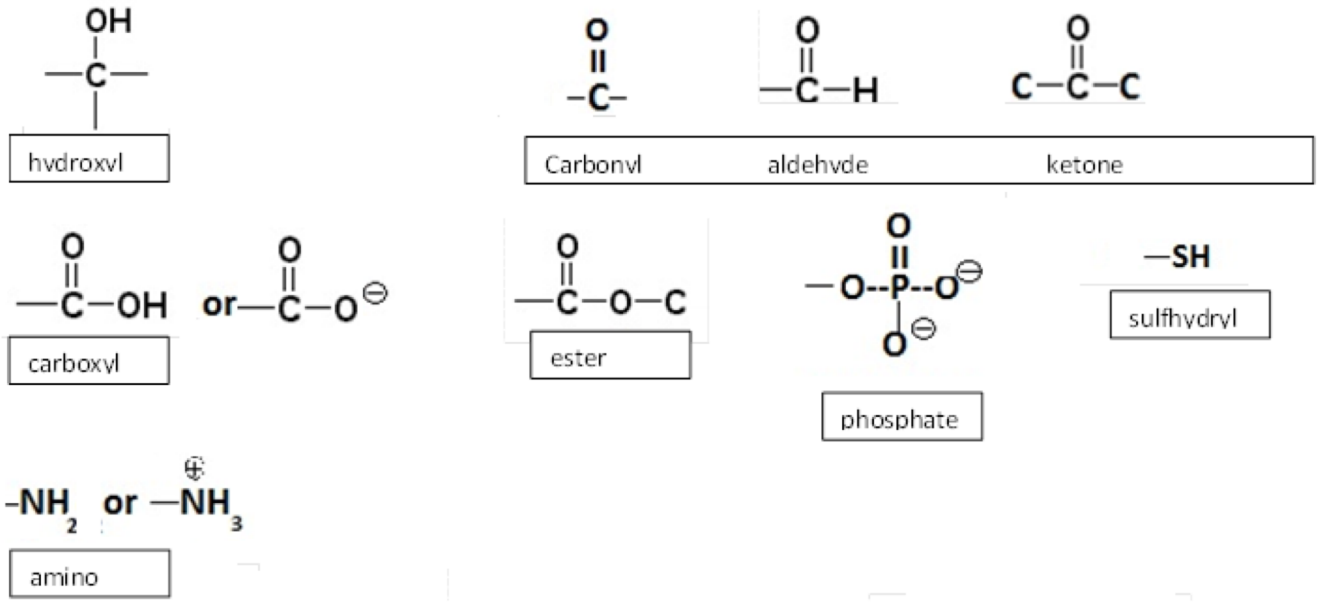
*phosphorus can also make 5 bonds; sulfur can also make 6 bonds.

You will build some molecules with the molecular kits provided. Use the following building blocks when buiding molecules: **Carbon** = black, **Nitrogen** = blue , **Oxygen** = red and **Hydrogen** = white. The connectors are used to make covalent bonds between the atoms.

By building simple molecules such as water and ammonia (shown before), you can see that molecules have definite three-dimensional shapes that are not easily seen from the way they are drawn on paper. Shape is very important for chemical reactions. In addition, it is important to consider what makes molecules react to form larger, more complex ones.

What makes molecules react with other molecules is the presence of specific groups of atoms bonded together. These are the ***functional groups***. Without these, complex molecules could not be formed, and life as we know it would not exist. Functional groups that you must know are summarized in Table 4-2.

Table 4-2: Names and structure of some functional groups



Various biochemical tests have been developed to identify the major types of macromolecules in living organisms. These tests are based on the chemical make-up of each type of macromolecule, including the presence of specific linkages within them and involve the use of positive and negative controls:

- A **positive control** contains the variable that we are testing for. The result of the positive control shows what a positive test looks like.
- A **negative control** does not contain the variable we are testing for. It usually contains distilled water, and does not react in the test. The results of this control show what a negative result looks like.

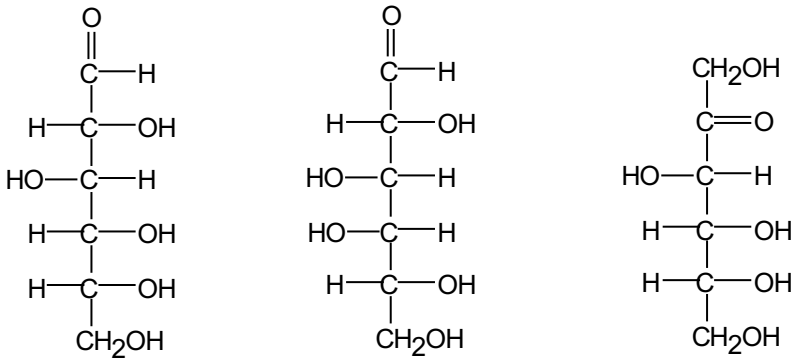
These controls are important because they indicate the specificity of the test. For instance, if starch and protein react similarly in a particular test, the test can not be used to distinguish starch from protein. Or if water and protein react the same way with a chemical, then the test can not be used to test for the presence of protein.

I. CARBOHYDRATES:

Carbohydrates are the sugars and their derivatives.

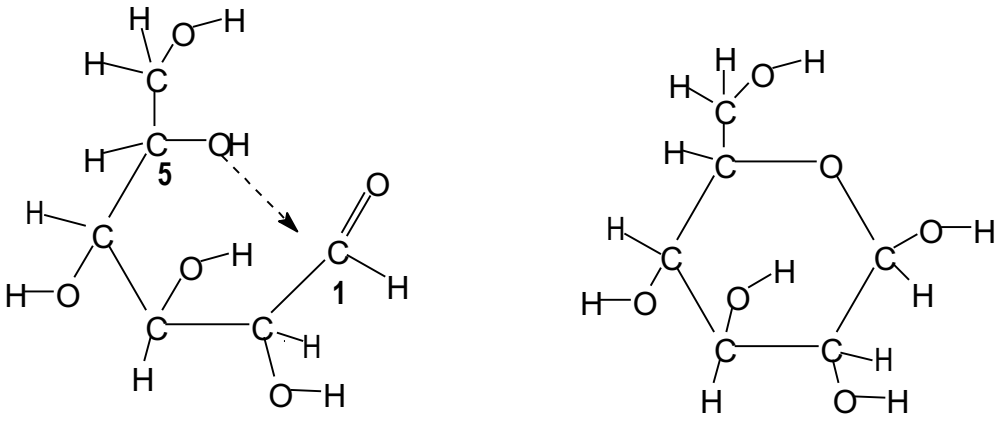
The simplest sugars are called the monosaccharides and they serve as the building blocks for more complex ones such as disaccharides, oligosaccharides and polysaccharides. Of the monosaccharides, there are three that you must remember by name: **glucose, galactose and fructose**. Their structures are shown below.

Activity 4-1: Circle all the Functional Groups in the Monosaccharides Below and Label them Accordingly.



glucose galactose fructose

In water, many 6-carbon sugars form cyclic structures by undergoing an intramolecular reaction between the carbonyl and the hydroxyl group in carbon #5 (when numbering the carbons in the chain give priority to the carbonyl so that it has the lowest possible number). This reaction is shown below for glucose.



Activity 4-2: In your group, make **TWO glucose molecules in the cyclic (ring) form** using the molecular kits provided. Find in these cyclic structures the only carbon that is bonded to two oxygens. This is called the ***anomeric carbon***.

Show your completed glucose molecules to your instructor.

Instructor Initials: _____

The anomeric carbon is important because it is involved in the formation of the more complex carbohydrates by a reaction that involves the union of two monosaccharides. The general name for this reaction is "***dehydration synthesis*** or ***condensation***". [The reverse of this reaction is called ***hydrolysis***. It consists of breaking a bond by adding a molecule of water. This reaction takes place during digestion: when large molecules must be broken down so they can be absorbed.]

Of course, which two monosaccharides are joined and how, will determine which disaccharide is formed. There are three disaccharides that you must know by name and by the units that form them. These are:

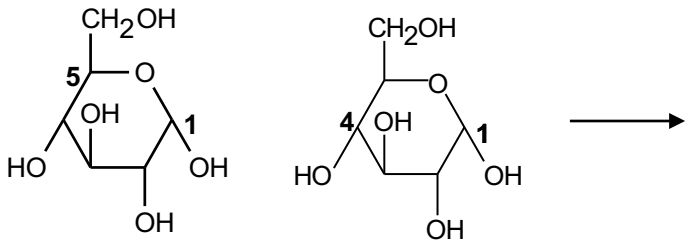
sucrose (table sugar) = glucose + fructose
maltose = glucose + glucose
lactose = galactose + glucose



The particular way in which these are put together has an effect on how they taste to us.

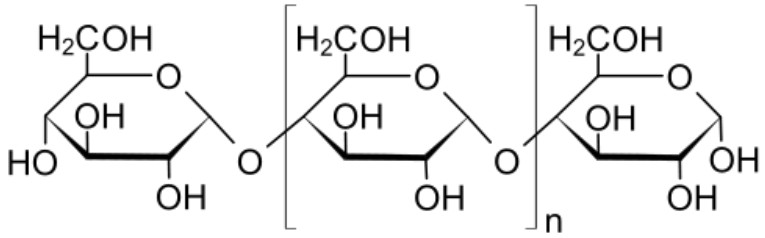
For example, the synthesis of maltose involves a reaction between the anomeric carbon of one glucose unit and the hydroxyl group at carbon #4 of another glucose. The new bond that is formed to form the disaccharide is called a **glycosidic linkage**.

Activity 4-3: Carry out this reaction using the two glucose monomers that you built. Then, draw this disaccharide (maltose) in the space provided below. Label the glycosidic linkage on your drawing of maltose.



Starch

If the whole class connected all the maltose molecules built in the previous activity, we would form the beginning of a molecule that could become the polysaccharide starch (which has thousands of glucose monomers joined together):



Iodine Test for Starch

Starch is a carbohydrate consisting of many glucose molecules tied together in a long chain. Many plant cells store their excess sugar produced by photosynthesis in the form of starch. The presence of starch in a sample can easily be demonstrated by its reaction with a solution of iodine. Iodine interacts with the coiled structure of starch, changing its color from yellowish-brown to blue-black.

Activity 4-4: Performing the Iodine test

Caution: If you have a known allergy to iodine, allow your lab partners to handle this test.

1. Obtain 3 clean, numbered test tubes and a spot plate (for samples 4 and 5).
2. Add 20 drops of each of the substances indicated in Table 4-4 to the appropriate tube. For samples 4 & 5, place a small amount of the dried potatoes and one piece of cereal on the spot plate, NOT in a test tube.
3. Add 5 drops of the *"Lugol's Iodine solution"* (may also be labeled IKI) to each sample, including the 2 on the spot plate. Record color changes in each tube on Table 4-4.

Table 4-4 Iodine Test

Sample	Contents	Initial Color of substance	Final color after adding Iodine	Conclusion (+ = starch present; - = no starch)
1	glucose solution	_____	_____	_____
2.	starch solution	_____	_____	_____
3	deionized water	_____	_____	_____
4	potato pieces	_____	_____	_____
5	breakfast cereal	_____	_____	_____

II. LIPIDS:

Lipids are compounds that share a basic property: they are insoluble in water. Lipids include the fats (source of energy), phospholipids (main components of cell membranes), sphingolipids (present in the myelin sheath of neurons in our body), cholesterol (present in cell membranes; precursor of steroid hormones) and others. In this exercise we will pay more attention to the fats.

Fats (or triglycerides) are large molecules made of 1 molecule of glycerol and 3 fatty acids. Fatty acids are long chains of carbon and hydrogen ending with a carboxyl functional group. They can be *saturated* (with only single bonds in the long chain of carbons) or *unsaturated* (containing at least a double bond in the long chain of carbons).

Examples:

Stearic acid: CH_3-$(CH_2)_{16}$COOH * Type: _____

Linoleic acid: CH_3-$(CH_2)_4$-CH=CH-CH_2-CH=CH-$(CH_2)_7$-COOH Type: _____

** Note that the carboxyl group is often abbreviated as -COOH*

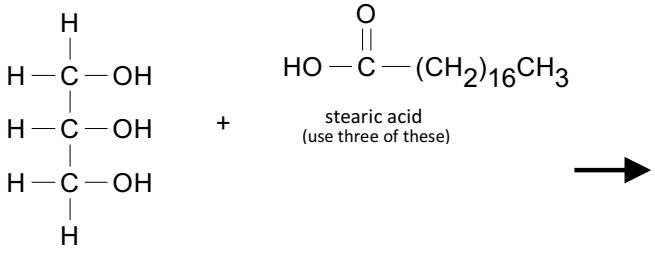
Saturated fatty acids can stack up very easily. Because of this, they tend to be solid at room temperature. These types of fatty acids are commonly found in fats from animal sources (e.g. lard). Many unsaturated fatty acids cannot stack up easily because the double bond introduces a bend in the molecule. Because of this, these fats tend to be liquid at room temperature and are called "oils". Common sources for these are plants (e.g. corn, canola, sunflower oils).

Activity 4-5: Look at the demonstration of lipids in the lab and note the "bend" in the unsaturated fatty acids compared to the "straight" saturated fatty acids.

The synthesis of a molecule of fat involves dehydration synthesis between the hydroxyl groups of glycerol and the carboxyl groups of *three* fatty acids. Of course, which fatty acids you use and where you put them on the glycerol will determine which specific fat you make.

Activity 4-6:

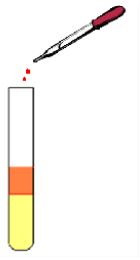
- Look at the drawing of glycerol and a saturated fatty acid containing 18 carbons below.
- Connect them by performing a dehydration synthesis reaction between the carboxyl group of the fatty acid and a hydroxyl group in glycerol. What bond is created?
- Below, draw "tristearin" (the fat made by combining a glycerol with ***three*** stearic acids.)



The Sudan Test for Lipids

When a lipid is mixed with water, two layers are formed. If a non-polar dye, such as the Sudan dye, is added to such a mixture, the lipid layer will selectively absorb the dye. Thus, a positive Sudan test consists of observing 1) two layers, and 2) the presence of the dye on the top (lipid) layer.

Activity 4-7: Performing the Sudan Test



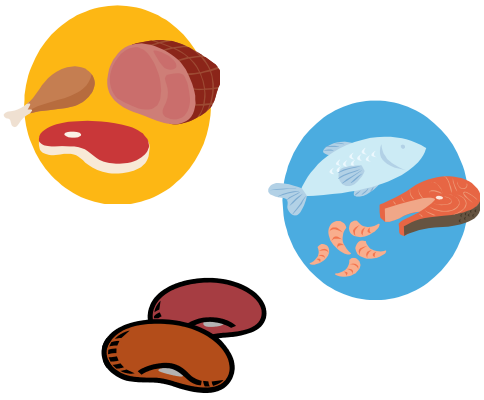
1. Obtain 3 clean tubes and number them with a permanent marker.
2. Add 2 mL of water to each tube.
3. Add 30 drops of each of the substances listed in Table 4-5 to the appropriate tube. (Make sure to shake well the salad dressing before adding it to your tube.)
4. Add 7 drops of the Sudan reagent to each tube.
5. Very carefully, mix each tube vigorously on the vortex machine (do not put your finger on the top of the tube!) to allow the contents to mix well.
6. Wait for the solution to settle (about 2 minutes) before observing to draw conclusions.

Note: If you get an emulsion in any of the tubes, you can add 20 more drops of water & re-mix to break it.

Table 4-5: The Sudan Test

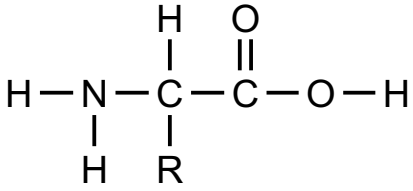
Sample	Contents	Two layers? (Yes or No)	Sudan on top layer? (Yes or No)	Conclusions (+ = lipid present - = lipid absent)
1	salad dressing	_____	_____	_____
2	vegetable oil	_____	_____	_____
3	deionized water	_____	_____	_____

III. PROTEINS:



Protein are large chains of building blocks called *amino acids*. Proteins have various functions and roles in living things: catalysts (enzymes), structural support, muscle contraction, hormones, transport, antibodies and storage containers (e.g. ferritin stores iron).

Any amino acid has a basic skeleton, which is shown below:



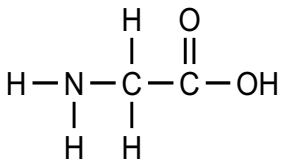
There are two functional groups that you can recognize in every amino acid (of the functional groups presented in Table 2). The R group of amino acids is what makes amino acids different from each other.

Activity 4-8: Name the two functional groups found in all amino acids:

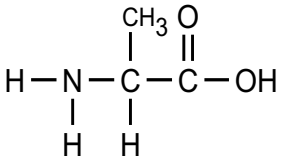
_____AND_____

Below are six of the twenty different amino acids commonly found in living organisms. These amino acids are all drawn from different perspectives, but you should be able to recognize the two functional groups they all have in common and how those two functional groups are connected to a central carbon atom. All amino acids also have one hydrogen atom covalently bonded to this central (alpha) carbon. **The unique part of each of these amino acids is what we call the "R group" or the "side chain."**

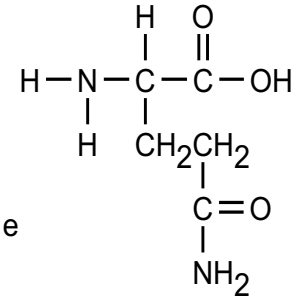
Activity 4-9: Find and circle the "R" group in each of the amino acids shown below.



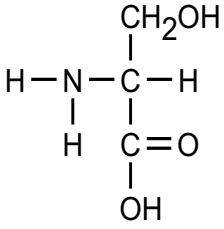
Glycine



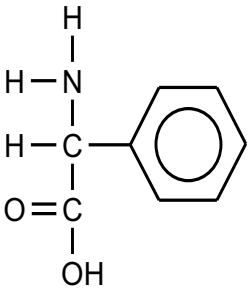
Alanine



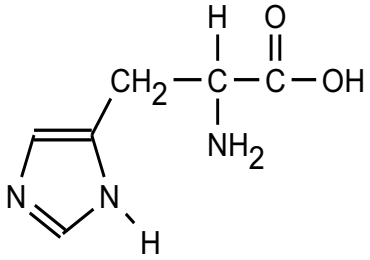
Glutamine



Serine

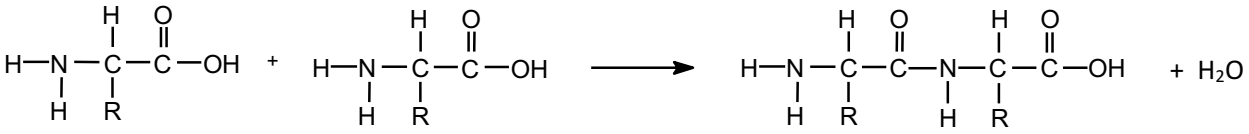


Phenylalanine



Histidine

Amino acids react by dehydration synthesis to form **peptides**. When only two react, a dipeptide is form. Many amino acids joined together constitute a polypeptide, or protein. The formation of a peptide bond follows the general pattern shown below. Notice that dehydration synthesis occurs between the *carboxyl group* of one amino acid and the *amino group* of another amino acid.



Activity 4-10:

- Build a molecule of Glycine and one of Alanine with your molecular model kit.
- Join them together as described in the previous reaction.
- Draw the resulting dipeptide in the space provided below and label the peptide bond.

The specific sequence of amino acids in a polypeptide chain is what scientists call the "**primary structure** of the protein". This sequence of amino acids in a protein is not a random event. It involves a series of sophisticated events in which information stored in the genes of the cell is used to build all the proteins of that cell.

The old system of abbreviating peptide chains uses three letters to abbreviate each amino acid, for example, the amino acid Glycine is abbreviated Gly, Serine is Ser, Alanine is Ala and so on. Thus a chain of amino acids consisting of Serine, Alanine, Glycine, Proline, Phenylalanine and Tyrosine is abbreviated Ser-Ala-Gly-Pro-Phe-Tyr.

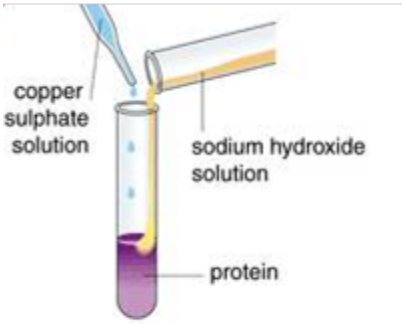
Peptides are read from left to right, and it is understood that the left-most amino acid has a free amino group and the right-most amino acid has a free carboxyl group. The new system used to abbreviate peptide chains uses one letter. More on this later in the semester.

The Biuret test for Proteins

The presence of proteins in a sample can be detected by the Biuret test . The Biuret reagent (made with copper (II) sulfate, or $CuSO_4$, is normally light blue. If a sample contains protein, the light blue color changes to violet when a complex is formed between the Cu^{2+} ion in the Biuret reagent and at least four peptide bonds. Free amino acids, therefore, would not allow for the reaction to occur.

Activity 4-11: Performing the Biuret test

1. Obtain 5 clean test tubes and number them.
2. To each of the numbered tubes, add 2 mL of the material to be tested, as listed in Table 4-6 below.



Caution! Be careful handling the next step. NaOH is a strong base and will burn your skin or eyes upon contact. You should wear eye protection while performing this test, and while washing test tubes.

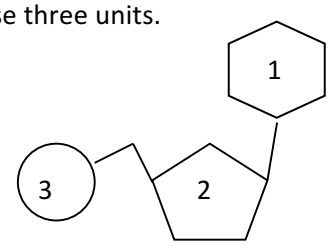
3. Add 2 mL of 2.5% NaOH to each tube and mix well.
4. Add three drops of $CuSO_4$ (Biuret's reagent) to each tube and mix well.
5. Record the final color and conclusions in Table 4-6.

Table 4-6: Biuret Test

Tube	Sample	Final color	Conclusion (+ = protein present - = protein absent)
1	amino acid solution	_____	_____
2	milk	_____	_____
3	egg albumin	_____	_____
4	protein solution	_____	_____
5	deionized water	_____	_____

IV. NUCLEIC ACIDS:

The 2 types of nucleic acids are DNA (deoxyribonucleic acid) and RNA (ribonucleic acid). These molecules are important because they are involved in the storage and the interpretation of the genetic makeup of an organism. The building block for nucleic acids is called a **nucleotide**. Any nucleotide has 3 basic units: 1) a nitrogenous base, 2) a 5-carbon sugar and 3) a phosphate group. The following skeleton represents the organization of these three units.

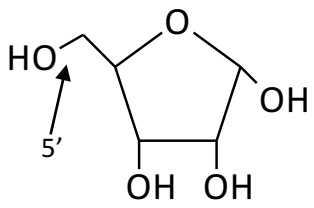


1) Nitrogenous bases: Are molecules that have several nitrogen atoms. You should know their names and in which nucleic acid they appear. The following table summarizes this.

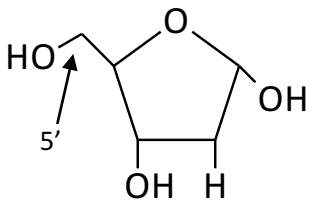
Name of Nitrogenous Base	Abbreviation	Found in:
Adenine	A	DNA,RNA
Guanine	G	DNA,RNA
Cytosine	C	DNA,RNA
Thymine	T	DNA
Uracil	U	RNA

2) Pentose (5-carbon sugar). In RNA this sugar is RIBOSE. In DNA it is DEOXYribose.

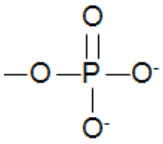
Activity 4-12: Find and circle the difference between these two sugars:



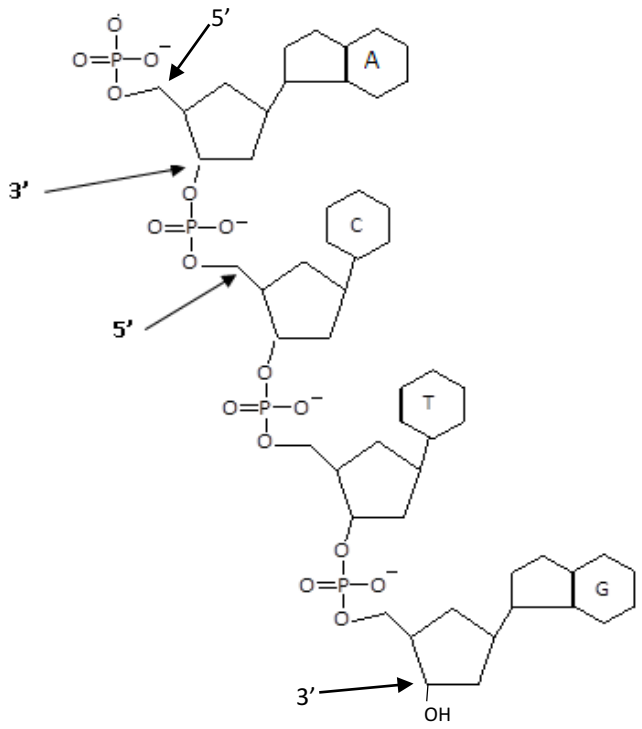
Ribose



Deoxyribose

3) A phosphate group  is attached to carbon #5 (5') of the sugar.

A long chain of nucleotides constitutes a nucleic acid chain. Nucleotides are joined together by dehydration synthesis occurring between the hydroxyl group in carbon #3 (3') of the sugar and the phosphate group in carbon # 5 (5') of another nucleotide, as shown on the next page:



Nucleic acids can be single chains or double chains. **DNA in chromosomes is a double chain**. RNA can be either double or single stranded. When two chains of nucleic acids are to pair up, they do it in a particular way. The two strands are held together by bonds between the nitrogenous bases of the two strands. The sugar and the phosphate group are towards the outside of the double chain.Not any two nitrogenous bases can pair up.

DNA	RNA
Adenine = Thymine	Adenine = Uracil
Cytosine ≡ Guanine	Cytosine ≡ Guanine

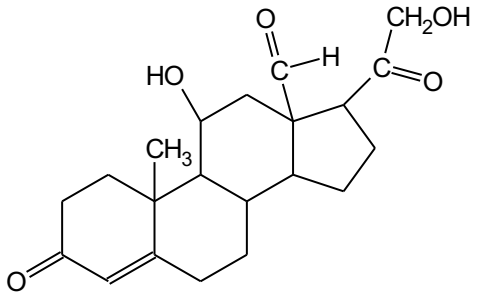
Because the structure of nucleic acids is so complicated, scientist write the sequence of nucleotides in a chain by using the first letter in the name of the nitrogenous bases in the sequence. For example, if one strand of DNA is

T-A-C-G-G-A-T-G-C-C-C-T-T-A-T-C-G-G-C, the complementary strand is
A-T-G-C-C-T-A-C-G-G-G-A-A-T-A-G-C-C-G

Activity 4-13: Observe the model of DNA in the lab. Find a nucleotide and its three components.

End of Lab Questions:

1. This is the hormone aldosterone, which is produced by the adrenal glands, located on top of your kidneys. It contains the four fused carbon rings characteristic of all steroids, like cholesterol and the sex hormones. ***Circle and label all the functional groups in this hormone.***



2. Two monosaccharides are linked together by a _____ linkage to form a _____.

3. Which of these are disaccharides? *Circle all that apply.*

a. sucrose	c. galactose	e. fructose
b. glucose	d. maltose	f. lactose

4. A molecule of fat is made of one _____ molecule and three _____ molecules, each of which can be saturated or unsaturated.

5. What is the basic difference between saturated and unsaturated fats?

6. Sugars are soluble in water but lipids are not. True or False? _____

7. A _____ is made of amino acids joined together by covalent bonds. These covalent bonds are called _____ and are formed by dehydration synthesis between the _____ functional group of one amino acid and the _____ functional group of another amino acid.

8. The building blocks of nucleic acids are called _____ .These monomers are, themselves, made of these three groups: _____ sugar, a _____ and a _____ group.

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Lab 5: Spectrophotometry

How Color and Light Absorption Are Measured

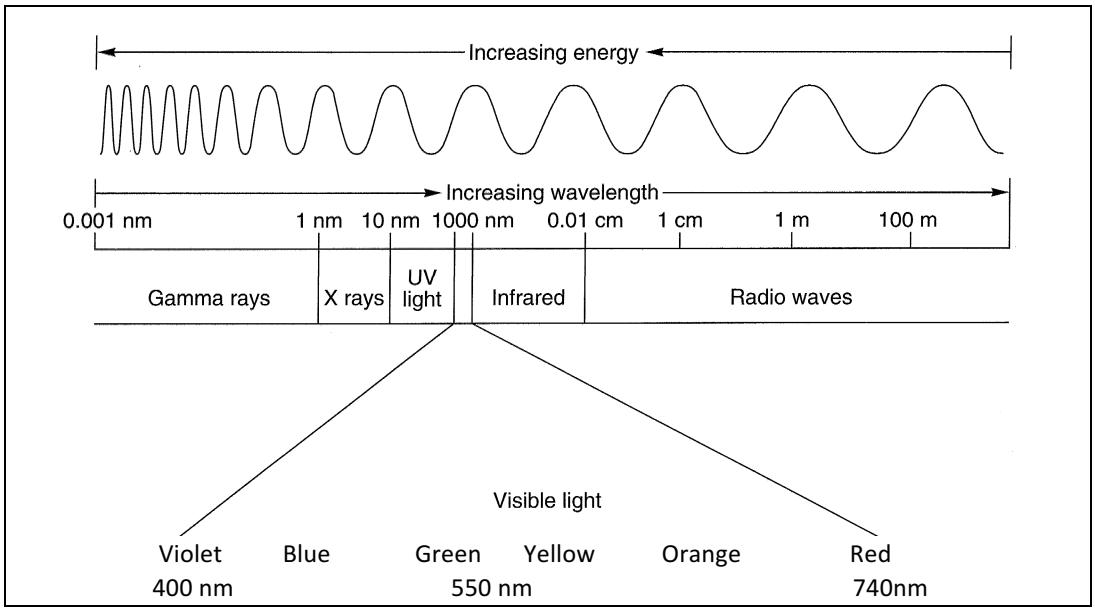
Objectives

- Understand the concept of absorbance and basic principles of spectrophotometry
- Learn how to operate the SpectroVIS spectrophotometer
- Practice running an enzyme assay with the spectrophotometer
- Understand the relationship between the change in absorbance with time and the enzyme reaction rate

Pre-Lab Reminder: *Before arriving to this scheduled lab, read the entire lab (background, procedure, and questions), and use that information to complete the pre-lab in eCampus.*

Background Information

Biologists routinely determine the presence, and measure the concentration of dissolved chemicals using a **spectrophotometer**. Spectrophotometry is based on the principle that atoms, molecules, or even chemical bonds, absorb a unique pattern of wavelength of light. Fig. 5-1 below shows the different wavelengths of electromagnetic radiation. *Notice that in the visible spectrum, different wavelengths have different energies and different color.*



In the visible spectrum, the cells in our eyes interpret these wavelengths as different colors. A colored solution, such as an oxidized guaiacol solution, appears that way because some of the light entering the solution is absorbed by the colored substance. A clear solution will allow almost all of the light to pass through. The **absorbance (A)** (amount of light absorbed by the solution) can be determined by using a spectrophotometer. The spectrophotometer quantitatively measures what fraction of the light passes through a given solution, and indicates on the absorbance scale the amount of light absorbed compared to that absorbed by a clear solution. **The darker the solution, the greater its absorbance.**

Inside the spectrophotometer there is a light that shines through a filter (which can be adjusted to control the wavelength of light), then passes through the sample and onto a light-sensitive phototube. The phototube produces an electric current proportional to the amount of light striking it. The absorbance meter measures how much light has been blocked by the sample and thereby prevented from striking the phototube. A clear tube of water or other solvent is the **blank** and is used to calibrate the instrument to zero absorbance. A solution that contains a small amount of a colored substance might show an absorbance of 0.1, a solution with a moderate amount might show an absorbance of 0.4, and so forth. In fact, in the lower portion of the absorbance scale, the amount of light absorbing particles in solution is directly proportional to the absorbance reading so that **a graph of absorbance versus concentration will give a straight line**. This very useful relationship is known as **Beer's Law**.

Activity 5-1: How Wavelength Relates to Absorption

1. Obtain 4 regular test tubes and label them 1, 2, 3, & 4.
2. Fill each tube with the following:

Tube #	Distilled Water	Blue Dye Concentrate
1	10.0 mL	*none*
2	9.7 mL	0.3 mL
3	9.4 mL	0.6 mL
4	9.1 mL	0.9 mL

3. Mix well each solution with the Vortex mixer.
4. Set Up the Vernier SpectroVIS Spectrophotometer:
   A. Turn the spectrophotometer on by: *Note: There is no on/off switch, it is software-controlled*
      a) Connect the USB cable to the computer
      b) Open the LoggerPro 3.8.2 software by doubl- clicking the icon on computer desktop.
   B. Calibrate the spectrophotometer by:
      a) Fill a cuvette about ¾ full by pouring in the solution from **Tube #1** (*distilled water is the "blank" because the blue dye was dissolved in this water in the other samples*).
      b) Insert the cuvette in the sample chamber, making sure that the clear side of the cuvette is in the path of light (facing the arrow sign ►).
      c) On the top menu, click "Experiment." Scroll down to "Calibrate" and select "Spectrometer: 1".
      d) Wait 60 seconds for the lamp to warm up.
      e) Then, click "Finish Calibration" and "OK". The spectrophotometer is now calibrated.
5. Setting the spectrophotometer for data capture and **determining the wavelength of maximum absorbance** for the blue dye solution.
   A. On the top bar, 2 places left of the 'Collect' icon, find the 'Configure Spectrophotometer"icon. 
   B. Select "Absorbance vs. Wavelength" as the Collection Mode.
   C. Click "OK" and a graph should appear with the wavelength (nm) on the x-axis and absorbance on the y-axis.
   D. Fill a cuvette ~3/4 full with the first blue dye solution (**Tube #2**) and place it in the spectrophotometer.
   E. Click "Collect," wait a few seconds, then click "STOP."
   F. The full chromatogram of the blue dye should be displayed to which which wavelengths of light are highly absorbed and which are not absorbed.
   G. Click on Autoformat if necessary. 

Answer these questions before moving on to the next activity:

1. What wavelength shows the maximum absorbance for the blue dye solution? _____

2. What color is being absorbed MOST by a solution that looks blue to our eyes? _____

3. What color is being absorbed LEAST by a solution that looks blue to our eyes? _____

Activity 5-2: Beer's Law in Practice

1. Set the spectrophotometer for data capture so we can observe how absorbance changes when the concentration of the solution changes.
   A. Place the cuvette containing the BLANK solution (Tube #1 contents) back into the sample chamber.
   B. On the top bar, 2 places left of the 'Collect' icon, find the 'Configure Spectrophotometer"icon. 
   C. Select "Absorbance vs. Concentration" as the Collection Mode.
   D. Type in "%" as the unit of concentration, but leave the other blanks with the default.
   E. Look at the column showing a list of wavelengths: click "Clear Selection," then select the wavelength choice that is closest to **the maximum absorbance for this blue dye** solution (as identified in the previous activity).
   F. Click "OK" and confirm that a graph appears in the main window with concentration on the x-axis and absorbance on the y-axis.
2. <u>Read the absorbance of the blue dye samples</u>:
   A. With the cuvette containing the **blank** (water from Tube #1) still in the chamber (making sure the clear side is in the path of light), click "Collect." 

   B. Wait until a red dot appears on the screen and then click "Keep." 

   C. A menu will pop-up asking for concentration of the sample. Type "0" (zero) and click "OK." **Do NOT click "STOP" yet!**
   D. Remove the blank cuvette and insert the cuvette containing Tube #2's blue dye solution and watch for a new red dot to appear. If you don't see it, adjust the scale of the axes.
   E. When the red dot stops moving, click "Keep" and enter 3.0% as the concentration.
   F. Repeat Steps D and E with a cuvette ¾ full of the solution in Tube #3, entering 6.0% as the concentration.
   G. Repeat Steps D and E with a cuvette ¾ full of the solution in Tube #4, entering 9.0% as the concentration.
   H. Click "Stop: on the top bar menu. 

3. Analyze the Data:
   I. Click the "Linear Fit" icon on the top bar menu. 

   J. A "best fit" line will appear over your data points. If your sample preparation (pipetting) and calibration were done correctly, your line should look like it runs through or very close to all four of your data points (0%, 3%, 6%, and 9%). A box will pop up showing information about your line. For this test, the "correlation" value shows you how closely your data fits into the straight line – the closer that number is to 1 the better your results, so >0.98 is pretty good.

4. Record your data and correlation in Table 5-1.

Sample Concentration	**Absorbance** *(does not have units)*	**Linear Correlation**
0%		
3%		
6%		
9%		

QUESTIONS

1. Which SAMPLE had the HIGHEST CONCENTRATION of dye? _____
2. Which SAMPLE had the HIGHEST ABSORBANCE READING? _____
3. What is the relationship between dye concentration and absorbance of light?

   The higher the concentration of dye, the higher / lower the absorbance.
   (circle one)

CHECKPOINT: Have your instructor check your data and answers before moving on to the next activity. *Instructor Initials:*_____

Activity 5-3: Enzyme Lab Practice Exercise

In your next lab, you will be performing an experiment to look at factors that affect the reaction rate of peroxidase, an **enzyme** extracted from turnips. This enzyme reacts with hydrogen peroxide and organic substrates like a molecule called guaiacol. When guaiacol and hydrogen peroxide bind to peroxidase, the guaiacol becomes oxidized and changes in color (to brown). This color change allows us to follow the reaction with the aid of the spectrophotometer.

Put simply, how FAST the sample turns brown is a measure of how FAST the reaction is going (also known as the reaction rate). Because a colored substance is absorbing light, we measure an increase in absorbance (formation of brown color) over TIME. The rate (change in absorbance over time) is equivalent to the reaction rate because it is showing us how fast the product was appearing in the tube.

The enzyme lab involves a lot of measuring and careful attention to detail. It will require that you know exactly what you are doing before you begin the experiment. In order to help you focus on the concepts during the actual enzyme lab, we are going to practice the set-up and measurements, and then run a practice test today.

Step 1: Sample Preparation

1. Obtain a small beaker or cup of each of the following solutions. *You only need about 20 mL of each solution. Make sure that each beaker or cup is properly labeled.*
   - Turnip Extract
   - pH 5 buffer
   - 10 mM H_2O_2 (this is hydrogen peroxide, NOT water)
   - 25 mM guaiacol
2. Obtain these pipets: two 1-mL, one 5-mL and one 10-mL.

3. Using the china marker on your tray, label 3 test tubes from 1 to 3. These tubes will be used for the following purposes:
   - #1: "Blank," sample with no H_2O_2 (to be used in calibrating the spectrophotometer).
   - #2: Substrates only, no buffer or enzyme
   - #3: Turnip extract (source of enzyme) and buffer (to stabilize the enzyme), no substrates

   The intent is to keep the reaction from starting until immediately before we place the sample into the cuvette and measure the absorbance. If we mix the enzyme and substrates early, the reaction will begin right away, and we will miss our chance to measure the rate.

4. Prepare these tubes by adding solutions to each tube as directed by Table 5-2. All amounts shown are in milliliters. *Take care to avoid mixing your pipettes - cross contamination is likely to introduce errors into your experiment and mess up your results.* DO NOT CROSS CONTAMINATE!

Table 5-2 Mixing table for enzyme rehearsal. (All volumes in milliliters)

Tube	Buffer (pH5)	H_2O_2	Extract	Guaiacol	*Expected Final Volume*
1/blank	6.0	---	1.0	1.0	8.0 mL
2	---	2.0	---	1.0	3.0 mL
3	4.0	---	1.0	---	5.0 mL

Step 2: Identifying the Best Absorption Wavelength

5. Similarly to the earlier instructions on how to use the spectrophotometer, calibrate the spectrophotometer as you did before:
   A. Fill a clean cuvette about ¾ full by pouring in the solution from Tube #1 (*NOT water!*).
   B. Insert the cuvette in the sample chamber, making sure that the clear side of the cuvette is in the path of light (facing the arrow sign ►).
   C. Click "Experiment" and scroll down to "Calibrate" and select "Spectrometer: 1".
   D. Wait 60 seconds for the lamp to warm up.
   E. Then, click "Finish Calibration" and "OK". The spectrophotometer is now calibrated.
6. Set this "BLANK" cuvette aside, but do not pour out the solution.
7. Now we need to create a solution that is already brown (end of the reaction, when lots of brown product has been made). To do this, obtain a separate medicine cup and mix 2 mL guaiacol, 2 mL hydrogen peroxide, and 3-4 drops (NOT milliliters, just drops!) of turnip extract. Swirl and watch it for a couple minutes and it should turn a brown/orange color. Pour this solution into a new cuvette.
8. On the top bar, click the "Configure Spectrophotometer" icon. 
9. Select "Absorbance vs. Wavelength" as the Collection Mode.
10. Click "OK" and a graph should appear with the wavelength (nm) on the x-axis and absorbance on the y-axis.
11. Place the cuvette of brown/orange solution into the spectrophotometer.
12. Click "Collect," wait a few seconds, then click "STOP."
13. The full chromatogram of the brown solution (oxidized guaiacol) should be displayed to which which wavelengths of light are highly absorbed and which are not absorbed.
14. Click on Autoformat if necessary. 

15. What wavelength shows the maximum absorbance for the brown solution?

16. Why is this wavelength different from the one most absorbed by the blue dye solution?

Step 3: Measuring Reaction Rate in a Spectrophotometer – Practice makes perfect!

17. Adjust the settings for data capture –**absorbance change over time = reaction rate**:
    A. Place the cuvette containing the BLANK solution (Tube #1 contents) back into the sample chamber.
    B. Click "Configure Spectrophotometer."
    C. Select "Absorbance vs. **Time**" as the Collection Mode.
    D. Look at the column showing a list of wavelengths: click "Clear Selection," then select the wavelength choice that is closest to the **maximum absorption wavelength for the brown** (oxidized) guaiacol solution.
    E. Click "OK" and confirm that a graph appears in the main window with TIME on the x-axis and absorbance on the y-axis.
    F. Back on the top bar, click "Experiment." Scroll down to "Data Collection" and set the experiment length to 120 seconds (this is the amount of time we'll watch the product being made). Leave everything else as the default values. Click "Done."

READ THE ENTIRE NEXT PART <u>BEFORE</u> DOING:

<u>Important points to keep in mind:</u>

- The reaction starts when the substrates (tube 2) and enzyme (in tube 3) come in contact. This is **immediately upon mixing** the tube contents, so don't start mixing until you are ready!
- The computer software "collect" button should be started 20 seconds after the INITIAL mixing, so we are recording the reaction from 20 seconds AFTER it began until 140 seconds AFTER it began. Do NOT stop the software when the TIMER in your group reaches 120 seconds – the computer needs to record until it stops on its own.
- **WHY?** The reaction rate we are recording does not include the first 20 seconds because we can't get the sample into the spectrophotometer without some delay and we want the delay to be CONTROLLED (the same for all samples). The standard 20 seconds is enough for *most* students to easily mix and place their sample into the spectrophotometer consistently.

18. In your lab groups,
    - TIMER: one person keeps track of time on a digital clock with seconds
    - MIXER: one person will mix the solutions, pour them into the cuvette, and insert the cuvette into the spectrophotometer (will need to be done very quickly, so choose someone with steady hands!)
    - ORGANIZER: one person who handles the software and instructions, keeping you on track
19. The TIMER will tell the MIXER when to start mixing tubes 2 and 3. The TIMER must be watching the clock for 20 seconds from this start time. While the TIMER keeps track of the time, the MIXER must:
    - Fully mix the contents of tubes 2 and 3. The best method is to: pour all of tube 2 into tube 3, then pour it all back into tube 2, then pour some into a clean cuvette until it is ¾ full.

- Have a Kim Wipe next to the spectrophotometer and wipe the sides of the cuvette to remove fingerprints and drips.
- Insert the cuvette (clear side facing the arrow sign ►) into the spectrophotometer and say "Ready!"

20. WAIT until the TIMER announcements 20 seconds, then the ORGANIZER clicks "Collect" 

on the software. **If you did it right, collection should begin a few seconds AFTER the "Ready!" announcement from your mixer. Do NOT start collecting until the TIMER announces the 20 seconds time is up.** *Note: If the MIXER is not finished and the cuvette is not already in place when the 20 seconds time is up, you are too late and you'll need to start over by cleaning the test tubes, making the solutions again, etc.*
21. The TIMER does not need to keep track of time anymore (after "Collect" has been clicked) – the computer software does the rest of the timing.
22. Everyone should watch the computer screen to ensure that the absorbance the increasing as the time passes. If your line is slanting upward as it appears, you're in good shape because the brown product is being made! ☺
23. When the computer finishes collecting the absorbance (after two more minutes), immediately remove the cuvette from the spectrophotometer; write down any color observations about the sample (this must be done immediately because the color keeps changing with time).
24. Discard the solution in the special waste bottle provided, rinse the cuvette several times with soapy water and then regular water. Do NOT try to scrub or brush the cuvette – scratches absorb light and mess up results!
25. Click the "Linear Fit" icon on the top bar menu. 

26. A "best fit" line will appear, along with the box of information about the line. The value "m" tells you the SLOPE of the line, which is the absorbance change over time.
    - Because the absorbance increased as product was formed, the slope IS the reaction rate.
    - The units can be written as **abs units / second**, but are more accurately written as **s^{-1}** or **1/s** because absorbance doesn't actually have a "unit."

Your peroxidase-catalyzed reaction rate (with units!): _____

References for the photos and procedure in this lab manual:

2. Dolphin, W.D., Exercise 4 (Properties of Enzymes) in: *Biology Laboratory Manual*, 4th edition, 1997, WCB Publishers
3. Eberhard,C., *General Biology Lab Manual*, 1996, Harcourt
4. Vernier Biology Laboratories

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Lab 6: Properties of Enzymes

Turnip Peroxidase, A Case Study

Objectives

- Name the class of macromolecules to which peroxidase belongs and the monomers that make it up.
- Name the substrates and products of the peroxidase catalyzed reaction.
- Explain the role of guaiacol in this experiment.
- Define enzyme, activation energy, active site, pH, and denaturation.
- Distinguish between oxidation/reduction, activation energy/catalysis, substrate/product, and hydrogen peroxide/peroxidase.
- Describe how temperature, pH, enzyme concentration, and substrate concentration affect the reaction rate.
- Explain why peroxidase is a necessary enzyme for all aerobic or oxygen-using cells.

Pre-Lab Reminder: *Before arriving to this scheduled lab, read the entire lab (background, procedure, and questions), and use that information to complete the pre-lab in eCampus.*

Background Information

The thousands of chemical reactions that occur in a cell at any given time do not occur randomly, but are highly under the control of biological catalysts called **enzymes**. A catalyst is a substance that speeds up a chemical reaction without being consumed by the reaction. Enzymes speed up reactions by lowering the **activation energy** of a reaction, which is that initial amount of energy necessary to bring reactants together with the proper amount of energy and in the proper orientation so that the products can be formed.

Most enzymes are proteins with particular primary structures dictated by genes. As proteins, upon their synthesis, enzymes assume particular shapes. This shape, especially in its **active site**, determines its catalytic effects. The active site of each enzyme binds to specific molecules – for example, the enzyme sucrase binds to sucrose but not to lactose, even though both are disaccharides. The reactant molecule that binds with to the active site of an enzyme (and undergoes chemical modification during the reaction) is called the **substrate** of that enzyme. Some enzymes bind to two substrates to form one or more products.

Certain enzymes have metallic ions (such as Cu^{2+}, Fe^{2+}, Mn^{2+}) as part of their active site These metal ions are called **cofactors**. Sometimes a cofactor is organic in nature and not an ion (e.g. some vitamins); in that case the cofactor is properly called a **coenzyme**.

The binding between enzyme and substrate(s) consists of weak, non-covalent chemical bonds. Because these bonds are week, this **enzyme-substrate complex** exists for only a few milliseconds. During this instant, the covalent bonds of the substrate(s) either come under stress or are oriented in such a way that facilitates the formation of a different molecule or molecules. This reduces the activation energy and leads to formation of the product(s) faster than if we waited for the substrate(s) to form this orientation on their own.

After the reaction, the product leaves the enzyme's active site and can be used in the cell. The enzyme is unchanged by the reaction and may enter the catalytic cycle again if more substrate molecules are available.

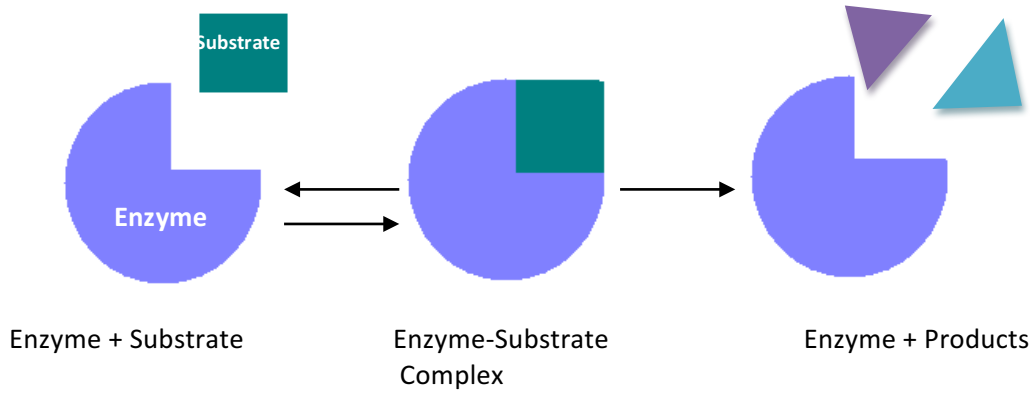

Fig. 6-1 A pictorial representation of an enzyme-catalyzed reaction.

An individual enzyme may convert about a thousand substrate molecules in one second. Their fast rates combined with the fact that they are not used up during the reaction means that enzymes are only needed in small amounts. Eventually enzymes wear out, break apart and lose their catalytic capacity. Cellular enzymes called "proteases" degrade inactive and damaged enzymes back apart to release the individual amino acids. This degradation of old enzymes is important to cells because it allows them to continually build new enzymes and other proteins. Our free amino acid pool is supported by digestion of the proteins we eat as well, especially for the "essential amino acids" that humans can only obtain from our dietary intake of protein.

The amount of a particular enzyme in a given cell is determined by a balance between its gene expression (process leading to synthesis of the particular protein from its gene on the cell's DNA), any regulating cofactors or other modifications, and how fast it is degraded after synthesis. Cells tightly regulate the concentration and activity level of ALL their enzymes because when no enzyme is present, the chemical reaction catalyzed by that enzyme does NOT occur (or occurs much too slowly to be useful to the cell). Like a store manager, cells need to regulate enzyme concentration and activity based on need. Imagine having 20 employees at the Walmart checkout counters when no customers were shopping, and none when hundreds of customers were there to shop. Not very efficient, is it?

Enzyme and Substrate Concentration: Generally, if the concentration of the enzyme increases, the rate of the reaction will also increase. This is true up to a certain point. The balance of enzyme and substrate concentration plays a significant role in the rate of the reaction: adding more enzyme will not increase the speed of a reaction if there is not enough substrate to be converted into product.

Environmental Conditions: The **pH** (a measure of the concentration of H^+ in an aqueous solution) and high salt concentrations affect the **shape** of enzyme molecules by interfering with ionic bonds that are necessary to maintain their tertiary structure. This, in turn, affects the substrate-binding efficiency of the enzyme. **Temperature**, within the physiological limits of 0 to 40°C, affects the frequency with which the enzyme and its substrates collide and, hence, affects the rate of substrate binding. Extremely high temperatures typically cause enzyme **denaturation**, rendering the enzyme

completely inactive. All factors that influence binding obviously affect the rate of enzyme-catalyzed reactions. Some of these factors will be investigated during this laboratory.

During this experiment you will study a type of enzyme called **peroxidase**, which is a large protein containing "heme" (iron-based organic cofactor, or coenzyme) as part of its active site. Peroxidase is a good experimental enzyme because it is easily prepared and assayed (tested). Turnips, horseradish roots, and potatoes contain a large amount of this enzyme. For this experiment, we extracted the peroxidase from turnips.

The normal function of peroxidase is to rid the cell of a toxic molecule called **hydrogen peroxide** (H_2O_2), which is produced as by-product in certain metabolic reactions. It is critical to remove this H_2O_2 before the cells become damaged by it.

We will carry out a peroxidase reaction "*in vitro*" using our own substrates designed to give us a specific, measurable product. We will monitor enzyme activity by observing the RATE an oxidized product is produced by reaction of the substrates we provide. A dye called **guaiacol** (molecule extracted from the guaiacum tree of Central and South America and the Caribbean) is an available substrate of peroxidase. When it binds to peroxidase along with hydrogen peroxide, the catalyzed reaction oxidizes the guaiacol as the hydrogen peroxide is reduced to water.

The complete reaction is as follows:

$$4\ H_2O_2 + \underset{\textbf{COLORLESS}}{4\ \text{guaiacol (reduced)}} \xrightarrow{\text{peroxidase}} 8\ H_2O + \underset{\textbf{BROWN}}{\text{tetraguaiacol (oxidized)}}$$

The oxidized product is called **tetraguaiacol**, and it is a brown color. We can quantitatively measure the amount of brown color as a measure of the amount of product at any given moment in time. To measure the reaction rate (how fast the product is made), the enzyme and substrates can be mixed in a tube and immediately placed in a spectrophotometer. As the brown color accumulates, the absorbance at ~500 nm will increase. **The reaction rate is the speed of increase in absorbance**, so by plotting absorbance versus time, the SLOPE of the best fit line from these data points is a direct measure of the enzyme-catalyzed reaction rate. *The procedure for using a spectrophotometer was explained in the previous lab and you should take a few minutes to review those instructions before proceeding.*

Factors Affecting Enzyme Activity

In this laboratory experiment, each team (table group) will tackle two of the following independent variables: enzyme concentration, temperature, pH, and the amount of inhibitor. All results will be shared on the board in the front of the room and should be recorded in your lab manual. *Please see note at the end about how to share data with your classmates.*

To perform each of the tests on the experimental variables, you will be following the same basic procedure. Please read over the instructions for all of the variables being studied in the lab, whether or not this is the variable to which your group has been assigned, and make sure you understand the instructions before you continue on with the experiment. This is the same basic procedure that you used in the Enzyme Lab Practice (Lab. 5)

For each of the following experimental variables, use the Mixing Table for each variable to set up the tubes. After you have all of the tubes set up with the appropriate volumes of solutions, you are ready to begin the experiment. In each test, tube 1 is always the blank/control for calibrating the spectrophotometer. The testing samples will always involve mixing a pair of tubes, one with substrates and one with the enzyme and buffer. Therefore, for all the other numbered tubes in the table, you will always be mixing tubes 2 & 3 together, tubes 4 &5 together, tubes 6 & 7 together, and tubes 8 & 9 together (when applicable). Remember to mix the solutions in the test tubes fully before pouring the contents into the cuvette.

The peroxidase extract has been prepared for you. It is a solution extracted from blended turnips, so it contains hundreds of different types of enzymes, not just peroxidase. We will not see the effect of any of these other enzymes because we are providing substrates that specifically fit into the peroxidase active site.

Activity 6-1: The Effect of Enzyme Concentration

Note: Changing the amount of turnip extract changes the amount of peroxidase!

1. Obtain a small beaker or cup of each of the following solutions. *You only need about 20 mL of each solution. Make sure that each beaker or cup is properly labeled.*
   - Turnip Extract
   - pH 5 buffer
   - 10 mM H_2O_2 (this is hydrogen peroxide, NOT water)
   - 25 mM guaiacol
2. Obtain these pipets: two 1-mL, one 5-mL and one 10-mL.
3. Using the china marker on your tray, label 7 test tubes from 1 to 7. Tubes 3, 5 and 7 will differ in the amount of turnip extract, providing different concentrations of the peroxidase enzyme during the experimental runs (reactions measured in the spectrophotometer).
4. Add solutions to each tube as directed by Table 6-1. Amounts shown are in mL. *Take care to avoid mixing your pipettes - cross contamination is likely to introduce errors into your experiment and mess up your results.* DO NOT CROSS CONTAMINATE!

Table 6-1 Mixing table for the effect of enzyme concentration test. (All volumes in milliliters)

Tube	Buffer (pH5)	H_2O_2	Extract	Guaiacol	*Expected Final Volume*
1/blank	6.0	---	1.0	1.0	8.0 mL
2	---	2.0	---	1.0	3.0 mL
3	4.5	---	0.5	---	5.0 mL
4	---	2.0	---	1.0	3.0 mL
5	4.0	---	1.0	---	5.0 mL
6	---	2.0	---	1.0	3.0 mL
7	3.0	---	2.0	---	5.0 mL

5. Calibrate the spectrophotometer as you did before:
   A. Fill a clean cuvette about ¾ full by pouring in the solution from Tube #1.
   B. Insert the cuvette in the sample chamber, making sure that the clear side of the cuvette is in the path of light (facing the arrow sign ►).
   C. Click "Experiment" and scroll down to "Calibrate" and select "Spectrometer: 1".
   D. Wait 60 seconds for the lamp to warm up.
   E. Then, click "Finish Calibration" and "OK". The spectrophotometer is now calibrated.

6. Now adjust the settings for data capture –**absorbance change over time = reaction rate**:
   A. Click "Configure Spectrophotometer." 
   B. Select "Absorbance vs. Time" as the Collection Mode. Leave all blanks as they are.
   C. Look at the column showing a list of wavelengths: click "Clear Selection," then select the wavelength choice that is closest to **500 nm** (may not be exact).
   D. Click "OK" and confirm that a graph appears in the main window with TIME on the x-axis and absorbance at 500 nm on the y-axis.
   E. Back on the top bar, click "Experiment" again. This time, scroll down to "Data Collection" and set the experiment length to 120 seconds (this is the amount of time we'll watch the product being made). Leave everything else as the default values. Click "Done."

READ THE ENTIRE NEXT PART <u>BEFORE</u> DOING:

<u>Important points to keep in mind:</u>

- The reaction starts when the substrates (tube 2) and enzyme (in tube 3) come in contact. This is immediately upon mixing the tube contents, so don't start mixing until you are ready!
- The computer software "collect" button should be started 20 seconds after the INITIAL mixing, so we are recording the reaction from 20 seconds AFTER it began until 140 seconds AFTER it began. Do NOT stop the software when the TIMER in your group reaches 120 seconds – the computer needs to record until it stops on its own.
- **WHY?** The reaction rate we are recording does not include the first 20 seconds because we can't get the sample into the spectrophotometer without some delay and we want the delay to be CONTROLLED (the same for all samples). The standard 20 seconds is enough for *most* students to easily mix and place their sample into the spectrophotometer consistently.

7. In your lab groups,
   - TIMER: one person keeps track of time on a digital clock with seconds
   - MIXER: one person will mix the solutions, pour them into the cuvette, and insert the cuvette into the spectrophotometer (will need to be done very quickly, so choose someone with steady hands!)
   - ORGANIZER: one person who handles the software and instructions, keeping you on track
8. To test the reaction rate using 0.5 mL of enzyme extract, the TIMER will tell the MIXER when to start mixing tubes 2 and 3. The TIMER must be watching the clock for 20 seconds from this start time. While the TIMER keeps track of the time, the MIXER must:
   A. Fully mix the contents of tubes 2 and 3. The best method is to: pour all of tube 2 into tube 3, then pour it all back into tube 2, then pour some into a clean cuvette until it is ¾ full.
   B. Have a kim wipe next to the spectrophotometer and wipe the sides of the cuvette to remove fingerprints and drips.
   C. Insert the cuvette (clear side facing the arrow sign ►) into the spectrophotometer and say "Done!"
9. When the TIMER announcements 20 seconds, the ORGANIZER needs to click "Collect" on the software. **This should be several seconds AFTER the "Done!" announcement! Do NOT start collecting until the TIMER announces the 20 seconds time is up.** *Note: If the MIXER is not finished and the cuvette is not in the spectrophotometer when the 20 seconds time is up, you need to start over by cleaning the test tubes, making the solutions again, etc.* 

10. The TIMER does not need to keep track of time anymore (after "Collect" has been clicked) – the computer software does the rest of the timing.

11. Everyone should watch the computer screen to ensure that the absorbance the increasing as the time passes. If your line is slanting upward as it appears, you're in good shape because the brown product is being made! ☺
12. When the computer finishes collecting the absorbance (after two more minutes), immediately remove the cuvette from the spectrophotometer; write down any color observations about the sample (this must be done immediately because the color keeps changing with time).
13. Discard the solution in the special waste bottle provided, rinse the cuvette several times with soapy water and then regular water. Do NOT try to scrub or brush the cuvette – scratches absorb light and mess up results!
14. Repeat steps 8-13 using Tubes 4 & 5 to test the reaction rate using 1.0 mL of enzyme extract. **Important:** ***When the ORGANIZER clicks "Collect" this time, it will ASK YOU what you want to do with your previous "run." You MUST choose "Store Latest Run" or the computer software will delete your data for the previous sample.***
15. Repeat steps 8-13 using Tubes 6 & 7 to test the reaction rate using 2.0 mL of enzyme extract. Make sure to choose "Store Latest Run" again!
16. At the end of the 3 reaction "runs," you should have 3 lines (each with a different slope/ steepness) on your absorbance vs. time plot.
17. Click the "Linear Fit" icon on the top bar menu and select all the runs. 

18. Three "best fit" lines will appear, along with three boxes of information, one for each line.
19. Drag the boxes around the screen until you can easily see which box goes with which line. Run 1 will be your 0.5 mL enzyme extract, Run 2 will be your 1.0 mL enzyme extract, and "Latest Run" will be your 2.0 mL enzyme extract (assuming you did them in the correct order).
20. As before, the value "m" in each box tells you the SLOPE of that line, which is the absorbance change over time.
    - Because absorbance increased as product was formed, the slope IS the reaction rate.
    - The units can be written as **abs units / second**, but are more accurately written as $\mathbf{s^{-1}}$ or **1/s** because absorbance doesn't actually have a "unit."
21. I strongly recommend that at least one member of your group use their phone to take a photo of the organized data on the computer screen (screen prints usually don't work on these computers).

Table 6-2 Reaction rates with differing enzyme concentrations.

Sample	Volume of Enzyme Extract	Reaction Rate (1/s)	Notes/Observations
0.5x	0.5 mL		
1.0x	1.0 mL		
2.0x	2.0 mL		

Activity 6-2: Temperature Effects on Peroxidase Activity:

1. Four water baths need to be set up: **~0°C** (an ice slurry)**, 40°C, 60°C,** and **100°C** (boiling water).
2. Refer back to the procedure for the effect of enzyme concentration for the solutions you need and how to set up your samples and how to start each experimental run. The instructions below

will ONLY describe the specific differences needed to evaluate how temperature affects peroxidase activity.

3. To test the effect of temperature, you'll need 11 test tubes, numbered 1 through 11, filled with the reagants listed in Table 6-3. **Read the instructions below BEFORE you add the solutions to the listed tubes.**
4. The temperatures listed in the table are to indicate which test sample each tube belongs to, NOT which temperature to place the tubes.
   A. For **23°C**: after adding the solutions, keep tubes 4 & 5 on the table rack, at room temp.
   B. For **0°C, 40°C, and 60°C**: after adding the solutions, place BOTH tubes in each pair (such as tubes 2 & 3 for 0°C) into the water bath for that temperature. Start a timer to leave them incubating at their specific temperature for >15 minutes before using any of these samples.
   C. For **100°C**: You need to create the "boiled extract" before you can add the solutions to tube 11. To create the boiled extract, label a new test tube with "12" to signify it is NOT one of your 11 numbered tubes below. Add 3.0 mL extract to Tube #12. Place this tube in the boiling water bath for 5 minutes. After 5 minutes, remove the tube and let it cool. It is then ready for you to add 1.0 mL of this boiled extract to Tube # 11. *Tubes 10 & 11 do NOT need to be heated to 100°C themselves - keep them at room temperature the entire time.*

Table 6-3 Mixing table for temperature experiment (**all values in milliliters**)

Temp.	Tube	Buffer (pH5)	H_2O_2	Extract	Boiled Extract	Guaiacol	*Expected Final Volume*
	1/blank	6.0	---	1.0	---	1.0	8.0 mL
0°C	2	---	2.0	---	---	1.0	3.0 mL
0°C	3	4.0	---	1.0	---	---	5.0 mL
23°C	4	---	2.0	---	---	1.0	3.0 mL
23°C	5	4.0	---	1.0	---	---	5.0 mL
40°C	6	---	2.0	---	---	1.0	3.0 mL
40°C	7	4.0	---	1.0	---	---	5.0 mL
60°C	8	---	2.0	---	---	1.0	3.0 mL
60°C	9	4.0	---	1.0	---	---	5.0 mL
100°C	10	---	2.0	---	---	1.0	3.0 mL
100°C	11	4.0	---	---	1.0	---	5.0 mL

5. As with set-up, refer back to the procedure for the effect of enzyme concentration for how to calibrate the spectrophotometer using the blank solution in tube 1.
6. The room temperature pair (tubes 4 & 5) can be tested first because they do not require pre-incubation. **Follow steps 8-13 from Activity 6-1** to test this temperature pair.
7. For **0°C, 40°C, and 60°C**: after each pair has incubated at the desired temperature for at least 15 minutes, you can test the samples. To ensure the temperature is correct at the start of the run, do NOT remove the tubes from their water bath until immediately before you are ready to mix them together and start the run. Begin with tubes 2 & 3 (0°C), then the 40°C pair, then the 60°C pair.
8. Remember for each experimental run that you need to chose **"Store Latest Run"** to keep the data from your previous runs, then **follow steps 17-21 from Activity 6-1** to obtain your reactions rates. In this experiment, the ORDER you tested the samples is very important, so make sure you know which line is which! If you tested 23°C first, the "runs" will not be in order of increasing temperature.

Table 6-4 Reaction rates with differing temperatures.

Temperature	Reaction Rate (1/s)	Notes/Observations
0°C		
23°C		
40°C		
60°C		
100°C		

Graph-Making Tip: *It is often difficult to figure out how to get the degree symbol when typing directly into Excel. ☺ If you copy and paste each temperature (with the degree symbol) from Microsoft Word directly into the Microsoft Excel worksheet, the degree symbol will come with it.*

Activity 6-3: How pH Affects Peroxidase Activity

1. Four special buffers need to be collected (in test tubes with special, colored caps): **pH 3, 5, 7, & 9**.
2. Refer back to the procedure for the effect of enzyme concentration for the solutions (do NOT use the pH 5 buffer from the large bottle for this experiment) and how to set up your samples and how to start each experimental run. The instructions below ONLY describe the specific differences needed to evaluate how pH affects peroxidase activity.
3. To test the effect of pH, you'll need 9 test tubes, numbered 1 through 9, filled with the reagants listed in Table 6-5. **Read the instructions below BEFORE you add any solutions to listed tubes.**
4. The pH values listed in the table are to indicate which test sample each tube belongs to:
   A. None of these samples should use the pH 5 buffer from the large bottle.
   B. Instead, when directed to add a certain volume of buffer, add the buffer indicated by filling the pipet with one of the solutions in the special pH buffers (the ones in test tubes with different colored caps).

Table 6-5 Mixing table for pH experiment (**all values in milliliters**)

pH	Tube	Buffer	H_2O_2	Extract	Guaiacol	*Expected Final Volume*
pH 5	1/blank	6.0 mL of pH 5	---	1.0	1.0	8.0 mL
pH 3	2	---	2.0	---	1.0	3.0 mL
pH 3	3	4.0 mL of pH 3	---	1.0	---	5.0 mL
pH 5	4	---	2.0	---	1.0	3.0 mL
pH 5	5	4.0 mL of pH 5	---	1.0	---	5.0 mL
pH 7	6	---	2.0	---	1.0	3.0 mL
pH 7	7	4.0 mL of pH 7	---	1.0	---	5.0 mL
pH 9	8	---	2.0	---	1.0	3.0 mL
pH 9	9	4.0 mL of pH 9	---	1.0	---	5.0 mL

5. As with set-up, refer back to the procedure for the effect of enzyme concentration for how to calibrate the spectrophotometer using the blank solution in tube 1.
6. The test each pair of tubes in sequence as directed in **steps 8-14 of Activity 6-1**.
7. Remember for each experimental run that you need to chose **"Store Latest Run"** to keep the data from your previous runs, then **follow steps 17-21 from Activity 6-1** to obtain your reactions rates. Remember to pay attention to the ORDER you tested the samples.

Table 6-6 Reaction rates with differing pH levels.

pH Level	Reaction Rate (1/s)	Notes/Observations
pH 3		
pH 5		
pH 7		
pH 9		

Activity 6-4: The Effect of an Inhibitor on Peroxidase

Hydroxylamine ($HONH_2$) is a molecule with a 3D structure similar to hydrogen peroxide (H_2O_2, but picture it in the bond order: HOOH). This molecule binds with the iron atom in the heme group at the active site of peroxidase, but does not react with guaiacol at all. Because this binding is likely to prevent hydrogen peroxide from entering the site, how do you think the presence of hydroxylamine will affect enzyme activity and the rate of reaction? This experiment will investigate that question.

1. Create a series of extract solutions in labeled test tubes (A, B, & C) containing differing amounts of inhibitor as follows, treating all samples the same. After adding all solutions and mixing well, let these tubes sit at room temperature for 5 minutes before moving on to the next step.
   - Test Tube "A": 3 mL extract (no inhibitor added), mix well
   - Test Tube "B": 3 mL extract plus 2 drops of 1% hydroxylamine, mix well
   - Test Tube "C": 3 mL extract plus 4 drops of 1% hydroxylamine, mix well
2. Refer back to the procedure for the effect of enzyme concentration for how to set up your samples and how to start each experimental run. The instructions below ONLY describe the specific differences needed to evaluate how this inhibitor affects peroxidase activity.
3. To test the effect of the inhibitor, you'll need 7 test tubes, numbered 1 through 7, filled with the reagants listed in Table 6-7. **Read the instructions below BEFORE adding solutions to the tubes.**
4. The inhibitor amounts listed in the table are to indicate which test sample each tube belongs to:
   - None of these samples should use the turnip extract from the large flask.
   - Instead, when directed to add a certain volume of extract, add the extract from one of the "treated" extracts as indicated by filling the pipet with one of the solutions in the test tubes labeled A, B, or C.

Table 6-7 Mixing table for inhibitor experiment (**all values in milliliters**)

Inhibitor Volume	Tube	Buffer (pH 5)	H_2O_2	Treated Extract	Guaiacol	*Expected Final Volume*
0 drops	1/blank	6.0 mL	---	1.0 of Tube A	1.0	8.0 mL
0 drops	2	---	2.0	---	1.0	3.0 mL
0 drops	3	4.0 mL	---	1.0 of Tube A	---	5.0 mL
2 drops	4	---	2.0	---	1.0	3.0 mL
2 drops	5	4.0 mL	---	1.0 of Tube B	---	5.0 mL
4 drops	6	---	2.0	---	1.0	3.0 mL
4 drops	7	4.0 mL	---	1.0 of Tube C	---	5.0 mL

5. As with set-up, refer back to the procedure for the effect of enzyme concentration for how to calibrate the spectrophotometer using the blank solution in tube 1.
6. The test each pair of tubes in sequence as directed in **steps 8-14 of Activity 6-1**.
7. Remember for each experimental run that you need to chose **"Store Latest Run"** to keep the data from your previous runs, then **follow steps 17-21 from Activity 6-1** to obtain your reactions rates. Remember to pay attention to the ORDER you tested the samples.

Table 6-8 Reaction rates with differing inhibitor levels.

Inhibitor Volume	Reaction Rate (1/s)	Notes/Observations
0 drops		
2 drops		
4 drops		

Data Analysis

1. The data you collected directly on the computer software showed a graph of absorbance vs. time. However, when you graph your data with Microsoft Excel, you will NOT be placing time on the x-axis or absorbance by itself on the y-axis.

   1A) For the effect of pH, what was the **independent** variable? Why was "time" NOT the independent variable?

   1B) For the effect of pH, what was the **dependent** variable? Why was "absorbance" NOT the dependent variable by itself?

2. Does the activity of peroxidase vary with **temperature**? Were you able to determine an optimum temperature? If so, what was it? If not, what do you need to do as a follow up?

3. Does the activity of peroxidase vary with **pH**? Were you able to determine an optimum pH? If so, what was it? If not, what do you need to do as a follow up?

References for the photos and procedures in this lab:

1. Dolphin, W.D., Exercise 4 (Properties of Enzymes) in: *Biology Laboratory Manual*, 4th edition, 1997, WCB Publishers
2. Eberhard,C., *General Biology Lab Manual*, 1996, Harcourt
3. MadScientist network: www.madsci.org
4. Vernier Biology Laboratories

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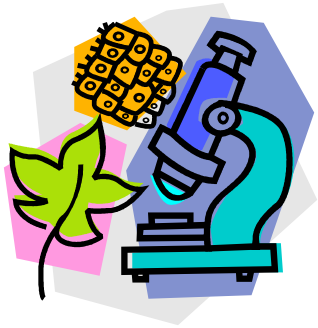
(to ensure each lab starts on a front side in 2-sided printing)

Lab 7: Cells

The Building Blocks of Life



Objectives

- Differentiate between prokaryotic and eukaryotic cells
- Observe cells from various forms of life
- Find similarities and differences among the cells observed
- Measure a plant, an animal and a bacteria cell

Pre-Lab Reminder: *Before arriving to this scheduled lab, read the entire lab (background, procedure, and questions), and use that information to complete the pre-lab in eCampus.*

Before Lab: For quick reference during today's lab, go back to Lab 2: Microscopy and copy your microscope's FOV diameters (in micrometers, NOT millimeters) into the table below.

Objective	*FOV diameter (µm)*
4x (scanning)	
10x (low power)	
40x (high power)	
100x (oil immersion)	

Background Information

Cells are considered the basic unit of living organisms because all living organisms are composed of cells, and cells perform all of the processes we collectively call "life". The development of this concept began with Robert Hooke in the 17th century. While observing slices of cork under the microscope, Hooke noticed that this tissue was made up of small units. Hooke called these units "cells" because they reminded him of the small cubicles that monks lived in. Over the next 300 years the cell theory emerged. The cell theory has three principles: A) All organisms are composed of one or more cells; B) the cell is the basic living unit of organization; and C) all cells arise from preexisting cells.

Although most individual cells are visible only with the aid of a microscope, some may be a meter long (e.g. nerve cells) or as large as a small orange (e.g. the yolk of an ostrich egg). Despite these differences, all cells are designed similarly and share fundamental features. All cells share three structural features: 1) All cells have a plasma membrane defining the boundary of the living material; 2) all contain a region of DNA (deoxyribonucleic acid), which stores genetic information; 3) all contain cytoplasm, which refers to everything inside the plasma membrane that is not part of the DNA region.

With respect to internal organization, there are two basic types of cells, **prokaryotic** and **eukaryotic**. The Greek word karyon means "kernel" which refers to the nucleus. Thus, prokaryotic means before a nucleus and, eukaryotic indicates the presence of a true nucleus. Organisms composed of prokaryotic cells are **archaebacteria** and **bacteria** such as *E. coli* and **cyanobacteria** (also called blue-

green algae). Prokaryotic cells are believed to be similar to the first cells, which arose on Earth 3.5 billion years ago. Organisms composed of eukaryotic cells are protists, fungi, plants, and animals. It is believed that eukaryotic organisms arose from prokaryotic ancestors.

Cytology is the study of cellular structure and function. The major tools of cytologists are light microscopy, electron microscopy, and cell chemistry. By studying the anatomy of a cell, we can find clues to how the cell works. In today's lab, you will study some of the features and variations among living cells to understand life processes of organisms.

PROKARYOTIC CELLS

Domain Archaebacteria: Archaebacteria are prokaryotes found in extreme environments (e.g. near volcanoes, sea-vents, and salty seashores). They will not be studied in this lab.

Domain Bacteria: Consists of the common bacteria and the cyanobacteria (sometimes called blue-green algae). You may see references to these organisms as eubacteria (a historical reference).

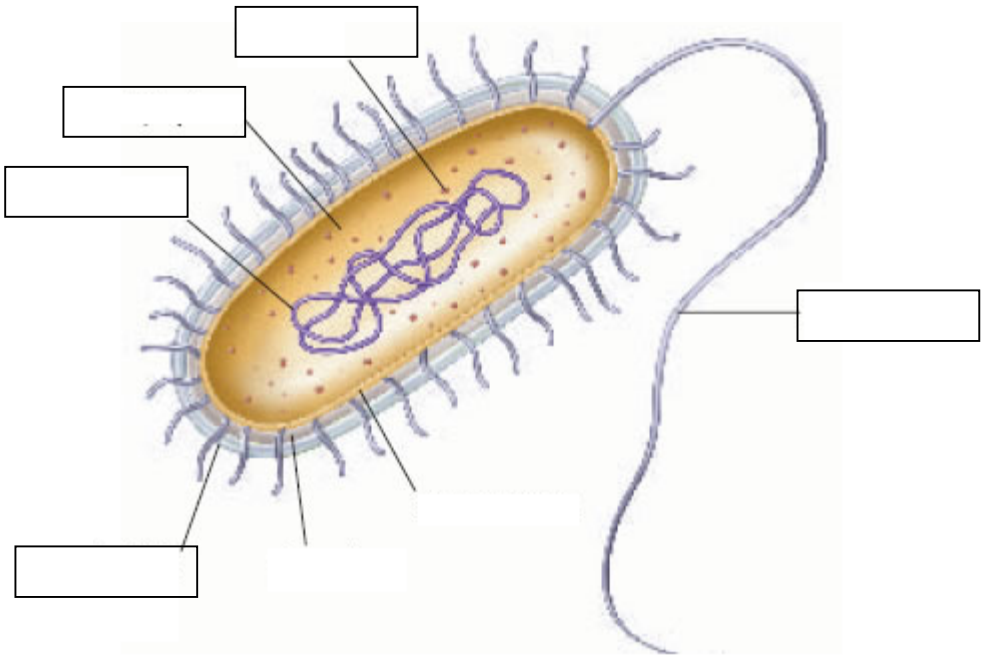


Fig. 7-1 A generalized bacterium cell.

Prokaryotes do not contain a membrane-bound nucleus or any other membrane-bound **organelles**. Organelles are organized structures of macromolecules having a specialized function and are suspended in the **cytoplasm**. The cytoplasm of prokaryotes is enclosed in a **plasma membrane** (cellular membrane) and is surrounded by a supporting **cell wall** covered by a gelatinous capsule. **Flagella** and hair-like outgrowths call **pili** are common in prokaryotes. Within the cytoplasm of prokaryotes are **ribosomes** (small particles involved in protein synthesis), **mesosomes** (internal extensions/folds of the plasma membrane), and **chromatin bodies** (concentrations of DNA). Prokaryotes do not reproduce sexually, but they have mechanisms for genetic recombination.

Activity 7-1: Preparing a wet mount slide

Throughout your biology classes, you will be asked to prepare fresh slides of various biological specimens, called "wet mounts." Some of these will be liquid (e.g. a slide of a blood sample), some may be solid and liquid (e.g. a piece of onion skin with a few drops of a dye).

1. Obtain a clean and dry microscope slide.
2. Place a drop of the cyanobacteria labeled *Oscillatoria* onto the center of your slide. *Make sure that your drop includes some of the green-looking stuff from the bottom.*
3. Obtain a clean coverslip. *Check that you don't have two coverslips stuck together; if you do, it may make it more difficult to focus it. If you see a "rainbow" while you shine light on the coverslip, there are two stuck together. Slide the two slips to separate them.*
4. Place the edge of the coverslip at one edge of your drop on the slide. (See Fig. 7-2), then slowly lower the coverslip onto the drop so that no air bubbles are trapped between the liquid and the coverslip.
5. Remove excess liquid on the sides of the coverslip with a KimWipe. Touching it gently will "wick" the extra water out from under the coverslip, so hold it there for 1-2 seconds, but no longer of you'll dry your sample out.
6. Place the slide on the stage (should be as low as it goes and with 4x objective in place) and follow the procedures in **Activity 7-2** to focus and view the sample with your microscope.

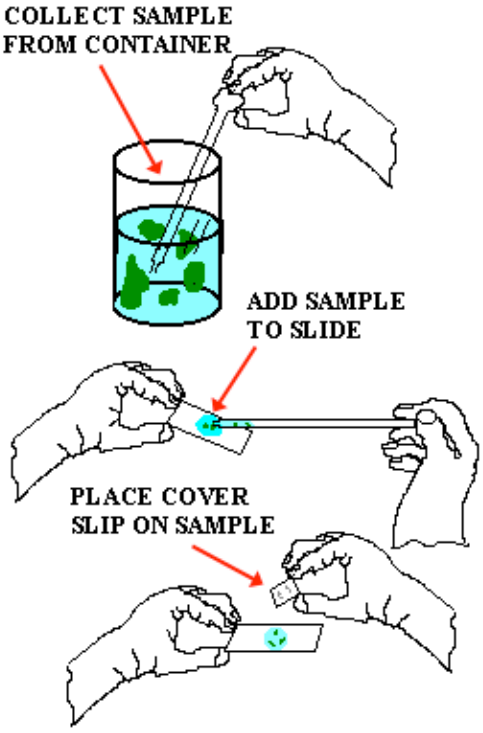


Fig. 7-2 Preparing a wet-mount slide

Activity 7-2: Cyanobacteria

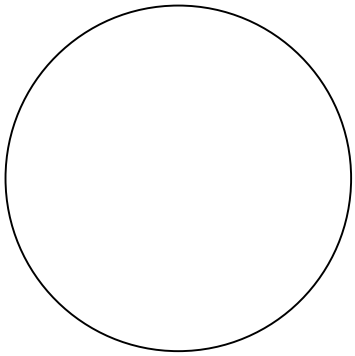
The largest prokaryotes are cyanobacteria, which are sometimes called blue-green algae. As described in the endosymbiotic theory, the ancestors of today's cyanobacteria are widely accepted to be the organisms that evolved into the chloroplasts in photosynthetic eukaryotes. Because they are prokaryotic, cyanobacteria do not contain any organelles, so the light-absorbing pigments (chlorophyll *a* and accessory pigments) are not in membrane-bound chloroplasts. Instead, these pigments are held in photosynthetic membrane folds that extend throughout the cell, called thylakoids. Cyanobacteria are often surrounded by a mucilaginous sheath.

Oscillatoria is a cyanobacteria in which individual bacterial cells live together in a colony, joined side-by-side to form a long filament. *Gleocapsa* is another type of cyanobacteria, in which the cells live in loosely arranged colonies. It is easier to identify individual cyanobacteria cells in the *Gleocapsa* colony because they are not tightly joined.

1. Observe the ***Oscillatoria*** in the wet mount slide you prepared in Activity 7-1, beginning with the scanning (4x) objective and working you way up to high power (40x).
2. When you observe each long green filament with the 40x objective, try to identify the cell wall barriers between individual cells along the filament.
3. **Remove and wash the slide - always do this after making a wet mount!**
4. Now, prepare a wet mount of the other provided cyanobacteria, ***Gleocapsa***.

5. After observing the *Gleocapsa* cells at 40x power, select an area with at least 2 or 3 *Gleocapsa* cells. Use the circle on the right to represent the entire field of view (FOV) and draw all of what you see under the microscope. Take care to draw the cells to scale - meaning if they took up 10% of the FOV when you were looking at them, they should take up 10% of the FOV in your drawing.
6. Point an arrow to one of the *Gleocapsa* cells, and show your calculations to estimate its size below (in micrometers):

   Size of one *Gleocapsa* cell:



Activity 7-3: Non-photosynthetic Bacteria (OPTIONAL)

Most bacteria are much smaller than cyanobacteria and do not contain chlorophyll. Yogurt is a nutrient-rich culture of bacteria. The bacterial cells composing most of the yogurt are *Lactobacillus*, a bacterium adapted to live on milk sugar (lactose). *Lactobacillus* converts milk to yogurt. Yogurt is acidic and keeps longer than milk. Historically, *Lactobacillus* has been used in many parts of the world by peoples deficient in lactase, an enzyme that breaks down lactose. Many Middle Eastern and African cultures use the more digestible yogurt in their diets instead of milk.

1. Create a wet mount with the yogurt by: Placing a small drop of water onto the center of your slide. Using a toothpick, rub a **tiny** dab of yogurt into the water on the slide and add a coverslip.
2. As you attempt to observe the Lactobacillus cells, remember that they are much smaller than the cyanobacteria, so look for super tiny gray rods (more like the size of dots.

Question

1. Why are *Lactobacillus* a different color and size than *Oscillatoria* and *Gleocapsa*?

EUKARYOTIC CELLS

Domain Eukarya includes plants, animals, fungi and protists. Eukaryotic cells contain membrane-bound **nuclei** (plural, nucleus = singular) and other organelles. Nuclei contain the genetic material of a cell and control metabolism. Cytoplasm forms the matrix of the cell and is contained by the plasma membrane. Within the cytoplasm are a variety of organelles. **Chloroplasts** are elliptical green organelles in plant cells. Chloroplasts are the site of photosynthesis in plant cells and are green because they contain chlorophyll, a photosynthetic pigment capable of capturing light energy. **Mitochondria** are organelles found in all eukaryotic cells (including plant cells!). These organelles are where aerobic respiration occurs. When viewed with a conventional light microscope, mitochondria are small, dark, and often difficult to see.

Eukaryotic cells are structurally more complex than prokaryotic cells. Although some features are common to both types (e.g. ribosomes, plasma membrane, DNA), eukaryotic cells contain several organelles not found in prokaryotic cells. In the following exercise you will investigate some of these organelles.

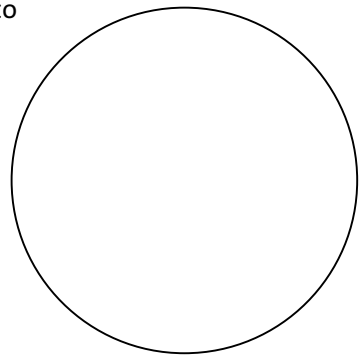
Kingdom Plantae

Activity 7-4: Structure of Plant Cells

Examine living *Elodea* cells and chloroplasts. *Elodea* is a common pond weed used frequently in studies of photosynthesis, cellular structure, and cytoplasmic streaming.

1. Remove a young leaf from the tip of a sprig of *Elodea*. The smaller the leaf section, the flatter it will sit under the coverslip, so avoid leaves with thick veins (or tear the edge off, leaving the vein behind).
2. Prepare a wet mount of the leaf by placing it into a drop of water on a microscope slide with the top surface facing up, and adding a coverslip. Make sure to keep the leaf wet.
3. Examine the leaf with your compound microscope.
   - The leaf will be composed of several layers of cells. The very top and bottom layers of the leaf will be protective epidermal layers, so ignore these non-photosynthetic cells and try to focus on a layer under the large, top layer of cells.
   - Each of the small, regularly shaped units you see is a cell, surrounded by a cell wall (a feature that distinguishes plant cells from animal cells). Cell walls are made primarily of cellulose. Cellulose is a complex carbohydrate made of glucose molecules attached end-to-end.
   - In plants, the plasma membrane lies just inside the cell wall and is attached in several places.
   - Observing the leaf cells at high power will allow you to examine the location and behavior of the chloroplasts in these leaf cells. You may even notice them streaming through the cytoplasm.
4. After observing the *Elodea* cells at high power, select an area with a clear view of one layer of leaf cells. Use the circle on the next page to represent the entire field of view (FOV) and draw all of what you see under the microscope. Take care to draw the cells to scale - meaning if they took up 10% of the FOV when you were looking at them, they should take up 10% of the FOV in your drawing. Make sure to show the position and size of the chloroplasts accurately, too!
5. Point an arrow to one of the *Elodea* cells, and show your calculations to estimate its size below (in micrometers):

   Size of one *Elodea* cell:



Questions

1. What three-dimensional shape are *Elodea* cells?

2. Switch back down to low power (10x) and examine various layers of cells by focusing up and down through the layers. Can you tell that this leaf is composed of more than one layer of cells?

Consider the spatial distribution of chloroplasts within the cells. They may be pushed against the margins of the cell by the large central vacuole containing mostly water and bounded by a vacuolar membrane. The vacuole occupies about 90% of the volume of a mature cell. Its many functions include storage of organic and inorganic molecules, ions, water, enzymes, and waste products. As the water level changes, the position of the chloroplasts often changes, too.

Activity 7-5: Plastids

Plastids are organelles where food is made and stored. You have already examined chloroplasts, a type of plastid in which photosynthesis occurs. Other plastids have different functions. We will examine **amyloplasts**, which are plastids that store starch, and therefore will stain darkly when exposed to iodine.

1. Use a razor blade to cut a very thin section of a potato. Make the section as thin as you can.
2. Place the potato slice in a drop of water on a microscope slide and add a coverslip. If the coverslip sits at an angle, your potato section is too thick on one or both ends. Cut the thicker side off of the section and try again until your coverslip lays relatively flat.
3. Stain the section by adding a drop of iodine to the edge of the coverslip and letting it diffuse UNDER the coverslip. If any iodine spills over the top, clean it carefully before placing the slide onto the stage. Allow the stain 5 minutes to diffuse into the potato.
4. Locate the small, clam-shaped amyloplasts within the cells. Notice the staining to indicate the presence of starch. High power magnification may reveal the eccentric lines distinguishing layers of deposited starch on the grains. Look carefully to observe the cell wall lines between cells (will not be stained, so may require adjusting the iris diaphragm to gain enough contrast to see).
5. After observing the stained potato cells at high power, select an area with a clear view of one layer of potato cells. Use the circle on the right to represent the entire field of view (FOV) and draw all of what you see under the microscope. Take care to draw the cells and amyloplasts to scale - meaning if something took up 10% of the FOV when you were looking at them, they should take up 10% of the FOV in your drawing.
6. Point an arrow to one of the potato cells, and show your calculations to estimate its size below (in micrometers):

   Size of one potato cell (make sure to estimate the CELL, not an amyloplast inside!):

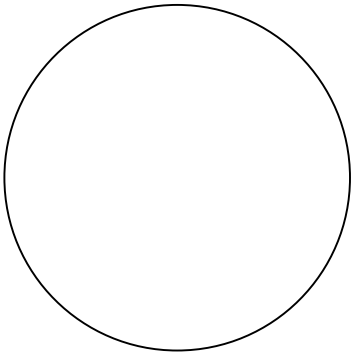
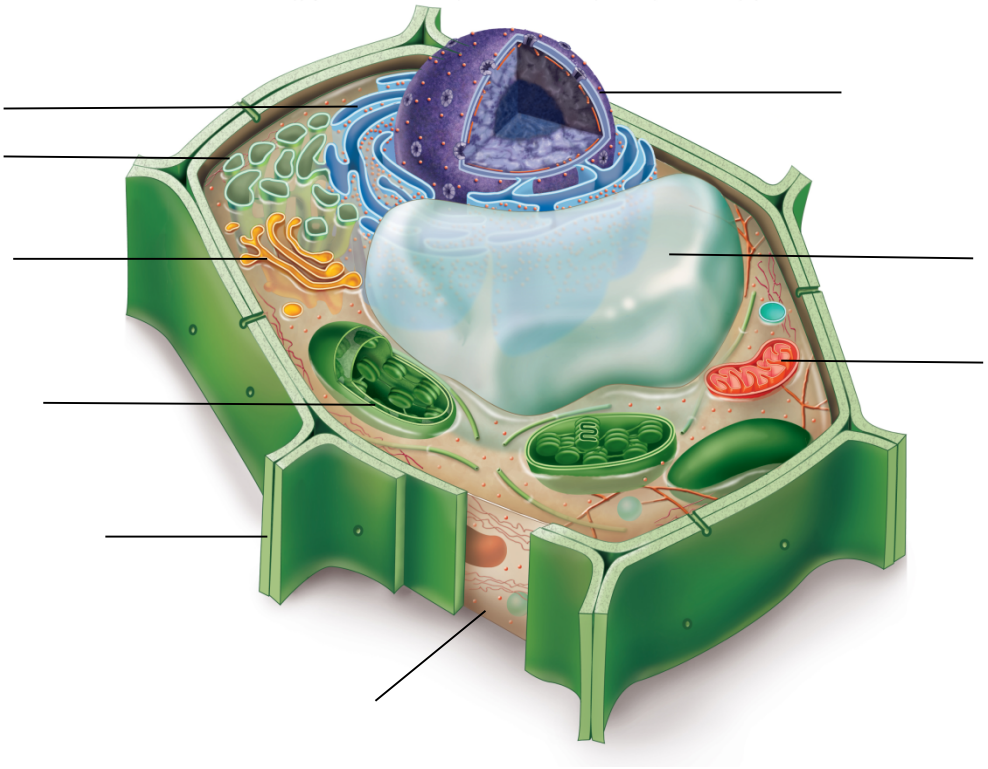


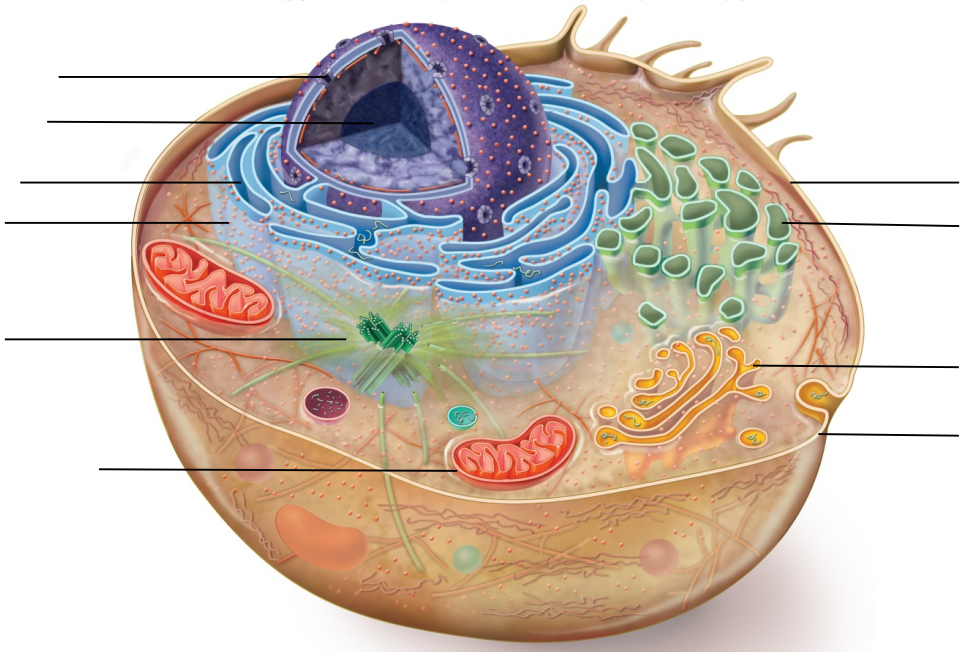
Fig. 7-3 A generalized picture of a plant cell. Can you identify the various cellular structures pointed?



Kingdom Animalia

Animals, like plants, are eukaryotes. They share many similarities, and also have several differences.

Fig. 7-4 A generalized picture of an animal cell. Can you identify the various cellular structures pointed?



Activity 7-6: Human Epithelial Cells

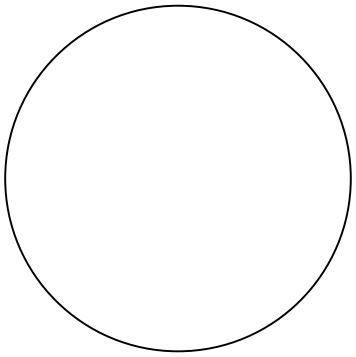
Human epithelial cells are sloughed from the inner surface of your mouth. They are flat cells with a readily visible nucleus.

1. To examine your own epithelial cheek cells, obtain a cheek cell slide from the side bench (do not use the slide from your lab table).
2. Place a TINY drop of water in the center of the slide.
3. Add a TINY drop of methylene blue to the water and mix.
4. Gently scrape the inside of your cheek with the broad, flat end of a toothpick (it should not hurt, but you should feel it) and then stir the scrapings into dye/water mix on the microscope slide.
5. Dispose of used toothpicks in the container of bleach solution on the side bench. If you use any paper towels or Kim Wipes to wipe anything that might have touched your cheek cells, place these in the biohazard trash, not the regular trash.
6. Carefully add a coverslip, wait 5 minutes for the stain to work, and then examine the cells using your compound microscope.

NOTE: After viewing the human epithelial cell slides, dispose of the entire slide (coverslip still on it) in the bleach solution on the side counter. **This is the ONLY slide that should be placed in bleach solution today!**

7. After observing the stained cheek cells at high power, select an area with a clear view of at least 2-3 epithelial cells. Use the circle below to represent the entire field of view (FOV) and draw all of what you see under the microscope. Take care to draw the cells and any visible organelles to scale!
8. Point an arrow to one of the cheek cells, and show your calculations to estimate its size below (in micrometers):

   Size of one epithelial cell:



Question
How do the size and shape of human epithelial cells differ from those of the Elodea cells that you examined earlier?

Kingdom Protista

Amoeba and *Paramecia* are members of the "kingdom" Protista, a large group of organisms that includes plant-like protists, animal-like protists, and fungi-like protists. The protists are not all descended from a single ancestor and are no longer classified as a true kingdom, but tend to be discussed as a single group even now because they are eukaryotic and mostly unicellular (a single cell

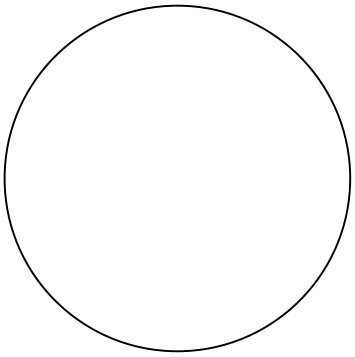
is the entire organism). Today, you will examine two of the animal-like protists, *Amoeba* and *Paramecia*, both of which are unicellular.

Activity 7-7: Amoeba

Amoeba are irregularly shaped protists with many internal organelles. *Amoeba* move via "amoeboid movement." Amoeboid movement occurs by means of **pseudopodia**, temporary protrusions of the cell – these extensions of the membrane move outward in one direction, pulling the other side of the cell inward as the cytoplasm streams into the extension. Amoeba also use pseudopodia to surround food particles, eventually closing around the food to create **food vacuoles**, where enzymes merge to digest the food (usually a live organism). Another important structure in *Amoeba* is the **contractile vacuole** that accumulates and expels water and waste products.

1. Examine *Amoeba* by looking at the live cultures already set up for you on a light microscope.
2. After observing the amoeba at low and high power, select the objective that allows you to see the ENTIRE cell and center one amoeba in the FOV.
3. Use the circle on the right to represent the entire field of view (FOV) and draw all of what you see under the microscope. Take care to draw the cells and any visible organelles to scale!
9. Show your calculations to estimate its size below (in micrometers):

   Size of one *Amoeba*:



Questions

1. Do *Amoeba* have a nucleus? _____ If so, were you able to see it? _____

2. Do *Amoeba* have chloroplasts? _____ If so, were you able to see them? _____

3. Do *Amoeba* have cell walls? _____ What physical characteristics did you observe that support your answer?

Kingdom Fungi

Fungi are among the most common and most important groups of organisms. Fungi are basically filamentous strands of cells that secrete enzymes and feed on the organic material on which they are growing. That organic material may be humus in the soil where mushrooms grow or a stale loaf of bread where mold thrives. It may be the skin between your toes inhabited by athlete's foot fungus or decaying animal on the forest floor being decomposed by fungi digesting the animal's dead tissue. Fungi not only cause disease; they are also important decomposers that recycle nutrients from dead organisms.

The basic structure of a fungus cell is the **hypha** (pl., hyphae) – a slender filament of cytoplasm and nuclei enclosed by a cell wall. A mass of these hyphae make up an individual organism and is

collectively called a **mycelium**. A mycelium can permeate soil, water, or living tissue; fungi certainly seem to grow everywhere. In all cases, the hyphae of a fungus secrete enzymes for extracellular digestion of the organic substrate. Then the mycelium and its hyphae absorb the digested nutrients. For this reason, fungi are called absorptive heterotrophs.

Activity 7-8: Bread Mold (OPTIONAL, time permitting)

Black bread mold (*Rhizopus stolonifer*) grows as cotton-like masses on bread, fruit and other organic material that is high in carbohydrates. Infection begins when a haploid spore germinates and grows into masses of filamentous hyphae that differentiate into rhizoids (these anchor the fungus to its substrate, secrete digestive enzymes, and absorb partially digested enzymes) and sporangiophores (aerial hyphae that produce sporangia containing spores at their tips).

1. Examine the bread mold **in the culture dish**. **Leave the glass plate on top** to prevent spores from being released into the air and examine the mold under the dissecting microscope.
2. Note the whitish mass of filaments growing over the surface of the bread. Each filament is called a hypha. Some hyphae grow upward and form small, black, globe-like structures called sporangia. Other specialized hyphae penetrate the bread.
3. Examine the prepared slide of bread mold under the microscope, which is set up as a demo on the side bench.

Activity 7-9: Yeast (OPTIONAL, time permitting)

1. Prepare a wet mount of the yeast suspension by placing a drop on a slide and adding a coverslip.
2. Observe the slide under a compound microscope. Look for nuclei and small food granules.
3. Note that some of the yeast cells exhibit small, rounded projections called buds. Yeast can reproduce sexually or asexually, and these buds are the product of the asexual reproduction process. When the division is complete, the bud will be released as an identical "daughter" cell.

Activity 7-10: Mushrooms (OPTIONAL, time permitting)

Mushrooms belong to a group of fungi called basidiomycetes. They are named for the basidiocarp, the fruiting body of a mushroom, or the structure that you think of as the mushroom. Mushrooms are characterized by plates or gills on the undersurface of the basidiocarp.

Examine the basidiocarp of the common commercial mushroom. Note the stalk and the cap. Examine the undersurface of the cap and locate the gills. If the mushroom is young, the gills may be covered by a thin membrane that extends from the stalk to the outer margin of the cap.

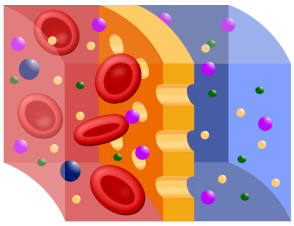
1. Carefully cut the mushroom to obtain one of the gills and mount it in a drop of water on a microscope slide and add a coverslip.
2. Examine the edges of the gill using the low power objective on the compound microscope.

References:

1. Perry et. al.: *Laboratory Manual for Starr and Taggart's Biology: The Unity and Diversity of Life*: 2002, Brooks/Cole
2. Vodopich & Moore: *Biology Laboratory Manual, 6th edition,* 2002, McGraw Hill

Lab 8: Membranes

Applying diffusion and osmosis principles



Objectives

- Define diffusion, passive transport, dialysis, osmosis, plasmolysis, hemolysis, isotonic, hypotonic and hypertonic
- Understand the relationship between diffusion rate and molecular weight
- Observe diffusion rates in systems with different ratios of surface area to volume
- Predict and observe the direction of diffusion of solutes across membranes
- Predict and observe the direction of diffusion of water across membranes
- Describe how the tonicity of a solution affects blood cells and plant cells

Pre-Lab Reminder: *<u>Before arriving to this scheduled lab</u>, read the entire lab (background, procedure, and questions), and use that information to complete the pre-lab in eCampus.*

Background Information

The plasma membrane separates the contents of living cells from their surroundings. This membrane acts as a physical barrier, regulating the passage of substances into and out of the cell. Cell membranes are made mostly of phospholipids, amphipathic substances that orient their polar phosphate heads towards the outside of the membrane and their non-polar fatty acid chains towards the inside of the membrane. Since the bulk of the plasma membrane is non-polar in nature, small non-polar substances can cross freely but polar substances cannot. Thus, the membrane is said to be "selectively-permeable" (or differentially permeable) since it "selects" what gets in/out of the cell. Proteins embedded in the membranes of living cells can serve as carriers, facilitating the passage of small polar substances.

a. b.

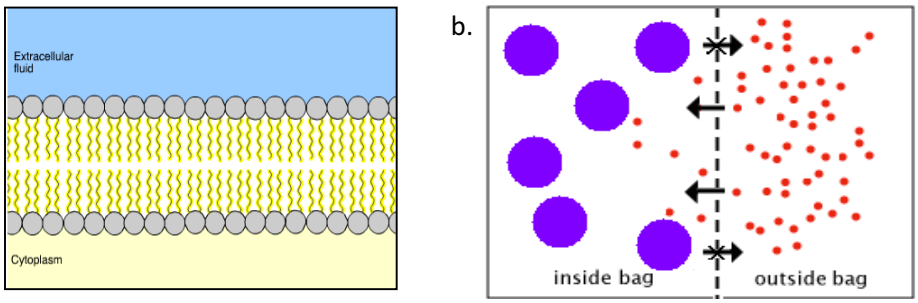


Fig. 8-1: a. The phospholipid bilayer of cell membranes screens substances according to polarity and size. b. A dialysis membrane (man-made membrane) may screen substances based on size.

Normal cell function involves the movement of many substances across the membrane. Simple diffusion is one way to exchange materials with the environment. Diffusion is the process by which substances spread from a place of higher concentration to a place of lower concentration. Diffusion is a spontaneous process that accounts for some of the movement happening across the cell membrane.

Dialysis

Dialysis refers to the diffusion of solutes across a selectively permeable membrane. A **solute** is a substance (ionic or covalent) dissolved in a **solvent**. The solute and the solvent together comprise a **solution**. A solution cannot be described as diffusing. The solute and solvent diffuse separately, and usually in opposite directions because their concentrations are in relation to each other. Therefore, it is important that you know which one you are talking about when you describe the ***direction*** of diffusion.

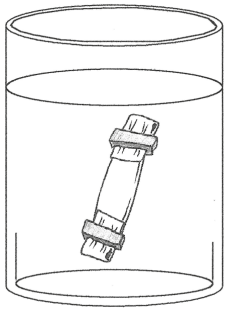
For example, in a 10% glucose solution, glucose is the solute that is dissolved in the solvent (water, unless otherwise indicated). The 10% indicates that there are 10 grams of the glucose for every 100 mL (or 100 g, depending on how the solution was made) total solution: 10 g / 100 mL = 0.10 = 10%. The glucose concentration is what we are referring to with 10%, so in simplified terms, you can estimate that the solvent concentration is the rest (90% in this case).

Two important factors are involved in the passage of solutes across a selectively permeable membrane:
1) whether the membrane is permeable to the solute, and
2) the concentration (or electrochemical*, if ionic) gradient.

Solutes move across membranes by diffusion:
diffusion is always down their concentration or electrochemical (if ionic) gradient.

** an electrochemical gradient consists of a gradient in concentration (more to less concentrated) as well as a gradient in charge (e.g. more positive towards more negative, or vice-versa)*

Artificial Cells – Dialysis Tubing



In today's lab, you will use dialysis tubing to prepare artificial cells for the study the diffusion of solutes and solvents across a selectively permeable membrane.

The dialysis membrane is composed of a plastic material with microscopic pores that allow the passage of small substances, while blocking larger ones. Though semi-permeable like a cell membrane, **dialysis tubing membranes only regulate passage of solutes or solvents by SIZE**. Polarity does not affect movement across these artificial membranes because they are not composed of phospholipids and do not contain transmembrane protein carriers or respond to differences in polarity. Similarly, large molecules cannot cross because there are no carrier proteins to move these molecules that can't fit through the membrane gaps on their own.

Activity 8-1: Observing Dialysis in Artificial Cells

In this activity you will use dialysis bags to prepare artificial cells for the study the diffusion of solutes across a selectively permeable membrane (dialysis). By observing changes in color during the experiment you will be able to make conclusions regarding the direction of dialysis and how size affects movement.

The color changes observed will be due to the use of two different dyes:

Phenolphtalein: an acid/base indicator that changes from colorless to pink in alkaline pHs

Iodine: reacts with starch producing a blue-black color

Your instructor will demonstrate the correct use of the dialysis bags and the orange clamps.

1. Obtain a piece of water-soaked dialysis tubing from the side bench.
2. Fold the end of one side twice. Close it securely with the orange clamp.
3. With a graduated cylinder or pipet, add 8 mL of water, and 3 drops of phenolphtalein to the bag.
4. Fold the other end of the bag twice and close it securely with an orange clamp.
5. Go to the sink and rinse the bag with water.
6. Fill a 250-mL beaker with 200 mL of water and add 10 drops of 1 M sodium hydroxide: **Caution: do not spill or touch the NaOH. It is caustic! Wear goggles!**
7. Immerse this bag in the prepared NaOH solution and leave it in the beaker for 30 minutes.

8. Prepare another dialysis bag and add 8 mL of a starch solution to the bag.
9. Fill a 250-mL beaker with about 200 mL water and 2 mL (about 40 drops) of Iodine.
10. Immerse this bag in the prepared iodine solution and leave it in the beaker for 30 minutes.

11. After 30 minutes, use Fig. 8-3 to draw arrows showing the direction of movement (if any) of the substances involved in each case.
12. Dispose of the dialysis bags (solutions in designated waste containers, bags in trash can) and rinse the clamps well with water.

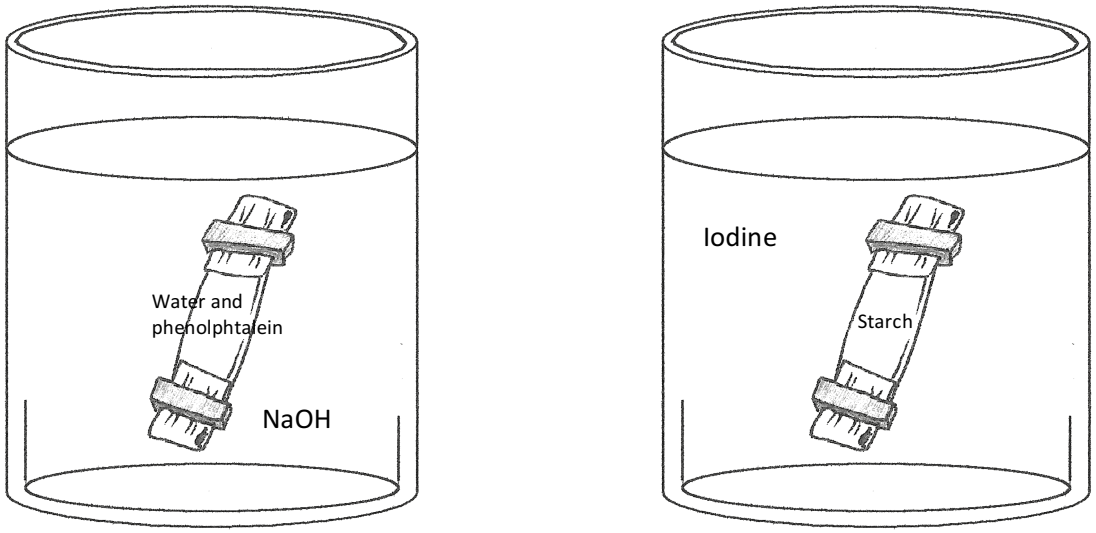


Fig. 8-3 Dialysis bags for solute diffusion experiment
Drawings by CPP and SPR,Spring 2004

Answer these questions:

a. Did NaOH enter the bag represented on the left of Fig. 8-3? Give evidence for that.

b. Did phenolphtalein move out of the bag? Give evidence for that.

c. Did Iodine move into the bag represented to the right on Fig. 8-3? Give evidence for that.

d. Did starch move out of the bag? Give evidence for that.

e. Give a reason for why a substance may NOT have diffused into or out of the bag:

Osmosis

Osmosis is the diffusion of solvent molecules (water for living cells) across a selectively permeable membrane. As with dialysis, osmosis involves the passive movement from a higher concentration to a lower concentration, but it refers only to the movement of water molecules, not the solute. Nevertheless, osmosis is affected by the amount of solutes in solution, or the tonicity. If two solutions separated by a selectively permeable membrane have the same concentration of solutes, they are said to be **isotonic**. If they have different concentrations, the one that is more concentrated is said to be **hypertonic** to the other, which is **hypotonic**. The hypotonic solution has less solutes – but more water – than the hypertonic solution. Thus,

Water moves by osmosis from a hypotonic solution to a hypertonic solution.
This is the opposite direction compared to solute diffusion,
but is still down its own concentration gradient.

Activity 8-2: Observing Osmosis in Dialysis Bags

The dialysis bags that you are using are impermeable to the disaccharide sucrose, but freely permeable to water. We will use these bags and various concentrations of sucrose to simulate osmosis in a cell.

1. Obtain 4 dialysis bags. Keep them moist while preparing them.
2. Label the clamps with the letters A, B, C and D with a permanent marker.
3. Using the same techniques as learned in Activity 8-3, prepare the bags by adding the following solutions to them:

Bag A: 8 mL 1% sucrose	Bag C: 8 mL 20% sucrose
Bag B: 8 mL 10% sucrose	Bag D: 8 mL 1% sucrose

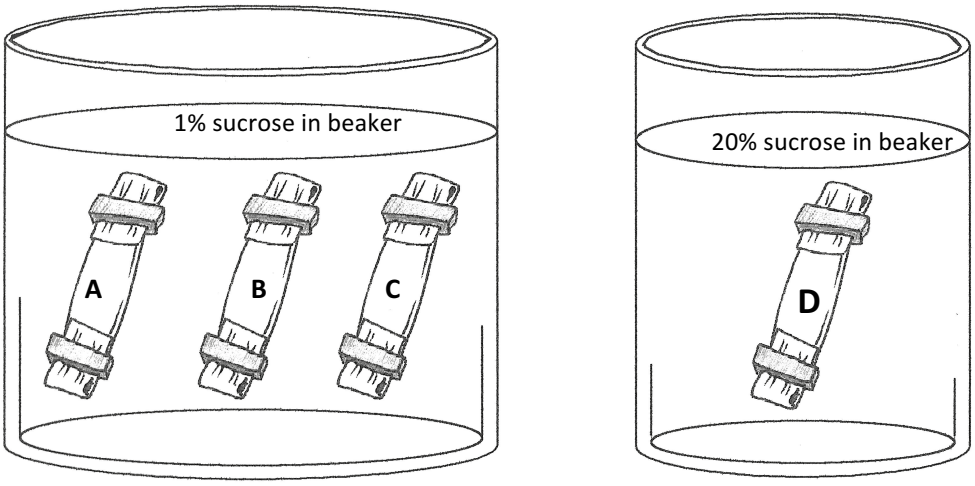


Fig. 8-4: Osmosis bags under various concentration gradients. (Drawings by CPP and SPR, Spring 2004)

4. One at a time, use a paper towel to gently pat the dialysis bag dry, then weigh it using the triple beam balance. Measure to the nearest tenth (1 decimal place). Record these initial weights in Table 8-1. **Take care to avoid mixing up the bags!**
5. Immerse Bags A,B and C in a large culture dish containing 1% sucrose solution.
6. Immerse Bag D in a 250-mL beaker containing enough of a 20% sucrose solution to cover the bag.
7. Allow all four bags to stay immersed in their solutions for at least 40 minutes.
8. After 40 minutes, remove the bags from the solutions one at a time.
9. For each bag, use a paper towel to pat the dialysis bag surface dry (those droplets will affect the weight!), then weigh the bag and its contents.
10. Record the weights in Table 8-1 and determine the change in weight during the 30 minutes.

Table 8-1 Weights of osmosis bags

	Initial weight (g)	Final weight (g)	Change in weight (g)	Gain or Loss of H_2O?
Bag A	_____	_____	_____	_____
Bag B	_____	_____	_____	_____
Bag C	_____	_____	_____	_____
Bag D	_____	_____	_____	_____

Answer the following questions:

a. Did osmosis occur in all bags? Why or why not?

b. Which bag(s) lost water? _____

c. Which bag(s) gained water? _____

d. Based on what you put into the bags and the beakers, which bag(s) was/were **hypotonic** to the outside medium? _____
Do your gain/loss results match what you expected for these bags? _____

e. Based on what you put into the bags and the beakers, which bag(s) was/were **hypertonic** to the outside medium? _____
Do your gain/loss results match what you expected for these bags? _____

Activity 8-3: Observing Hemolysis of Blood Cells (OPTIONAL)

Red blood cells are excellent models to show osmosis in living cells as affected by the tonicity of the surrounding solution. Since the outer boundary of a red blood cell is the fluid plasma membrane (no cell wall is present), exposing the cells to pure water (0.0% solute) would cause water to rush into the cell, resulting in the **lysis**, or bursting, of the cell. Such destruction of a red blood cell is called **hemolysis**. If on the other hand, red blood cells are exposed to a hypertonic solution (e.g. >1.0% NaCl), water will move out of the cells causing them to shrivel. This is called **crenation**. Of course, normal functioning of red blood cell depends on their roundish shape, so both of the situations described would be detrimental to the organism.

When blood cells are intact, they absorb light and cause a solution to be opaque (not transparent, so you cannot see through to the other side). Therefore, you will be able to tell if hemolysis has occurred because when ~75% of the cells are hemolyzed, the contents of the test tube become transparent (see through). If hemolysis does not occur, the mixture will remain opaque. ***Note**: transparent does NOT mean colorless – the solution will remain red.*

Caution: All blood-contaminated materials should be disposed of properly: all paper towels and plastic droppers should be discarded in the BioHazard-labeled trash bag; the test tubes and their contents must be discarded in the tub filled with bleach water (do NOT rinse the tubes – just place them directly in the bleach solution).

In this exercise you will expose red blood cells to 3 different concentrations of salt: 0.0%, 0.9% NaCl & 1.8% NaCl.

1. Obtain tubes containing 0.0%, 0.9% and 1.8% NaCl from the side bench.
2. Use a clean plastic pipette to add four drops of pig's blood to the test tube containing 0% solutes.
3. Immediately note the time.
4. Swirl (do not shake) the test tube to mix the contents well.
5. Observe the solution to determine if the contents become transparent and if so, how long it takes. Record the results in Table 8-2.
6. Repeat steps 2-5 to add blood to the other two solutions (0.9% NaCl and 1.8% NaCl). If the solution does not become transparent after 5 minutes, record the hemolylsis time as "N/A" or "did not hemolyze"
7. Go to the demonstration microscopes and observe the visual shape and condition of any remaining cells. The instructor will have set up slides of the same three solutions you tested in steps 2-6. Record your observations in Table 8-2.

Table 8-2 Hemolysis times for red blood cells in different NaCl solutions.

External Solution	**Time to Hemolysis ?**	**Microcopic Cell Condition** (as seen under microscope: normal/crenate/lysed)
0.0% NaCl		
0.9%NaCl		
1.8% NaCl		

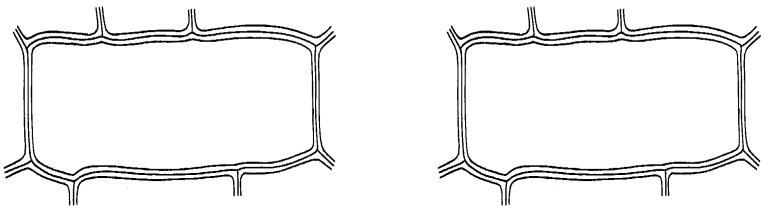
Activity 8-4: Observing plasmolysis in plant cells

Plant cells have a cell wall (made of cellulose fibers) in addition to the plasma membrane. The presence of the cell wall influences osmosis in plant cells: if exposed to pure water, the cell will gain water only up to a point. Lysis will not occur because the cell wall will prevent the cell from gaining any additional water or bursting.

As a plant cell gains water, **turgor pressure** builds up at the interface of the plasma membrane and the cell wall. Turgor pressure is the pressure that the plasma membrane exerts on the cell wall. At some point, the turgor pressure will prevent more water from entering the cell, even if the concentration of solutes favors the inward movement of water. When the cell is full of water it is said to be turgid. This is the preferred situation for plant cells.

If, on the other hand, a plant cell is exposed to a hypertonic solution, the cell will lose water to its surroundings, as expected. At some point, the plasma membrane separates from the cell wall due to the internal loss of volume. This event, separation of the plasma membrane from the cell wall, is called **plasmolysis**. As long as the solution is changed relatively quickly, this process can be reversed by exposing the cell to a hypotonic medium. Over time, however, the lack of water in the cytoplasm and organelles affects too many cellular processes and the cell dies.

1. Prepare a wet mount slide of an *Elodea* leaf and observe the cells under high power with your microscope. Notice the abundance of chloroplasts and the "full" appearance of the cell: the green contents of the cell extend completely towards the cell wall.
2. Remove the slide from the microscope.
3. Lift the coverslip and add 2 drops of 10% NaCl directly on top of the leaf section. Allow 1 minute for the hypertonic solution to affect the cells.
4. Put the coverslip back in place and observe with the high power (40x) lens.
5. Notice the appearance of the cells now - the cytoplasm is no longer pressed against the cell wall, and instead looks like a green ball near the center of each rectangle. *This is plasmolysis.*
6. Use the cell outlines in Fig. 8-5 to draw ONE normal (a) and ONE plasmolyzed (b) Elodea cell.



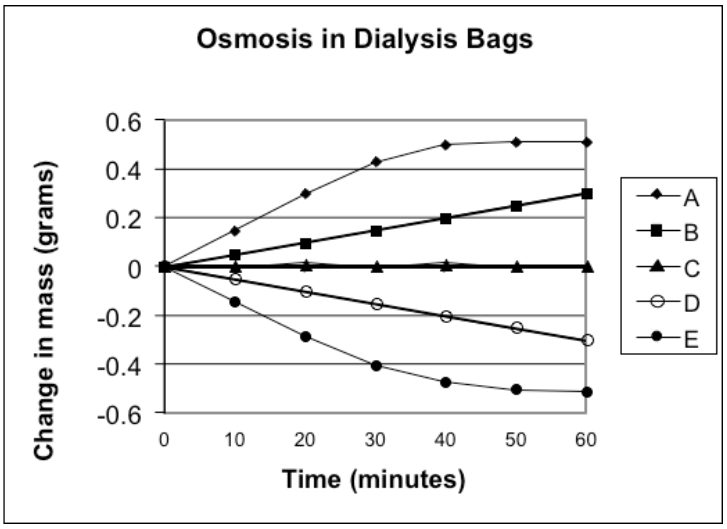
a) Normal cell b) Plasmolyzed cell

Fig.8-5 Sketch a SINGLE *Elodea* cell into each rectangle, using these boxes to represent the cell wall of each cell. Be as accurate as possible to show the size and shape of the plasma membrane and other structures inside.

7. OPTIONAL: remove the coverslip and rinse the leaf with plenty of water (drop water over it, wick it off, and repeat 4-5 times, finally finishing with two drops of distilled water. Observe the leaf cells again under the microscope. Did your cells recover?

Questions

1. Five dialysis bags (impermeable to sucrose) were filled with various concentrations of sucrose and then placed in beakers containing an external solution of 1 M sucrose. The initial weight of all bags was the same. Every ten minutes the bags were weighed, and the loss or gain in mass for each bag was plotted as a function of time.



   1A. Which bag was isotonic to the 1 M sucrose solution? _____

   1B. Which bag must have started with the highest concentration of sucrose? _____

   1C. Which bag must have started with the lowest concentration of sucrose? _____

   1D. Why does line A flatten out after 40-50 minutes? Explain.

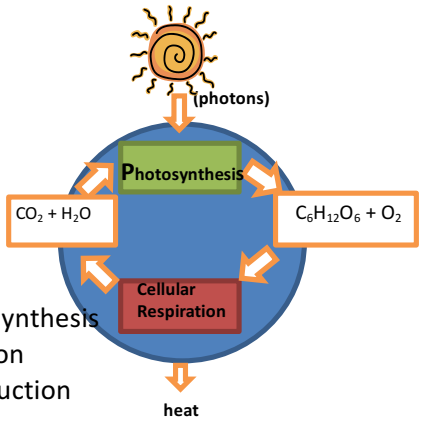
2. Two animal cells of similar size are placed in a 0.4% NaCl solution. Cell 1 enlarges in size for a while and then stops. Cell 2 continues to enlarge and bursts. What can you conclude from these observations? ***Hint:*** *Draw a picture of the situation and label everything you can before trying to evaluate the answer choices!*
   a) Cell 1 was hypertonic to Cell 2.
   b) Cell 1 was hypertonic to the solution, but Cell 2 was hypotonic to the solution.
   c) Cell 1 was hypotonic to the solution, but Cell 2 was hypertonic to the solution.
   d) Cell 2 was hypertonic to Cell 1.
   e) Cells 1 and 2 were isotonic to each other.

References

1. Vodopich & Moore: Biology Laboratory Manual, 6th edition, 2002, McGraw Hill

Lab 9: Respiration and Photosynthesis

a) The Effect of Exercise on the Rate of CO_2 Production
b) The Effect of Temperature on the Metabolic Rate of an Ectotherm
c) The Absorption Spectrum of Chlorophyll a



Objectives

- Know the overall equation of aerobic cellular respiration and photosynthesis
- Understand the difference between aerobic and anaerobic respiration
- Observe the relationship between exercise and carbon dioxide production
- Define metabolism, metabolic rate, ectotherm and endotherm
- Understand the interdependence of ambient temperature and metabolic rate in an ectotherm, and explain how enzymes of the respiratory pathway are related to this
- Get acquainted with technological devices to monitor and measure the production or uptake of CO2 during cellular respiration and photosynthesis
- Separate the pigments in a leaf extract using paper chromatography
- Obtain the absorption spectrum of chlorophyll *a*

Pre-Lab Reminder: *<u>Before arriving to this scheduled lab</u>, read the entire lab (background, procedure, and questions), and use that information to complete the pre-lab in eCampus.*

Background Information

The first law of thermodynamics states that energy can neither be created nor destroyed, only converted from one form to another. Because all living organisms have a constant energy requirement, they have mechanisms to gather, store, and use energy. Collectively, these mechanisms are called **metabolism**. A single, specific reaction that starts with one compound and ends up with another compound is a reaction. A sequence of such reactions is a **metabolic pathway**.

PART 1: Cellular Respiration

Carbohydrates are temporary energy stores. The process by which energy stored in carbohydrates is released to the cell is **respiration**. Both autotrophs and heterotrophs undergo respiration. Photoautotrophs, such as plants, use respiration to break down their carbohydrates (which they produced themselves by **photosynthesis**) to use the energy to build new cells and maintain cellular machinery. Heterotrophic organisms also break down carbohydrates to fuel these processes, but they obtain materials for respiration in two ways: by digesting plant material or by digesting the tissues of animals that have previously digested plants.

For aerobic respiration, the general equation is:

$$C_6H_{12}O_6 + 6\,O_2 \longrightarrow 6\,CO_2 + 6\,H_2O + \text{“available energy”}$$

Metabolic rate refers to metabolism energy per unit of time, or the rate at which an organism burns calories to maintain itself. The metabolic rate of an animal is affected by the external temperature, diet, age, time of day and year, level of activity and many other factors.

The body temperature of **ectotherms** (e.g. amphibians, reptiles, and insects) is largely determined by the temperature of their surroundings. Because of this, the metabolic rate of an ectotherm is closely linked to the external temperature. This is largely due to the fact that aerobic respiration, particularly the reactions of glycolysis and the Krebs cycle, are catalyzed by enzymes that are extremely sensitive to small changes in temperature. In fact, for ectotherms the relationship between metabolic rate and external temperature can be almost linear within a wide range of temperatures.

Most ectotherms control their body temperatures through behavioral adaptations. They actively select the most appropriate environment. High temperatures can be rapidly fatal for these organisms because critical enzymes of the respiratory pathway denature and cease to function. Low temperatures might reduce the metabolic rate so much that the animal's ability to find food, capture prey, or escape predators would be severely compromised. For these reasons, ectotherms tend to select temperatures close to 40^{o}C, where cellular respiration tends to be more efficient.

It is important to note that **endotherms** (mammals, birds) do not have the same response to temperature in terms of metabolic rate as ectotherms. The metabolic rate of endotherms is constant for a range of temperatures and increases at cold and high temperatures. These increases at the extreme temperatures are due to increased metabolic rate needed to trigger mechanisms that may help control the temperature (shivering, sweating).

Activity 9-1: The Effect of Exercise on the Rate of CO_2 production in humans

To examine the effects of "level of activity" on cellular respiration (and metabolic rate by extension), we'll use humans as a test subject to measure rate of CO_2 production before and immediately following exercise.

In this experiment, we'll use a pH indicator to help us measure CO_2 production. As expired air is bubbled through an alkaline (basic, pH >7) solution, the carbon dioxide in your breath will dissolve in the solution and produce **carbonic acid**, changing the pH of the solution from alkaline towards neutral (and then acidic if you continue breathing out).

The pH indicator Phenolphthalein is purple at alkaline pH levels (pH >7), but loses its color in neutral and acidic pH levels. Thus, if we exhale into an alkaline solution containing phenolphthalein, it should change color from purple to colorless.

The time required for this color change to occur is dependent upon the amount of carbon dioxide expired. Higher levels of CO_2 in each breath will cause the solution to become colorless more rapidly.

1. Prepare two 250 mL Erlenmeyer flasks as directed here:
   A. Measure out 100 mL of 0.01% NaOH solution in a graduated cylinder.
   B. Pour all 100 mL into one of the Erlenmeyer flasks.
   C. Directly into this solution, add 2 drops of phenolphthalein and mix by swirling for about 30 seconds (the solution should be an even shade of pinkish purple).
   D. Pour 50 mL of this purple solution into the graduated cylinder.
   E. Transfer the 50 mL from the graduated cylinder into the 2nd Erlenmeyer flask so that both flasks have exactly 50 mL of purple solution in them.

   NOTE: *Many flasks are different widths, so the 50 mL solutions may NOT be the same height!*

2. Designate one person in your group to be the test subject and another to be the official timer.
3. When directed to begin by the timer, the test subject should take a straw and blow continuously through the straw so that the exhaled air exits the straw directly over the purple liquid.
   IMPORTANT: Do NOT exhale into the straw for more than 5-10 seconds without pausing to take a new breath in! Do not talk during this breath, just inhale and return to blowing through the straw. While taking your breath, swirl the flask to ensure the solution is well-mixed. Keep track of time continuously (do not subtract the pauses to breathe and swirl – these are part of the controlled set up) until the solution in the flask loses its purple color.
4. Record the total time it took to go from starting purple to "colorless" for your group as Student #1 in Table 9-1 below.
5. When directed by the timer, the test subject should run in place (or up and down the hallway outside) for approximately 2 minutes. This exercise should increase your metabolic rate…
6. At the end of 2 minutes, repeat steps 3-4 with the second purple solution.
7. For the Student 2 column, obtain data on a 2nd student from a DIFFERENT TABLE in the lab room for comparison and record it below.

Table 9-1: Breathing exercise results

	Student 1: _____	Student 2: _____
Level of Activity	Time required (seconds) for solution to become colorless	Time required (seconds) for solution to become colorless
Resting/Inactive		
Post-Exercise/Active		

Questions

1. How does the time it took for the solution to become colorless correlate with the test subject's metabolic rate?

2. Did exercise increase metabolic rate? What does this have to do with ATP? With aerobic respiration rates? Why?

3. Did you notice any differences between the 2 test subjects? What about additional students in the class? Discuss your normal exercise and activity patterns – was there a correlation between how much a person's metabolic rate changed and individual differences such as how frequently they exercise? Record your notes here.

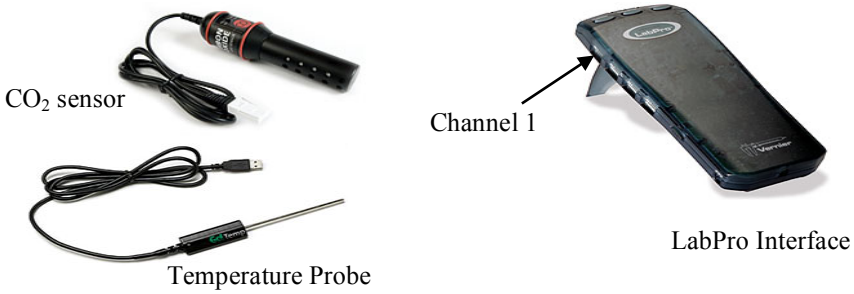
Activity 9-2: Measurement of Respiration in an Ectotherm

In this experiment we will be monitoring the change in the concentration of CO_2 in an animal chamber containing a type of lizard called "anole". [The *Anolis carolinensis* is commonly called 'the American chameleon' because of its capacity to change colors.] Since the anole respires, it is expected that the amount of CO_2 in the chamber will increase with time. A CO_2 sensor attached to the chamber will allow us to observe these changes in CO_2. Furthermore, by exposing the animal chamber to higher and lower temperatures, we will observe the effect of temperature on the production of CO_2 by the lizard.

IMPORTANT: Assign one group member to watch the temperature throughout the entire experiment. If the chamber temperature ever drops below 15˚C the chamber needs to be GENTLY moved away from the cold until it rises back above 16˚C. If the chamber temperature rises above 38˚C, the chamber needs to be GENTLY moved away from the heat until it drops back below 37˚C.

1. **Preparing the system:**

a. Fill an insulated ice bucket about ¼ full with ice and add water until it is slushy, NOT soupy.
b. Wedge the empty lizard chamber into the slush and let it sit for 5 minutes with the cover loosely in place.
c. Plug in and turn on a heating pad to high. Set it aside so it doesn't touch any cords or papers as it heats up.
d. Turn on the computer and monitor. Check that the CO_2 sensor is plugged into Channel 1 and the signal cable from the temperature probe is connected to Channel 2 of the LabPro Interface. Make sure the sensor switch on the CO_2 sensor is set to the low range (0-10,000 ppm)



e. Double click on the LoggerPro 3.8.2 icon on the monitor.
f. Wait until a graph of CO_2(ppm) vs. Time (s) appears.
g. Click on 'Experiment' again and scroll down to 'Data Collection'.
h. **Set the experiment length to 70 minutes and the sampling rate to 15 samples per minute.** Click on 'Done'.
i. Make sure that the sensor reads around 400 ppm at the start. If it's not, it may have to be recalibrated. Consult your instructor for this.

2. **Running the experiment**

Throughout the experiment, keep an eye on the lizard and record any changes in its behavior and appearance at the different temperatures.

a. Check the chamber temperature (on computer screen) and ensure that it is between 16˚C and 20˚C.
b. Very carefully grab a lizard (do not grab it by its tail!!) from the small cage and put it in the Vernier animal chamber. Make sure the chamber is tightly closed.

b. Wait 5 minutes for the lizard to recover from the transfer, and then click on Collect 

.

c. Allow the program to collect data for 4 minutes at COLD temperatures (16˚C to 19˚C). **Do NOT stop recording – KEEP COLLECTING DATA CONTINUOUSLY** as long as the lizard is in the chamber**.**

START time for COLD temperature rate = 0.0 minutes OBSERVATIONS:
STOP time for COLD temperature rate = 4.0 minutes

d. After 4 minutes with the chamber at the cold temperatures, GENTLY lift the chamber out of the slushy ice and place it onto a pile of 3-4 paper towels on the provided tray.
e. Continue to collect data and monitor the temperature as it rises towards the room's normal temperature.
f. When the temperature reaches 21˚C, mark the times below (add 4 minutes to the start time to determine when you will be finished and ready to move on to "warm") and allow the program to collect data for 6 minutes at MID temperatures (between 21˚C and 24˚C).

START time for MID temperature rate = _____ minutes OBSERVATIONS:
STOP time for MID temperature rate = _____ minutes

g. After 4 minutes with the chamber at the room temperature, GENTLY lift the chamber and place it onto the pre-warmed heating pad. Try to wrap the heating pad partway up the sides of the chamber to increase the internal heat transfer.
h. Continue to collect data and monitor the temperature as it rises.
i. When the temperature reaches 26˚C, mark the times below and allow the program to collect data for 4 minutes at WARM temperatures (between 26˚C and 29˚C).

START time for WARM temperature rate = _____ minutes OBSERVATIONS:
STOP time for WARM temperature rate = _____ minutes

j. After 4 minutes with the chamber at the warm temperatures, continue continue to collect data and monitor the temperature as it rises even more.
k. When the temperature reaches 31˚C, mark the times below and allow the program to collect data for 4 minutes at HOT temperatures (between 31˚C and 34˚C).

START time for HOT temperature rate = _____ minutes OBSERVATIONS:
STOP time for HOT temperature rate = _____ minutes

j. At the end of 4 minutes at the hot temperatures, click STOP to end the data collection, but keep the program open.
k. Carefully return the lizard to the professor's carrying chamber, then clean up the tray, discard the slushy ice, and unplug the heating pad (store it away from wires as it cools).

3. **Data analysis**

a. Click on "Linear Fit" 

on the top bar menu and select Run 1.
b. Click and drag the brackets over EACH of the 4 minute periods you marked above. First, for COLD temperature, drag the brackets over the range of 0.0 minutes to 4.0 minutes. This is the time on the CO_2 plot when the lizard was respiring at cold temperatures.

c. The slope of this area (m) represents the rate of CO_2 production of the lizard at cold temps. Record the slope and the median (middle) temperature during this time period in Table 9-2.

d. Now click and move the brackets over the 4 minute period for MID temperature (recorded in step f above). This is the time on the CO_2 plot when the lizard was respiring at MID temperatures.

e. The slope of this area (m) represents the rate of CO_2 production of the lizard at room temps. Record the slope and the median (middle) temperature during this time period in Table 9-2.

f. Repeat for the WARM and HOT temperature ranges and record those slopes in Table 9-2.

Table 9-2 Results of the Experiment with Lizard A (this is your group's data)

Condition	Median Chamber Temperature (˚C)	Respiration rate (CO_2 ppm/min)	Behavioral observations
COLD temperature			
MID temperature			
WARM temperature			
HOT temperature			

Table 9-3 Results of the Experiment with Lizard B (obtain data from a different lab group)

Condition	Median Chamber Temperature (˚C)	Respiration rate (CO_2 ppm/min)	Behavioral observations
COLD temperature			
MID temperature			
WARM temperature			
HOT temperature			

4. Interpretation and Conclusions

The **ectotherm** respiration experiment you performed today is your 3rd chance to practice graphing and analyzing your data (see the end of the lab for more instructions). You will also be writing a **complete, formal lab report over this experiment**. If you haven't already done so, make sure to download the lab report instructions from eCampus.

Question: If respiration slows down, production of the energy carrier molecule _____ also slows down. How does this affect the lizard's ability to move around, digest its food, and do all the other things that keep the lizard's cells alive?

PART 2: Photosynthesis (TIME PERMITTING)

Photosynthesis is the process by which light energy converts carbon dioxide and water to organic substances with subsequent release of elemental oxygen. It may well be the most important biological event sustaining life. Without it, most living things would starve, and atmospheric oxygen would become depleted to a level incapable of supporting animal life. Ultimately, the source of light energy is the sun, although on a small scale we can substitute artificial light.

The photosynthetic reaction is often conveniently summarized by the equation:

$$\text{light} + 6\,CO_2 + 6\,H_2O \xrightarrow{\text{chlorophyll}} C_6H_{12}O_6 + 6\,O_2$$

Only the light that is **absorbed** by chloroplasts can be used in photosynthesis. Organic compounds that absorb light of various wavelengths are called pigments. A chloroplast pigment extract has been prepared for you by soaking spinach leaves in cold acetone and ethanol. This extract contains the primary photosynthetic pigments that absorb light for photosynthesis, **chlorophylls *a* and *b***. Chlorophyll **"*a*"** has a distinct "blue-green" color, whereas chlorophyll "*b*" is "olive green". However, chlorophylls are not the only photosynthetic pigments; accessory pigments such as the **carotenes** (yellow-orange) also absorb light and transfer energy to chlorophyll "*a*". Your extract will probably show some yellow pigments. These are the **xanthophylls.**

In this activity, you will use **paper chromatography** to separate the pigments present in the lettuce leaf extract. Separation occurs due to the solubility of the pigment in the chromatography solvent and the affinity of the pigments for adsorption to the paper surface. When a solution of pigments is applied to strips of paper, the pigments adsorb onto the fibers of the paper. When the tip of the paper is immersed in a solvent, the solvent is adsorbed and moves up through the paper.

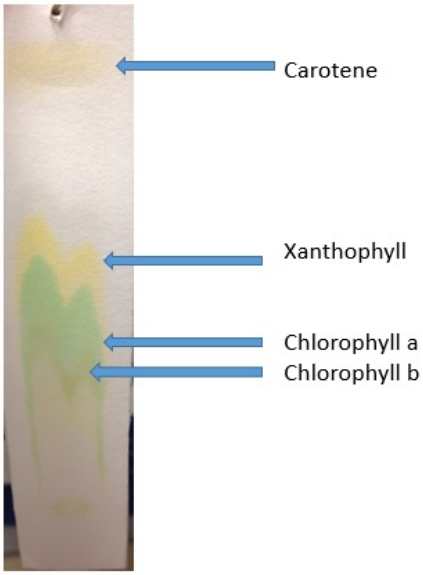
As the solvent moves through the spot of pigments, the pigments dissolve in the moving solvent. However, the pigments can't always keep up with the moving solvent – some pigments move almost as fast as the solvent, whereas others move more slowly. This differential movement of pigments results from each pigment's solubility and characteristic tendency to stick (i.e. be adsorbed) to the cellulose fibers of the paper. A pigment's molecular size, polarity, and solubility determine the strength of this tendency; pigments adsorbed strongly move slowly, whereas those adsorbed weakly move fastest. Thus, each pigment has a characteristic rate of movement, and the pigments can be separated from each other. The finished product, showing separated pigments, is called a chromatogram.

Activity 9-3: Paper Chromatography of Photosynthetic Pigments

1. Obtain a strip of chromatography paper for your lab instructor. Handle the paper by its edges so that oil on your fingers does not contaminate the paper.
2. Use a pencil to mark a line across the paper approximately 2 cm from the tip of the paper. Now mix the leaf extract gently and use a capillary tube to apply **ONE SMALL SPOT** of leaf extract over the pencil mark. Allow the stripe to dry and repeat this application at least 20 times. For this separation to work, you must start with an extremely concentrated application of extract on the paper. Also, the smaller the spot, the better the separation.

3. Place the chromatography strip in a test tube containing 2 mL of chromatography solvent (9 parts petroleum ether **:** 1 part acetone). Position the chromatography strip so that the tip of the strip (but not the stripe of the plant extract) is submerged in the solvent. This should be done by hooking the strip of paper to the hook in the cork (tube's stopper).
4. Place the tube in the test tube rack and watch as the solvent moves up the paper. Keep the tube capped and undisturbed during solvent movement.
5. Remove the chromatography strip just before the solvent front reaches the top of the strip.

PHOTOSYNTHETIC PIGMENTS: ABSORPTION SPECTRUM



The particular wavelengths of light that are absorbed by a substance form a pattern called its **absorption spectrum**. The spectrum is determined by illuminating a solution of the substance with each wavelength of light in turn and measuring the absorption in each case. Using visible light, you will determine the visible spectrum for a mixture of pigments extracted from chloroplasts. The spectrum can then be plotted as a graph of absorbance versus the wavelength or color of light used.

Activity 9-4: Absorption Spectrum of chlorophyll a

1. Compare your chromatogram with the one shown to the right.
2. Cut the section of your paper chromatogram containing *chlorophyll a*. Avoid getting any of the other pigments as you cut.
3. Cut the 'chl *a*' band into smaller pieces and place these in a small plastic cup. Add 2 mL of ethanol and wait about 5 minutes for the solvent to extract the pigment from the paper.
4. Calibrate the spectrophotometer as you did for the enzyme lab:
   a. Make sure that the USB cable is connected to the computer.
   b. Double click on the LoggerPro 3.8.2 icon on the computer desktop.
   c. Fill a cuvette (about ¾ full) with the blank solution (ethanol).
   d. Insert the cuvette in the sample chamber, making sure that the clear side of the cuvette is in the path of light.
   e. On the top menu, click on Experiment. Scroll down to "Calibrate" and select "Spectrometer:1"
   f. Wait 60 seconds for the lamp to warm up.
   g. Click on "Finish Calibration" and on "OK". The spectrophotometer is now calibrated.
5. Set the machine for data capture:
   a. On the top bar, find the "Configure Spectrophotometer"  icon.
   b. Select "Absorbance vs. Wavelength" as the Collection Mode.
   c. Click OK. A graph should appear with wavelength(nm) on the x-axis and absorbance on the y-axis.
6. Fill a cuvette about ¾ full with the solution containing the *chlorophyll a* pigment.
7. Click "Collect"  and wait a few seconds, and then click "STOP" 
8. The full chromatogram of *chlorophyll a* should be displayed. Click on Autoformat if necessary. 

9. Show your absorption spectrum of *chlorophyll a* to the instructor, then answer the questions below while discussing the spectrum with your group.

Questions:

1. What colors of light are MOST absorbed by *chlorophyll a* ? _____
2. What colors of light are LEAST absorbed by *chlorophyll a* ? _____
3. How does the absorption spectrum relate to the colors you SEE when you look at a leaf or the *chlorophyll a* solution?

4. Based on the absorption spectrum, do you think green light wavelengths are the best colors to help a plant do photosynthesis? Why or why not?

References for photos and equipment

1. Hunt, Stephen; Qubit Systems Inc., Kingston, *Ontario Instructor's Manual* (2000) and personal communications (Summer 2001).
2. Vernier Biology Laboratories

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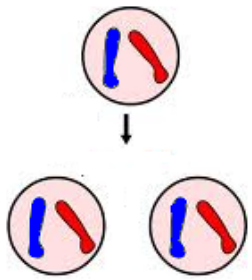
Lab 10: Cell Cycle and Mitosis



Objectives

- Know the phases of the cell cycle and the events that occur in each
- Understand differences between mitosis in animal and plant cells
- Identify cells in the various steps of mitosis

Pre-Lab Reminder: *Before arriving to this scheduled lab, read the entire lab (background, procedure, and questions), and use that information to complete the pre-lab in eCampus.*

Background Information

The complex series of events that encompasses the life span of an actively dividing cell is referred to as the **cell cycle**. Each cell divides to give rise to two entirely new individuals, and it is remarkable that each of us began life as one cell and developed into such a complex animal, the human. Our first cell has all the hereditary information that we will ever need or get.

In higher plants and animals, **fertilization**, the fusion of egg and sperm nuclei, produces a single-celled **zygote**. The zygote divides into two cells, these two into four, and so on to produce a multicellular organism. During cell division, each new cell receives a complete set of hereditary information and an assortment of cytoplasmic components.

Recall from Lab 7 (Cells) that there are two basic cell types, prokaryotic and eukaryotic. The genetic material of both consists of DNA (deoxyribonucleic acid). In prokaryotes, the DNA molecule is organized into a single circular chromosome. Prior to cell division, the chromosome duplicates. Then the cell undergoes fission, the splitting of a preexisting cell into two, with each new cell receiving a full complement of the genetic material.

In eukaryotes, the process of cell division is more complex, primarily because of the much more complex nature of the hereditary material. Here the chromosomes consist of DNA and proteins complexed together within the nucleus. Cell division is preceded by duplication of the chromosomes and usually involves two processes: **mitosis** (nuclear division) and **cytokinesis** (cytoplasmic division). Mitosis produces two nuclei, each containing identical chromosomes, whereas cytokinesis ensures that each new cell contains all the metabolic machinery necessary for sustenance of life such as ribosomes, free enzymes, and organelles.

In today's lab we will examine cell division in eukaryotic cells only.

Dividing cells pass through a regular sequence of events called the cell cycle (Figure 10-1). The cycle begins from the time a cell is formed by division and ends when it, too, divides. The cell cycle consists of two primary phases – the M (mitosis) phase during which the nucleus and cell are actively dividing, and **interphase**. Notice that the majority of the time is spent in interphase and that actual nuclear division – mitosis – is but a brief period of the cycle.

Interphase is comprised of three parts: the **G1**, **S** and **G2** periods. During the G1 period cytoplasmic growth takes place and organelles duplicate. During the S phase, the DNA is duplicated. During the G2 period, structures directly involved in mitosis, such as spindle fibers, are synthesized.

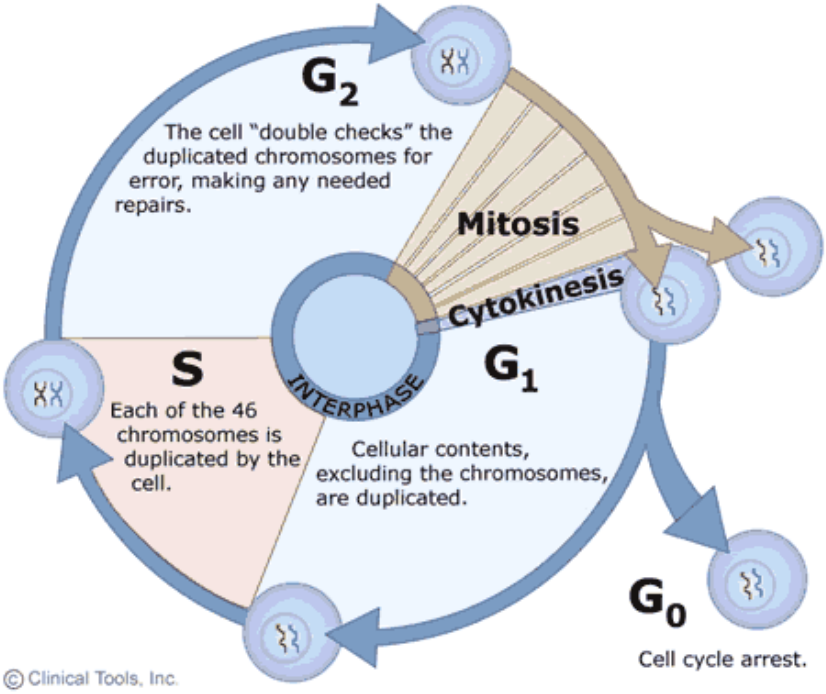


Fig. 10-1 The stages of the cell cycle: Interphase (G1, S and G2), Mitosis and Cytokinesis

The G1 period is the time from the beginning of a new cell to when it begins to replicate its DNA. The doubling of DNA during the S phase provides a full complement of DNA for the daughter cells that will result from the next mitotic division. The G2 period is a second period of growth and lasts from the end of DNA synthesis until the next cell division.

Unfortunately, because of the apparent relative inactivity that early biologists observed, interphase was given the misnomer "resting stage". In fact, we now know that interphase is anything but a resting period. The cell is producing new DNA, assembling proteins from amino acids, and synthesizing or breaking down carbohydrates. In short, interphase is a very busy time in the life of the cell. The G1 and G2 periods comprise the vast majority of a cell's life primarily because they are the time when the cell is accomplishing all these tasks of life.

Cell Division in Plant Cells

Nuclear and cell divisions in plants are, for the most part, localized in specialized regions called **meristems**. Meristems are regions of active growth. A meristem contains cells that have the capability to divide repeatedly.

Plants have two types of meristems: apical and lateral. Apical meristems are found at the tips of plant organs (stems, roots) and increase in length. Lateral meristems, located beneath the bark of woody plants, increase girth. In this section, you will examine structures related to the cell cycle in the apical meristem of onion roots.

Activity 10-1: Mitosis in an Onion Root Tip

1. Obtain a slide of a longitudinal section of an *Allium* (onion) root tip. This slide has been prepared from the end of an actively growing root. It was "fixed" by chemicals to preserve the cellular structure and stained with dyes that have an affinity for the structures involved in nuclear division. There are three sections of an onion root tip on this slide. You may want to search all three sections or focus your study on one.

2. First, focus with the low-power objective of your compound microscope to get an overall impression of the root morphology.

3. Concentrate your study in the region about 1-mm behind the actual root tip. This region is the apical meristem of the root. Keep in mind that the cell cycle is a continuous cycle; however we separate events into different stages as a convenient way to study cell division, but with living organisms it is often difficult to say definitely when one phase begins and another ends.

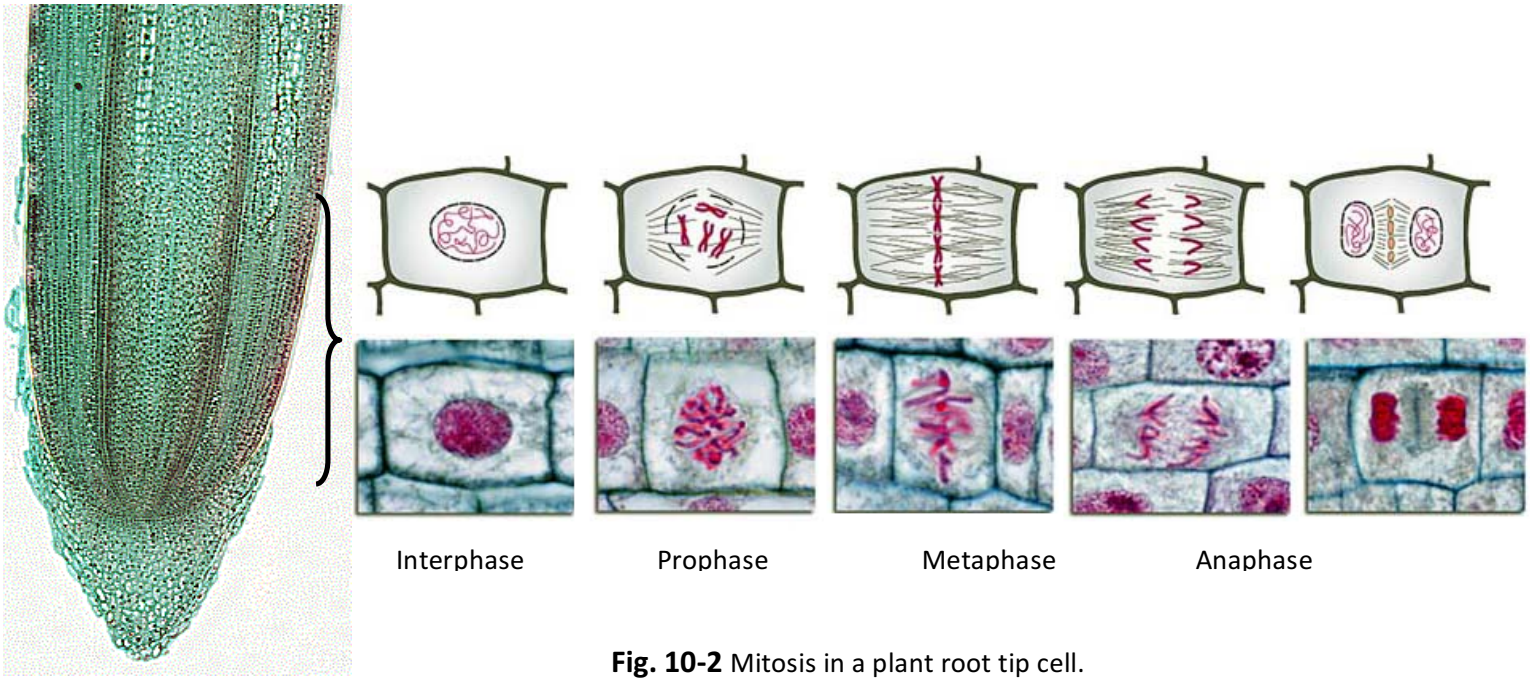


Fig. 10-2 Mitosis in a plant root tip cell.

Interphase

- Use the 10x objective to scan the apical meristem. Note that most of the nuclei are in interphase: each nucleus has a granular appearance and is colored throughout with no chromosomes visible.
- Switch to the 40x objective, focusing on a single interphase cell. Note the distinct nucleus, with one or more nucleoli, and the chromatin dispersed within the bounds of the nuclear envelope.

Mitosis in Onion Cells

Prophase

During prophase the chromatin condenses, rendering the duplicated chromosomes visible as threadlike structures. At the same time, microtubules outside the nucleus are beginning to assemble

into spindle fibers. Collectively, the spindle fibers make up the spindle, a three-dimensional structure widest in the middle and tapering to a point at the two poles (opposite ends of the cell). The spindle is not visible during prophase.

- Using Fig. 10-2 as reference, find a nucleus in prophase.

The transition from prophase to metaphase is marked by the fragmentation and disappearance of the nuclear envelope. At about the same time, the nucleoli disappear. Scientists now agree that an intermediate stage, **prometaphase** is identifiable between prophase and metaphase, in which the nuclear envelope is completely dissolved and the chromosomes are beginning to form attachments to the spindle, but are not yet lined up at the mid-plate.

Metaphase

When the nuclear envelope is no longer distinct, the cell is in metaphase. Identify a metaphase cell by locating a cell with the duplicated chromosomes, each consisting of two sister chromatids, lined up midway between the two poles. This imaginary midline is called the spindle equator. The spindle has moved into the space the nucleus once occupied. The microtubules have become attached to the chromosomes at the kinetochores, groups of proteins that form the outer faces of the centromeres.

- Using Fig. 10-2 as reference, find a cell in metaphase.

Anaphase

During anaphase sister chromatids of each chromosome separate, each chromatid moving toward an opposite pole.

- Find an <u>early</u> anaphase cell, recognizable by the slightly separated chromatids.
- Notice that the chromatids begin separating at the centromere. The last point of contact before separation is complete is at the ends of the "arms" of each chromatid, which straggle behind like spaghetti noodles facing the mid-plate as they are pulled toward the poles from their centromeres.

Although incompletely understood, the mechanics of chromatic separation is based on action of the spindle-fiber microtubules. **Once separated, each chromatid is referred to as an individual daughter chromosome.** Note that now the chromosome consists of a single chromatid.

- Using Fig. 10-2 as reference, find a cell in <u>late</u> anaphase.

Telophase

When the daughter chromosomes arrive at opposite poles, the cell is in telophase. The spindle disorganizes. The chromosomes expand again into chromatin form, and a nuclear envelope re-forms around each newly formed daughter nucleus.

- Using Fig. 10-2 as reference, find a cell in telophase.

<u>Cytokinesis in Onion Cells</u>

Cytokinesis, division of the cytoplasm, usually follows mitosis. In fact, it often overlaps with telophase. Find a cell undergoing cytokinesis in the onion root tip. In plants, cytokinesis takes place

by **cell plate** formation. During this process, Golgi body-derived vesicles migrate to the spindle equator, where they fuse. Their contents contribute to the formation of a new cell wall, and their membranes make up the new plasma membranes. In most plants, cell plate formation starts in the middle of the cell.

- Observe the prepared slide of the onion root tip and find a cell undergoing cytokinesis. With your light microscope, the developing cell plate appears as a line running horizontally between the two newly formed nuclei.

Activity 10-2: Duration of the Phases of the Cell Cycle

An onion root tip preparation can be thought of as a snapshot capturing cells in various phases of the cell cycle at a particular moment in time. The frequency of occurrence of a cell cycle phase is directly proportional to the length of a phase. You can therefore estimate the amount of time each phase takes by tallying the proportions of cells in each phase.

The length of the cell cycle for cells in actively dividing onion root tips is approximately 24 hours, with mitosis lasting for about 90 minutes. ***Note**: These are cells in the actively dividing region of a plant, and they still spend over 20 hours of each day in interphase!*

1. Use the software program from *The Biology Project* (www.biology.arizona.edu) to determine the type and number of cells in each of the stages of mitosis plus interphase.
2. Once you complete the program, copy your tally results on Table 10-1.
3. Calculate the time spent in each stage based on a 24 hour cell cycle by dividing the number of cells in each stage by the total number of cells counted. This gives you the percent of total cells in each stage.
4. Multiply the fraction obtained in step 2 by 24 to determine duration of each in a 24-hr cycle.

Although these calculations are only a rough approximation of the time spent in each stage, they do illustrate the differences in duration of each stage in the cell cycle.

Table 10-1 Time duration of interphase and mitotic phases in onion root cells

Phase	Number of Cells	Duration of Phase (hrs)
Interphase	_____	_____
Prophase	_____	_____
Metaphase	_____	_____
Anaphase	_____	_____
Telophase	_____	_____
Total Counted	_____	

The Cell Cycle in Animal Cells

Animals do not have specialized regions to which cell division is limited, whereas plants have meristems where divisions may take place continually. Indeed, divisions occur continually throughout many tissues of an animal's body, replacing worn-out or damaged cells. However, there are some tissues that divide more rapidly than others.

Fertilization of an ovum by a sperm produces a zygote. In animal cells, the zygote undergoes a special type of cell division, **cleavage**, in which no increase in cytoplasm occurs between divisions. A ball of cells called a blastula is produced by cleavage. Within the blastula, repeated nuclear and cytoplasmic divisions take place. Cleavage is a time of extremely rapid cell division (compared to other developmental stages), with much shorter than normal interphase periods because the cells do not need to wait for growth or extensive metabolic activities between cell divisions.

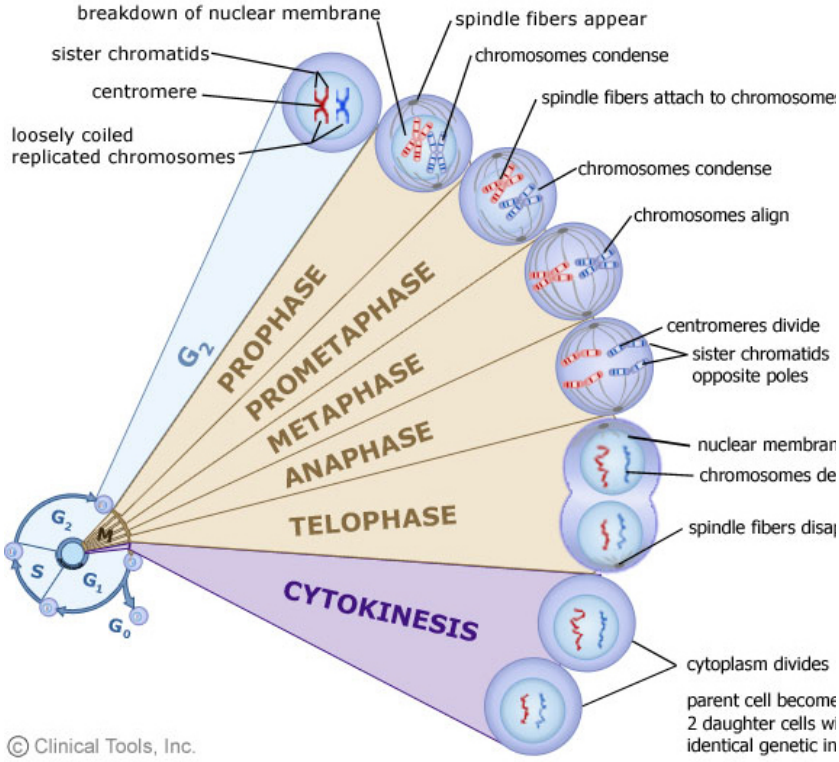


Fig. 10-3 Mitosis in an animal cell.

Activity 10-3: Mitosis in Animal Cells: Whitefish Blastula

With several important exceptions, mitosis in animals is remarkably like that in plants.

1. Obtain a slide labeled "whitefish blastula".
2. Scan it with the low-power objective and then at medium power. This slide has numerous thin sections of a blastula. Select one section and then switch to the high-power objective for detailed observation.
3. Locate a cell in interphase. As you observed in the onion root tip, note the presence of the nucleus containing DNA as chromatin. Note also the absence of a cell wall.

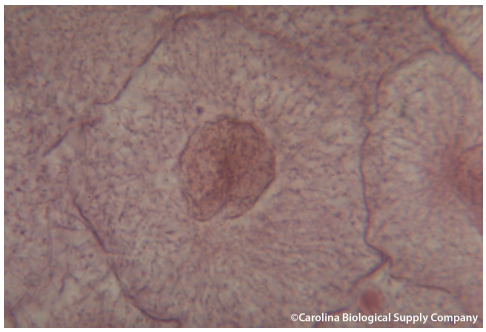


Fig. 10-4 An animal cell in Interphase.

Mitosis in Fish Cells

Prophase

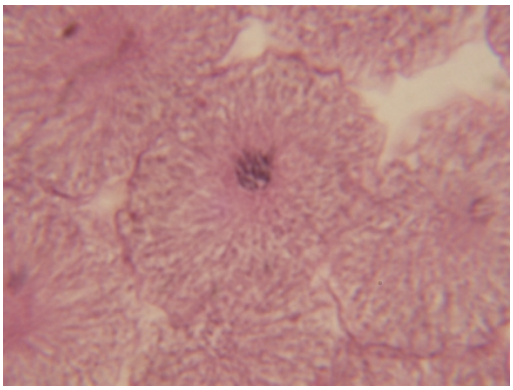


Fig. 10-5 An animal cell in prophase.

The first obvious difference between mitosis in plants and animals is found in prophase. Unlike the onion cells, those of whitefish contain centrioles. As seen with the electron microscope, centrioles are barrel-shaped structures consisting of nine radially arranged triplets of microtubules.

One pair of centrioles is present in the cytoplasm in the G1 stage of interphase. Centrioles start to duplicate during the S phase of interphase and complete their duplication process upon mitotic entry.

During prophase, the chromosomes become visible as the chromatin condenses. One new and one old centriole migrates to each pole. Although centrioles are too small to be resolved with your light microscope, you can see a starburst pattern of spindle fibers that appear to radiate from the centrioles. Other microtubules extend between the centrioles, forming the spindle as the cells move into **prometaphase**.

- Find a prophase cell and identify the spindle and starburst cluster of fibers around the centriole.

Metaphase

As was the case in plant cells, during metaphase the spindle fiber microtubules become attached to the kinetochore of each centromere region, and the duplicated chromosomes (each consisting of two sister chromatids) line up on the spindle equator.

- Locate a metaphase cell.

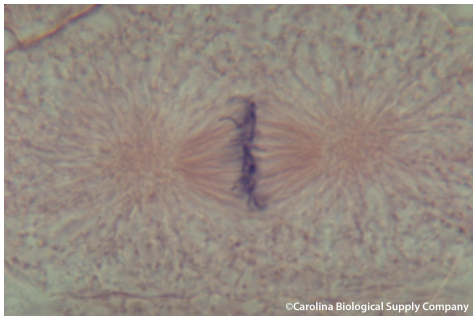


Fig. 10-6 An animal cell in Metaphase

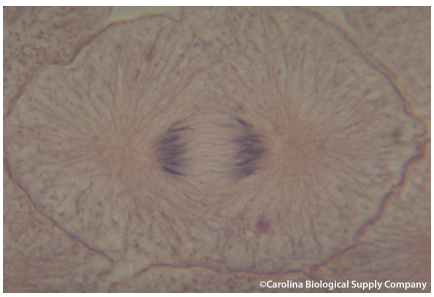


Fig. 10-7 An animal cell in Anaphase

Anaphase

Again, similar to that observed in plant cells, anaphase begins with the separation of sister chromatids into individual (daughter chromosomes.

- Observe a blastula cell in anaphase.

Telophase

Telophase is characterized by the arrival of the individual (daughter) chromosomes at the poles. A nuclear envelope forms around each daughter nucleus. Find a telophase cell. Is the spindle still visible? Is there any evidence of a nuclear envelope forming around the chromosomes?

- Find a cell in telophase.

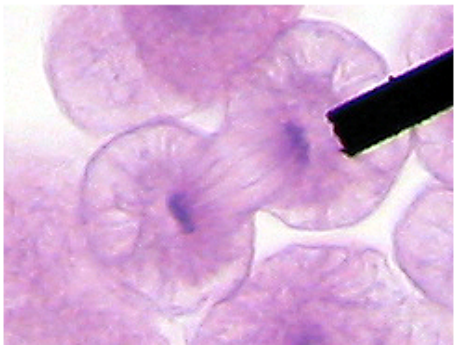


Fig. 10-8 An animal cell in Telophase.

Cytokinesis in Animal Cells

A second major distinction between cell division in plants and animals occurs during cytoplasmic division. Cell plates are absent in animal cells. Instead, cytokinesis takes place by furrowing.

To visualize how furrowing takes place, imagine wrapping a string around a balloon and slowly tightening the string until the balloon has been pinched in two. In life, the animal cell is pinched in two, forming two discrete cytoplasmic (cell) entities, each with a single nucleus.

- Find a cell in the whitefish blastula undergoing cytokinesis.
- The telophase cell that you found in the telophase section may also show an early stage of cytokinesis.

References

1. DNA replication kit: *Carolina Biological Supply Company*, 2700 York Road, Burlington, North Carolina
2. Perry et. al*.: Laboratory Manual for Starr and Taggart's Biology: The Unity and Diversity of Life*, 2002, Brooks/Cole
3. Skavaril, R., Finnen, M., Lawton, S., *General Biology Lab Manual, Investigations into Life's Phenomena*, 1993, Harcourt College Publishers
4. Stern, K., Jansly, S., Bidlack, J., Student Art Notebook for Introductory Biology, 9th edition, McGraw-Hill, 2003
5. Vidwans, Smruti J., Wong, Mei Lie, and O'Farrell, Patrick H., *The Journal of Cell Biology*, Volume 147, Number 7, December 27, 1999, pages 1371-1378
6. http://www.biology.arizona.edu/cell_bio/activities/cell_cycle/cell_cycle.html
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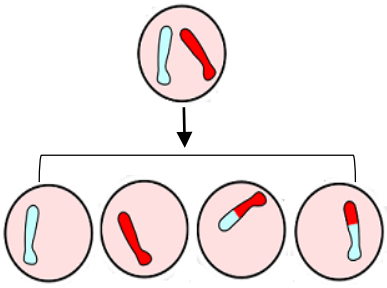
Lab 11: Meiosis

Preparing for Sexual Reproduction



Objectives

- Understand the function of meiosis
- Understand the difference between mitosis and meiosis
- Recall the key steps associated with the process of meiosis
- Understand how meiosis increases genetic diversity

Pre-Lab Reminder: *Before arriving to this scheduled lab, read the entire lab (background, procedure, and questions), and use that information to complete the pre-lab in eCampus.*

Background Information

The life cycle of most eukaryotic cells consists of a long interphase and a short period of mitosis. Interphase is the time when a cell performs its given function, i.e. specialized cells in a tissue making certain macromolecules (proteins, hormones, etc.). It is also during interphase that DNA replication takes place during S Phase. S phase is in the middle of interphase, between G1 ("Gap"/Growth 1) and G2 ("Gap"/Growth 2).

Mitosis is the process of nuclear division, when the chromosomes, which were replicated during S phase and now have sister chromatids, are distributed to the future nuclei of the daughter cells in order to produce two cells identical to the mother cell. After mitosis, cytokinesis results in cytoplasmic division, and the daughter cells split apart from each other. Mitosis is the mechanism cells undergo to reproduce, and thus allow the body to grow, (e.g. during the production of hair, during wound healing). It is well worth your while to quickly review mitosis in your textbook.

Meiosis

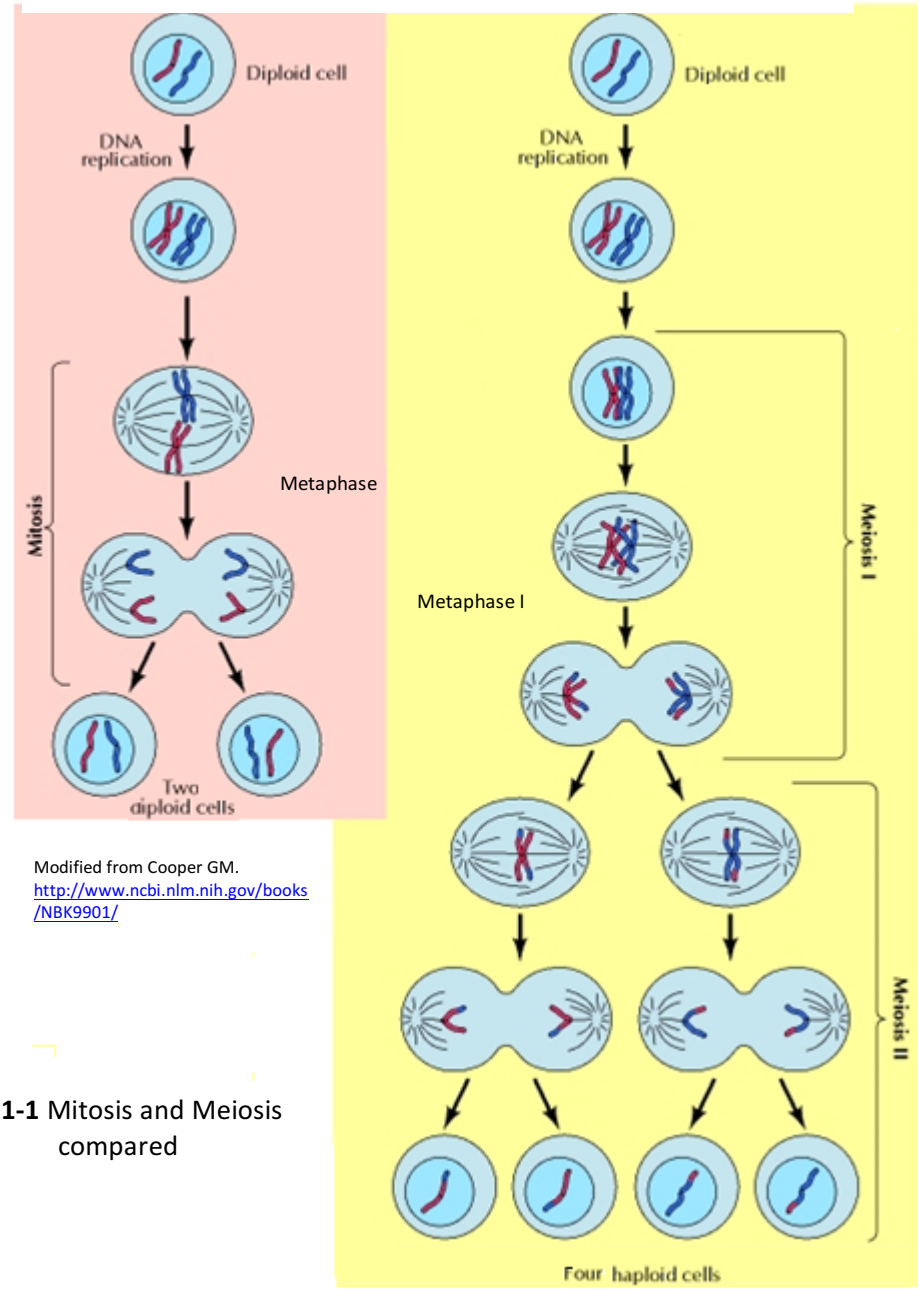
Like mitosis, meiosis is a process of nuclear division. The crucial difference is that meiosis results in a ***haploid*** cell in which there is only one copy of each chromosome (1n). In mammals, as an example, it is the process that produces reproductive cells, i.e. gametes. That is, there is a reduction in the ***ploidy*** of the cells from 2n (46 chromosomes in humans) to 1n (23 chromosomes in humans). On the other hand, mitosis can occur in either haploid or diploid cells. An example of mitosis in haploid cells is the male honeybee (drone bee) which develops from a haploid unfertilized egg.

Think about this: diploid organisms result from the fusion of gametes from their parents. Therefore, the gametes must be ***haploid (n)*** or the number of chromosomes in the children would be twice as many as in their parents with *every* new generation!

In animals the gametes are the result of meiosis, which accomplishes a reduction in the number of chromosomes and produces four haploid cells from one diploid progenitor. This requires not one but two cell divisions, which we call **meiosis I** and **meiosis II**. Plants, fungi, and many protists (e.g. algae) produce their gametes differently than animals.

In animals, meiosis is necessary ***only*** for the formation of gametes. For this reason meiosis only happens in the testes, where sperm cells are produced, and the ovaries, where egg cells (also known as ova, or the singular form ovum) are made.

Comparison between Mitosis and Meiosis



Modified from Cooper GM. http://www.ncbi.nlm.nih.gov/books/NBK9901/

Fig. 11-1 Mitosis and Meiosis compared

Activity 11-1: Building a diploid nucleus.

Chromosomes contain genes, which specify traits such as color, size, number, or presence of certain structures. Different individuals may have different versions of the genes. For example, people have genes for eye color, but some people have a version, or **allele**, of the gene that results in brown eyes, while others have an allele that produces blue eyes. In all people, however, the gene for eye color is in the same location of the chromosome. Because of this, diploid individuals will have, on the homologous chromosomes, either two copies of the same allele or one copy of each of two alleles.

Working in groups of four, you will assemble the chromosomes of a diploid cell using pop beads:

1. Put together two strands of beads by attaching eight beads of the same color on each side of a magnetic centromere. Now, assemble two similar strands using beads of the second color.

2. Assemble two more strands of each color, as above, but using three beads on each side of the centromere.

At this point you should have four long strands, two of each color, and four short strands, two of each color. Each of these strands represents one molecule of DNA –one chromosome-with each bead representing one gene.

3. Now, gather the chromosomes of a diploid cell during the G1 stage of interphase: place **<u>one</u> long, red, <u>one</u> long yellow, <u>one</u> short red and <u>one</u> short yellow strand** in the middle of your workspace. *Note: each chromosome is a single molecule because the cell has not undergone S phase.*

The long chromosomes are ***homologous*** to each other, as are the short ones. Imagine that the red chromosomes come from the father and the yellow from the mother.

4. Simulate DNA replication (S phase) by placing the remaining strands next to their identical copies on your bench: attach identical strands by their magnetic centromeres.

Now the cell has duplicated chromosomes, each composed of two sister chromatids. The alleles on each pair of sister chromatids are identical.

Looking at your beaded chromosomes:

How many <u>chromosomes</u> are represented by the four short sister chromatids? _____

How many <u>chromosomes</u> are represented by all eight sister chromatids? _____

What is the ploidy of the cell you created in Step 3 (diploid or haploid)? _____

Why did the ploidy of the cell NOT change after DNA replication in Step 4?

Activity 11-2: Comparing Mitosis and Meiosis I

Study Fig. 11-1, focusing on the metaphase stage of mitosis and metaphase I of meiosis: In meiosis I, the homologous chromosomes are side by side at the metaphase plate. Why are they NOT side by side in mitosis?

In meiosis I, each centromere has a spindle fiber attached only to one side. Why is it important that each chromosome in metaphase I of meiosis only be attached to ONE spindle pole? What would happen if a chromosome were attached to both?

Look at what happened right after metaphase in both processes:

After metaphase of mitosis, what has been separated, homologous chromosomes or sister chromatids? _____

After metaphase I of meiosis, what has been separated, homologous chromosomes or sister chromatids? _____

Activity 11-3: Meiosis I Generates Diversity

Ignoring crossing over ***for now***, read the instructions and manipulate your chromosomes through the stages of meiosis I, drawing each stage in Figure 11-2 as directed (on the next page).

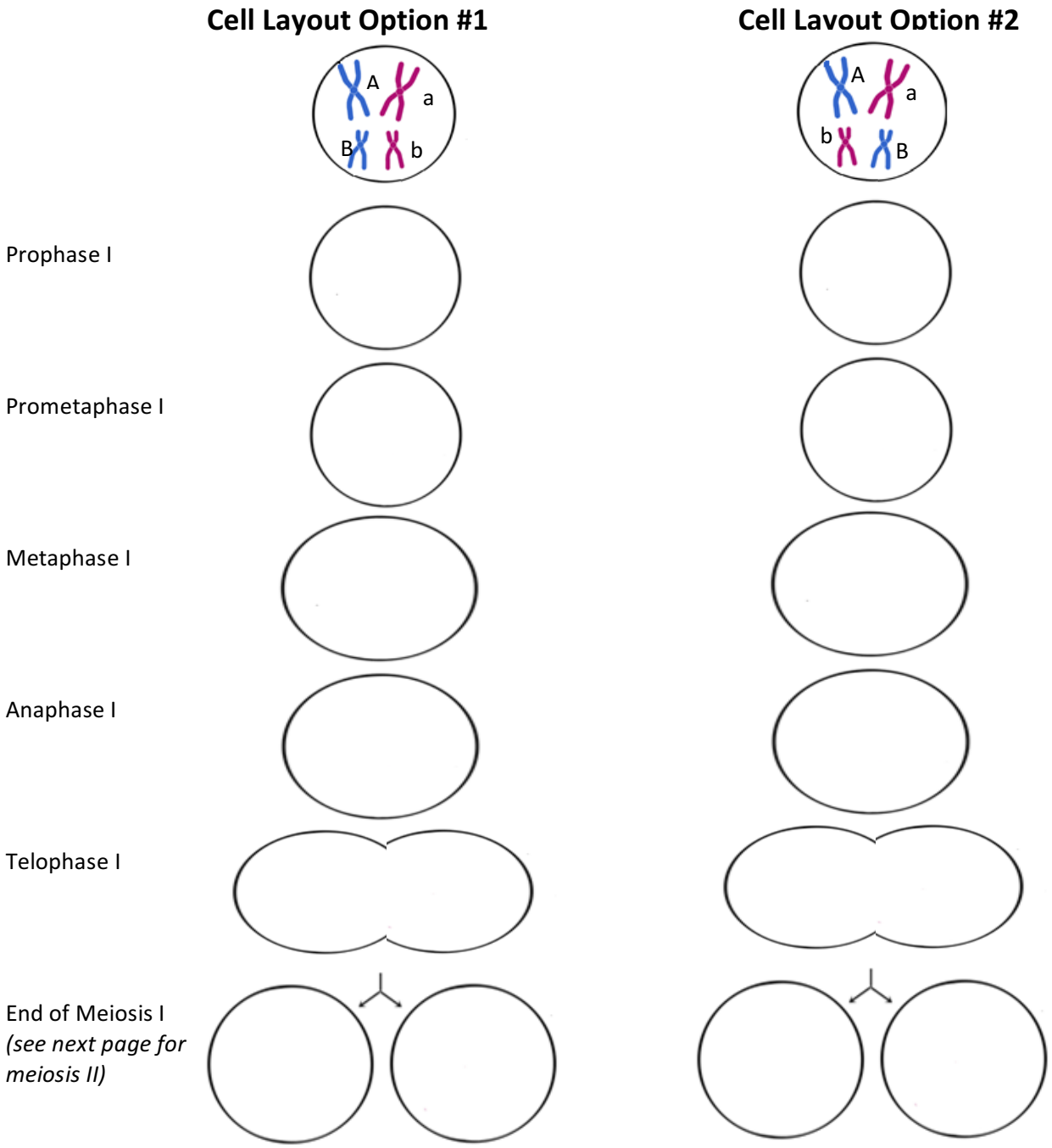
Notice the separated chromosomes remain composed of two sister chromatids each. This is very different from what happens in mitosis.

Imagine that on your homologous chromosomes, the red chromosomes from the father contained the allele "a" on the long chromosome and the allele "b" on the short one, while the yellow from the mother contained the allele "A" on the long chromosome and the allele "B" on the short one.

Figure 11-2 shows two views of the chromosomes (including the labeled alleles) as they would look in prophase I. Looking only at the LEFT column of cells for now, draw the chromosomes in each cell downward as they would appear in the meiosis I stage indicated. Maintain the position (A allele on left, B allele on left) that was provided in the top cell.

Next, looking at the RIGHT column of cells, draw the chromosomes again in each cell downward, this time maintaining the position of the chromosomes as provided in the top cell on the right (A allele still on left, but now the B allele is on the right).

Figure 11-2: *Following two homologous pairs Aa and Bb through Meiosis*



Questions

a) With 4 chromosomes in the starting cell, how many different types of cells could be produced at the end of Meiosis I (not considering crossing over yet)? _____

b) Is there any other way you could have arranged the chromosomes than the 2 layouts in Figure 11-2? _____ For example, could you have arranged for A and a to both go to the same cell by the end of telophase I? _____ What about B and b? _____

c) Are the daughter cells haploid or diploid? _____

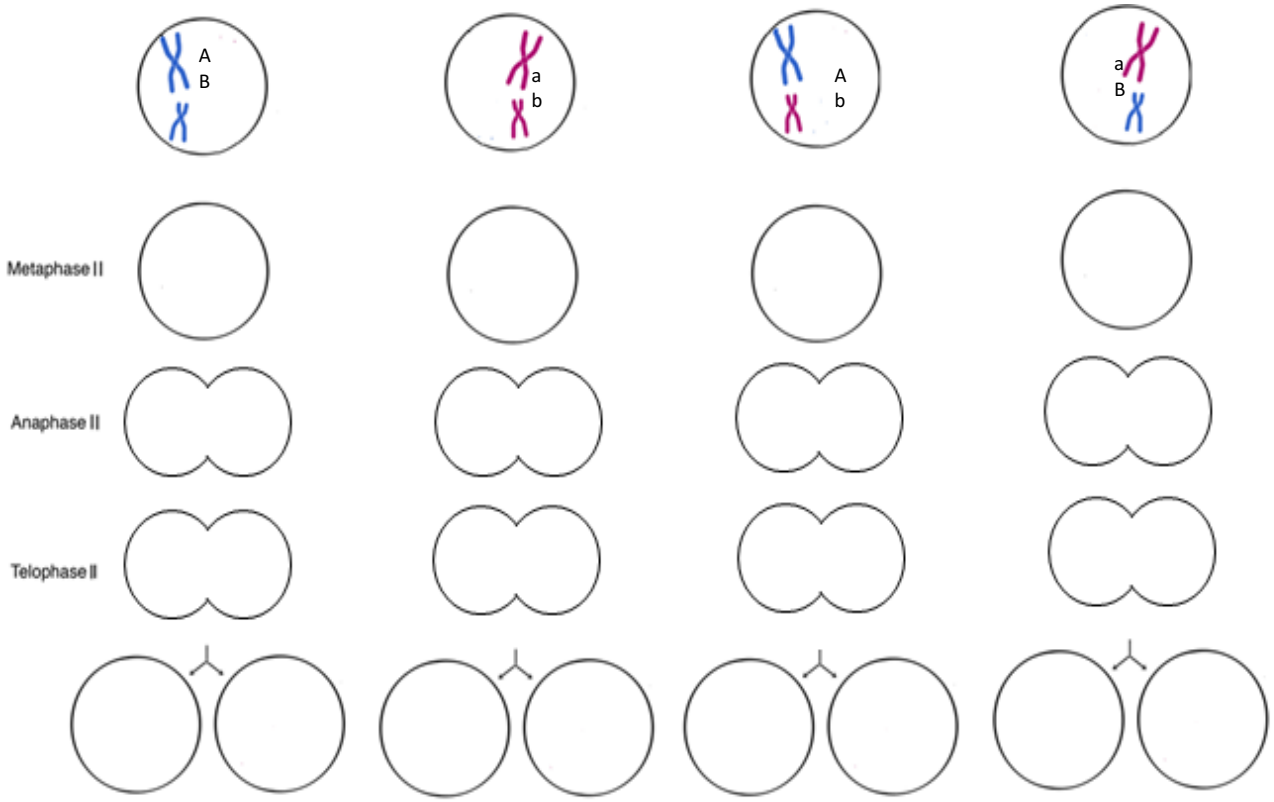
d) Do the daughter cells have the same set of gene loci (types of genes, such as one for hair color, one for muscle protein, etc.) as the original cell? _____ The same set of alleles (specific sequences, such as for brown hair vs. blonde at the hair color locus)? _____

e) Compare the genetic composition of the haploid cells produced by the 1st cell layout (left side) to those produced by the 2nd cell layout (right side). What is different and why?

Activity 11-4: Meiosis II is more similar to mitosis

Continuing to ignore crossing over ***for now***, manipulate your chromosomes through the stages of meiosis II, drawing each stage in Figure 11-3. Notice the similarities to the events of mitosis.

Figure 11-3: ***products of meiosis I in Layout 1*** ***products of meiosis I in Layout 2***



Questions

a) In Meiosis I, the _____ _____ separate, while in Meiosis II the

_____ _____ separate.

b) The cells produced at the end of meiosis I are phases are haploid / diploid *(circle one)*. The

cells at the end of meiosis II are haploid / diploid *(circle one)*.

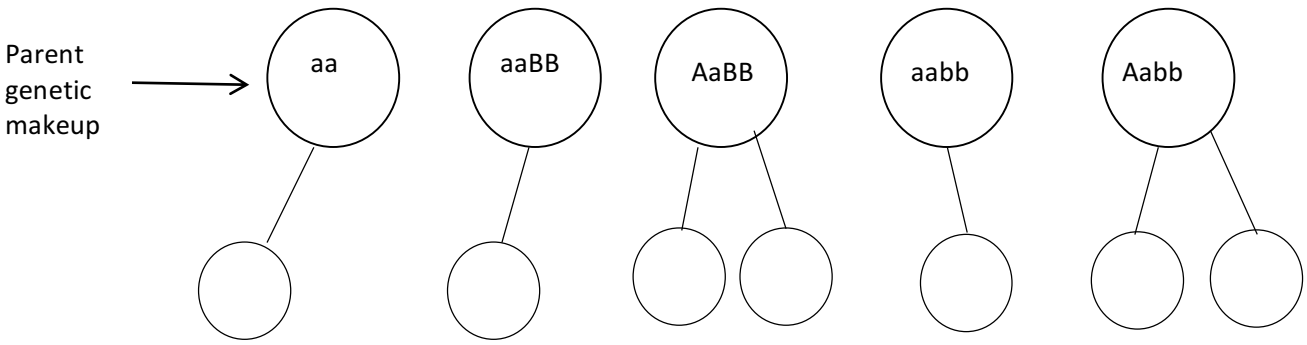
The number of different possible gametes in diploid organisms due to independent assortment of the chromosomes during meiosis can be calculated from the simple formula 2^n where n = the haploid number. For the 4 chromosomes we used, n = 2 (2 different type of chromosomes were present, with 2 copies of each in the diploid = 2n). Therefore, calculations suggest that we could produce $2^2 = 4$ different haploid cells at the end of meiosis II. Do your drawings agree with this calculation?

_____

For a human with 23 pairs of chromosomes (2n = 46), this number would be 2^{23} or 8,388,608 genetically different gamete cells !!

Activity 11-5: Predict the *possible* genetic makeup for haploid cells.

For each of the "parent" cells shown, predict the possible gametes that could be produced by meiosis from this parent (note: this involves independent assortment of chromosomes, which we'll cover in more detail in the next chapter and lab). To help you, the number of different gametes is already indicated by the number of different circles extending below each parent. For example, the parent shown with alleles "a" and "a" (written as "aa"), only one kind of gamete can be produced, so there is only one gamete circle extending below it.



BUT WAIT!! There's more!!! There is another way by which Meiosis generates diversity in the progeny!!

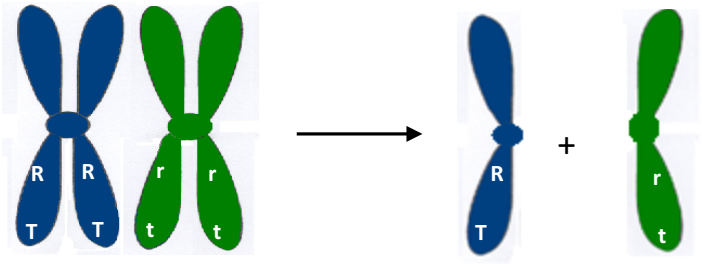
Activity 11-6: Prophase I and Crossing Over

In prophase I ***synapsis*** brings together the homologous pairs of chromosomes (observe Fig 11-1). Since each chromosome contains two sister chromatids, synapsis results in the formation of structures containing FOUR chromatids; these structures are called ***tetrads***.

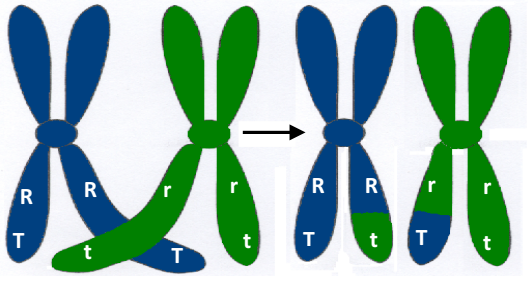
Re-set your colored chromosomes back to their prophase I state and form tetrads of each homologous pair; you should end up with two of them. During synapsis an important process shuffles the genes between homologous chromosomes. At random points the DNA of chromosomes go through a process termed crossing over at points called ***chiasma***. At these points, the DNA is cut on both homologues and segments of each chromosome switch from one homologue to the other. The DNA is then ligated in place. This is called ***recombination***, and can occur many times in every chromosome pair during prophase I.

The result is the production of new and unique combinations of alleles on a cell's chromosome. The resulting set of chromosomes is now different from the set inherited from the cell's parents.

Take a look at this simple example: Without crossing over, the independent assortment of the homologous chromosomes on the left would result in only **two** different possibilities in the gamete, R with T (RT) or r with t (rt).



Without crossing over, Rt and rT gametes would not be found because the R and T loci are on the same chromosome. But, if crossing over occurs (example shown below), these new combinations are possible:



How many different haploid cells would be produced now? _____

What is their genetic makeup? _____

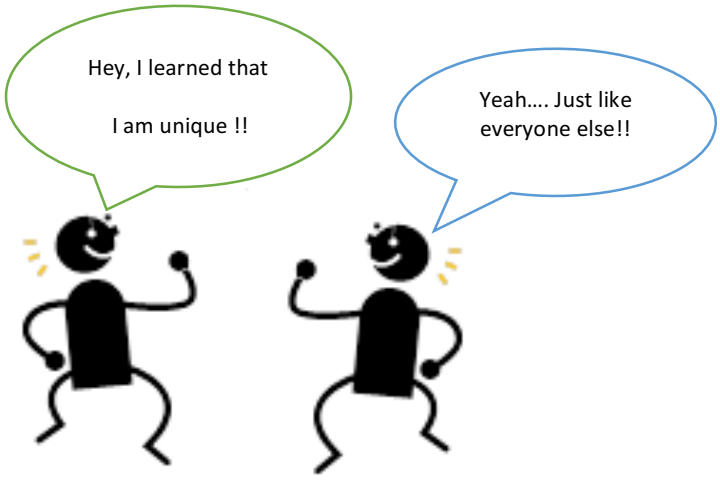
To visualize crossing over with your beaded chromosomes, break several beads (the same number) from the arms of two non-sister chromatids on the same tetrad and exchange their positions. You should now have chromatids with segments of different colors. Do this for both the long tetrad and the short tetrad.

Make sure you understand the terms synapsis, chiasma, and recombination.

Looking back at Activities 11-3 and 11-4, how does crossing over change the possibilities? In your drawings, you only considered one allele on each chromosome, so the effects of crossing over would not be seen in your list of possible alleles. In reality, though, every chromosome has thousands of genes, so the allele combinations that crossing over creates become almost infinite.

For example, if the number of different possible gametes in diploid organisms due to independent assortment of the chromosomes during meiosis can be calculated from the simple formula 2^n where n = the haploid number, a human male with 23 pairs of chromosomes (2n = 46) was able to produce 8,388,608 genetically different sperm cells without consider crossing over. Even if crossing over occurred only three times (over all 23 pairs) during meiosis, the number of possible sperm cells would be multiplied by 2^3, resulting in a total number of 2^{26} or 67,108,864 genetically different sperm cells!!

Then, to produce YOU, this highly unique (1 in over 67 million) sperm cell had to fuse with a highly unique (also 1 in over 67 million) egg cell. So…considering independent assortment of 23 pairs of chromosomes (n = 23) and 3 crossing overs during meiosis, the total number of different ways that human gametes can combine during **fertilization** exceeds four quadrillion $(2^{26})^2$ or 2^{52} !! This number of possible offspring from just ONE set of parents is greater than all the atoms in our solar system.! Indeed, you are UNIQUE!!



Of course, many of the alleles carried by humans are similar enough to the alleles every other human carries that we can't see any physical differences. That's why humans have all the same basic features and often only vary in a few key visible characteristics, so we aren't necessarily THAT different from our friends and neighbors even if our gene sequences are extremely unique…

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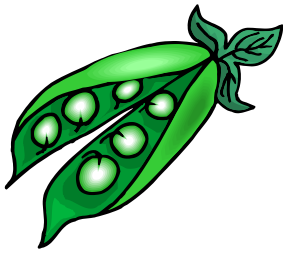
Lab 12: Mendelian Genetics

Working with Genetic Crosses



Objectives

- To become familiar with the language of Mendelian genetics.
- To become familiar with one- and two-trait crosses.
- To learn how to use Punnett squares to predict the probable outcome of crosses.
- To understand the concept of probability and its application in hybrid crosses.

Pre-Lab Reminder: *Before arriving to this scheduled lab, read the entire lab (background, procedure, and questions), and use that information to complete the pre-lab in eCampus.*

Background Information

PD-US

In 1866 Gregor Mendel, a Moravian monk living in the Austro-Hungarian Empire, published his results on crossing different varieties of the garden pea. He found that the appearance (**phenotype**) of the plants resulting from crosses depended on the genetic composition (**genotype**) of the parents, and that many traits seemed to be determined by only two possibilities: one inherited from the male parent and the other from the female parent. Although his discoveries remained obscure for many years, they are still the foundation for all we know about inheritance.

One-Trait Crosses

Garden pea plants have flowers with both male and female parts, so they can self-fertilize. This makes it easy to maintain varieties that are "**true-breeding**", meaning that for any number of generations all plants have the same phenotype as their parents. Mendel found that when he placed pollen (containing sperm) from a true-breeding white flower onto the pistil (containing egg) of a purple flower (and vice versa), all of the flowers in the next generation were purple. This type of genetic manipulation, where one true-breeding variety is "mixed" with another, is called a **parental cross**, and the resulting progeny are called **hybrids**. In his experiment Mendel found that the hybrids were all purple. This is why we say that when comparing white and purple, purple is **dominant** over white flower color.

Mendel obtained a more interesting result when he took the hybrid plants and either let them self-fertilize or crossed them to each other: in the progeny of this **hybrid cross**, ¾ of the plants were purple, but ¼ were white, even though both parents were purple. Today we can summarize these crosses as follows:

Table 12-1: Mendel's Crosses by Generation

Generation	Cross	Result
P	Purple X White	Produces generation **F_1**: All plants are purple hybrids.
F_1	Purple (hybrid) X Purple (hybrid)	Produces generation **F_2**: ¾ of plants have purple flowers. ¼ of plants have white flowers.

What Mendel had discovered is that "whiteness" does not disappear entirely in the F1 generation; rather, it was simply not observed. He reasoned that the white version of flower color was "masked" by purple. He called purple dominant and said that white was **recessive** (it "steps back" for a generation). The simplest explanation was that each plant could have two "genetic particles" for flower color; one inherited from the male parent and one from the female. If one purple particle is present, the flower is purple, even if the other particle is white. The only way to get a white flower is when both copies of the particle for color are white. These particles are now called **genes**, and the different versions of them are known as **alleles**.

Most eukaryotic organisms are diploid, meaning that they have two copies of each chromosome, one inherited from the male parent and the other from the female parent. Each pair of similar chromosomes is called a **homologous pair**, and each carries the same set of genes in the same order. Because the term homologous means similar, but not exactly the same, although they may carry the same genes, the exact sequence (version of each gene) may differ – these different **alleles** may produce different physical characteristics in the organism.

In the case of Mendel's flowers, the "true breeding" plants are what we call **homozygous**: both of the homologous chromosomes carry the same allele, either the white allele or the purple. This is why if we let them self-fertilize, these flowers can only produce offspring of the same color as themselves. When Mendel created the F_1 hybrids, these plants inherited one of each different allele, so the hybrids were **heterozygous**. In plants we usually indicate the name of an allele with a capital letter if it is dominant, and we use the same letter in lower case for the recessive allele at the same **gene locus**. We can now write the genetic composition, or genotype, of Mendel's plants like this:

PHENOTYPE	**GENOTYPE**
True-breeding purple	PP (both of the alleles say "purple")
True-breeding white	pp (both of the alleles say "white")
Hybrid purple	Pp (there is one of each allele)

The mechanics of how this works is entirely due to meiosis, which is a necessary process to obtain gametes, the reproductive cells of plants and animals. Remember that during gametogenesis in animals the **homologous chromosomes** are separated during the first division, Meiosis I. This results in daughter cells that contain **ONLY ONE OF THE TWO POSSIBLE ALLELES**. This process is the mechanism for the separation, or segregation, of alleles that Mendel discovered.

Activity 12-1: Identifying Gametes from Parent Genotype

If the gametes from a diploid individual only contain one of the two possible alleles, it is not difficult to determine the genotypes of the gametes that individual will make during meiosis. For the diploid genotypes indicated below, write all the possible genotypes of the gametes:

Diploid Genotype of Parent	**Expected Genotype of the Gametes (haploid)** *list all that could be produced*
RR ("homozygous dominant")	
rr ("homozygous recessive")	
Rr ("heterozygous")	

If you find that you are not sure about how the genotypes of the gametes are the result of the genotype of the diploid individual, you probably don't have a solid feel for the purpose and mechanics of meiosis. Don't let time go by without reviewing this subject, since the ability to solve genetics problems requires a good understanding of meiosis.

In people, the little finger can be straight or bent. Bent is the dominant allele, so we'll use the letter "B" to represent this allele.

1. What allele should be used to represent the allele for a straight little finger? _____
2. Now, imagine a man with a bent little finger. If you know he is homozygous, what is his genotype? _____
3. For the man in question #2, considering only the bent/straight little finger gene, <u>how many different</u> genotypes would you expect to find in sperm cells he produced? _____ What are the possible genotypes he could produce by meiosis? _____
4. A woman's little fingers are straight. Is she homozygous or heterozygous? _____ What is her genotype? _____
5. For the woman in question #4, considering only the bent/straight little finger gene, <u>how many different</u> genotypes would you expect to find in egg cells she produced? _____ What are the possible genotypes she could produce by meiosis? _____
6. If the man in question #2 and the woman in question #4 have a children together, what possible genotypes could their children have? _____ What possible phenotypes? _____

Activity 12-2: Solving One-Trait Crosses

An easier way to predict the outcomes of crosses is to use a geometric tool devised by English mathematician Reginald Punnett, known today as the **Punnett square**.

A Punnett square is set up by writing the genotypes of all the possible gametes from one parent along the top of a series of columns and the genotypes of all the possible gametes from the other parent down the left side of rows. Then, you imagine that each gamete from the top fuses with each gamete down the left – filling each box with the offspring that fusion would produce.

For example, let's set up a "parental cross" in which each parent is homozygous, but for opposite phenotypes. In this case, we'll make the female parent the homozygous recessive, with genotype bb. She would produce only one type of egg, "b," so we'll write the same genotype above each box. The male parent would then be homozygous dominant, with genotype BB, so his gametes are all "B." To

help keep track of which parent is which, I sometimes like to draw the symbol for female and male onto my Punnett square.

♂ \ ♀	b	b
B		
B		

In order to "solve" the Punnett square, we simply copy the alleles of the female parent down into the offspring boxes of both columns, and copy the alleles of the male parent into the offspring boxes across both rows. The diploid genotypes of the offspring are automatically generated inside the smaller squares.

One rule about writing genotypes, though, is that if there is a capital letter and a lower-case letter, the capital letter always goes first. So the solved Punnett square looks like this:

♂ \ ♀	b	b
B	Bb	Bb
B	Bb	Bb

This Punnett square, therefore, shows that of four theoretical children, all of them will be heterozygous for the trait we are studying.

Which parent will they resemble for this gene locus trait? _____

On the empty Punnett square provided on the next page, determine the outcome of a cross between two individuals, both of whom are like the children above, heterozygous for the "B" gene locus. As in the example above, start by writing the genotypes of the parent's gametes above and beside the square. Then fill in the squares inside the larger square.

List the predicted offspring genotypes: _____

List the predicted offspring phenotypes: _____

Out of the four theoretically possible types of offspring, what fraction will inherit the recessive **phenotype**? _____

What is the expected ratio of dominant phenotype to recessive phenotype? _____

Note: Write a ratio with a colon between the two numbers after reducing the comparison to the lowest common denominator. For example, if there were 2 offspring showing the dominant phenotype and 2 showing the recessive, the ratio would initially be 2:2, but you should reduce this and write it as 1:1. Always write the dominant phenotype number first, then the recessive.

The cross you just performed is an example of a **monohybrid cross**; that is, a cross between two hybrids. The reason we call it a monohybrid cross is that we are only concerned with one trait. In this case we are looking at the phenotype of the little finger and are not interested in other traits, such as hair color, height, etc.

Typically, in monohybrid crosses the result is that ¼ of the offspring is predicted to display the recessive phenotype and the other ¾, the dominant phenotype. This ratio of 3 dominant to 1 recessive is called the "phenotype ratio", and is written as 3:1. But, notice that of the three offspring expressing the dominant phenotype, two of them are expected to be heterozygotes. The cross predicts one homozygous dominant individual, two heterozygotes, and one homozygous recessive individual. This **genotype ratio** is therefore 1:2:1.

Activity 12-3: Test crosses

In Mendel's pea plants, the dominant flower color was purple, which we can indicate as P. The recessive color, white, can therefore be expressed as p. If you were given a random pea plant with purple flowers, how would you be able to tell if the plant was homozygous (PP) or heterozygous (Pp)?

The answer lies in the fact that even though purple flowers can be PP or Pp, white flowers can only be pp. So, the genotype of an individual expressing the recessive allele is always known. If you cross your plant with uncertain genotype to a recessive individual, you can use the results to discern the genotype of your plant. This is called a **test cross**.

To see why a test cross works, use the Punnett squares below to determine the outcome of two crosses to a white flower: on the left, hypothesize that the purple parent with uncertain genotype is actually homozygous, and on the right, hypothesize that the purple plant is heterozygous. For both crosses, the genotype of the white parent remains the same, so write its gametes across the top of both squares, placing the purple plant possibilities down the left side.

Hypothesis 1: Purple parent is homozygous.

Predicted Offspring and Ratios:

Hypothesis 2: Purple parent is heterozygous.

Predicted Offspring and Ratios:

Now that you have the predictions for your two hypotheses, all you would have to do is to actually cross your purple-flowered plant to a white-flowered plant and see which of the two hypotheses matches the real cross.

If you performed this cross and saw 7 offspring, 5 purple and 2 white, what would you conclude about the purple parent with uncertain genotype? Why?

Activity 12-4: A large monohybrid cross.

Obtain an ear of monohybrid cross corn. The object we call an "ear" is a collection of up to several hundred individual kernels; each kernel is a single fruit, containing a single seed inside.

The kernel's physical characteristics are the result of the OFFSPRING genotype from an individual cross: one pollen grain fertilized one ovule to produce each of the kernels on a single ear of corn. For this reason, the ear is a sort of population, and by counting the kernels on an ear of corn we can test the inheritance of a trait in many independent crosses.

The monohybrid cross ears are the offspring of a cross between two heterozygous parents, or hybrids. If you look back at Table 12-1, the history of this corn is the same: The P (parental) generation consisted of one parent that was homozygous for yellow kernels and one parent that was homozygous for purple kernels. The offspring of that cross – the F_1 generation – was allowed to self-pollinate to generate the F_2 generation, which is what produced the kernels on this ear of corn.

1. Count the total number of kernels on an ear of corn: _____
2. Count the number of yellow kernels on the same ear: _____
3. Subtract the total from the number of yellow to obtain the number of purple: _____
4. Now, divide the color with the LARGER number of kernals by the color with the SMALLER number of kernels to reduce the smaller number to 1. Round the other number to the nearest tenth (one decimal place) and write it as a ratio:

$$\frac{\text{\# kernels in color that was more common (greater \# counted)}}{\text{\# kernels in color that was less common (smaller \# counted)}} = \frac{\_\_\_\_\_}{1}$$

5. Which of the following possible ratios is closest to your actual data? _____
   a) 1 purple : 1 yellow
   b) 2 purple : 1 yellow
   c) 3 purple : 1 yellow
   d) 4 purple : 1 yellow
   e) 1 purple: 2 yellow
   f) 1 purple: 3 yellow
   g) 1 purple: 4 yellow
6. Why were your results NOT an EXACT match to the predicted 3:1 ratio of dominant to recessive?

7. Based on your data, which of the two colors is the dominant allele for corn kernel color?

Activity 12-5: Solving Two-Trait Crosses

In all of the examples above we have only been concerned with one trait. It is possible, however, to track the inheritance of more than one trait at a time. When one is investigating the inheritance of two traits at once, we are investigating two-trait crosses. You may hear others refer to the 2-trait cross as a **dihybrid cross**. Strictly speaking, a dihybrid cross is one between two individuals that are both heterozygous for both of two traits, meaning that they are double-, or di-hybrids. For example, a dihybrid might have genotype AaBb where the two gene loci are represented by different letters (A for the first gene locus and B for the second) and this individual is heterozygous for both (carrying one dominant and one recessive allele at each).

For example, look at the ear of dihybrid corn on your lab table. In this case you will notice that not only are the kernels of different colors, but also that some of the kernels are smooth and some are shriveled. The shriveled kernels have a recessive mutation that prevents the formation of starch, so they accumulate sugar instead. As before, this ear comes from the F_2 generation, meaning that its parents were hybrids for both kernel shape and color.

To practice identifying genotype for two loci at the same time:

1. List the possible genotypes for a corn kernel with smooth, purple kernels.

2. List the possible genotypes for a corn kernel with shriveled, purple kernels.

3. List the possible genotypes for a corn kernel with smooth, yellow kernels.

4. List the possible genotypes for a corn kernel with shriveled, yellow kernels.

Sample Problem: A corn plant is heterozygous for both the smooth/shriveled locus and for the purple/yellow locus. This corn plant self-fertilizes and produces offspring, so this is a true dihybrid cross. Let's work through the problem step-by-step.

The curious thing that Mendel observed from his dihybrid crosses is that two unrelated traits are usually inherited independently. For our above example that means that eye color and finger shape are not inherited together; they act without regard for one another. If you recall from Lab 11, the separation of homologous chromosomes during meiosis I, you may remember that the daughter cells can receive individual chromosomes from either parent. This was evident from the fact that daughter cells got a mixture of "red" and "yellow" chromosomes after meiosis I. This is what Mendel called the **Law of Independent Sorting**. Although he didn't know about meiosis, Mendel was able to deduce this behavior from the way two traits were inherited. Two-trait crosses are also solvable by using the Punnett square, but the key to success lies in being able to deduce the genotypes of the gametes from the parents.

Consider the genotype of the corn plant described above who is double heterozygous, or **PpSs**.

In terms of kernel color, this person's can produce gametes that receive either P or p, but not both. In terms of kernel shape, the gametes can receive either S or s, but not both. Therefore, when considering both traits together, the gametes can either get P and S, P and s, p and S, or p and s. Because the two gene loci are independent of each other, each set is equally likely.

We can write this as gametes of an individual with genotype PpSs could be: __PS , Ps , pS , ps__ .

A convenient way to remember this arrangement is by identifying the letters as "big" or "little". This way the four combinations are: big big, big little, little big and little little.

5. Solve the Punnett square for a cross between the two heterozygous corn "parents" (PpSs x PpSs) by writing the genotypes of the gametes along the top and down the left side of the square, then filling in the offspring squares by simulating the fusion of the gametes at the top and left for each box.

Analyzing the Results:

A. How many of the 16 progeny will be expected to have purple and smooth kernels? _____

B. How many will be expected to have purple and shriveled kernels? _____

C. How many will be expected to have yellow and smooth? _____

D. How many will be expected to have yellow and shriveled? _____

When these numbers are written as a ratio, you get the typical ratio of a dihybrid cross: **9:3:3:1**

This means that 9/16 have both of the dominant phenotypes, 1/16 has both of the recessive phenotypes, 3/16 will have one dominant and one recessive phenotype, and another 3/16 will have the opposite phenotype to that. As with the one-trait crosses, this result is a prediction, not the exact result you should expect from a cross.

Activity 12-6: A Large Dihybrid Cross.

1. Count the number of kernels of each of the four possible phenotypes on one ear of dihybrid cross corn and enter the numbers in the table below:

Purple, smooth	Purple, shriveled	Yellow, smooth	Yellow, shriveled
genotype: P _ S _	genotype: P _ ss	genotype: pp S _	genotype: all ppss

2. Calculate the actual phenotype ratio from your numbers by dividing all the counted values above by the smallest number (usually the # of yellow/shriveled):

Purple, smooth	Purple, shriveled	Yellow, smooth	Yellow, shriveled
genotype: P _ S _	genotype: P _ ss	genotype: pp S _	genotype: all ppss

3. According to the Punnett square, you expected 9:3:3:1. Your actual numbers are different from the results expected, right? Why do you think that is?

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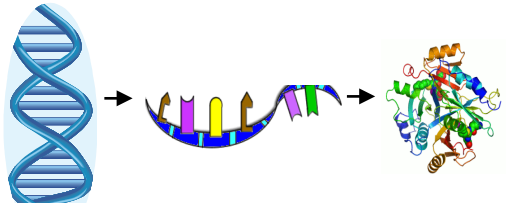
Lab 13: Gene Expression



Objectives

- Define and describe the process of transcription.
- Understand the role of RNA polymerase.
- Describe the function of the promoter and the termination region.
- Summarize the sequence of events that occur in translation.
- Describe the function of messenger RNA, transfer RNA and ribosomal RNA.
- Given a sequence of a gene, predict the peptide that will be made.

Pre-Lab Reminder: *Before arriving to this scheduled lab, read the entire lab (background, procedure, and questions), and use that information to complete the pre-lab in eCampus.*

Background Information

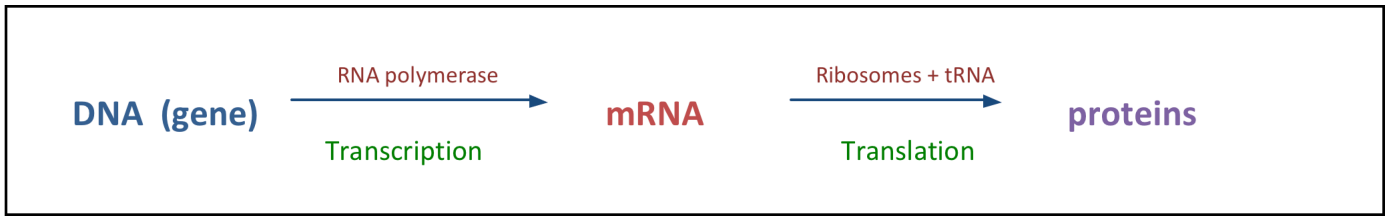
In living cells, deoxyribonucleic acid (**DNA**) carries the genetic information (genes) leading to the synthesis of proteins. This process is mediated by an intermediate molecule called the messenger RNA (**mRNA**).

The process by which an mRNA molecule is made from a nucleotide sequence in DNA is called **transcription**, and is catalyzed by the enzyme **RNA polymerase**. This process takes place in the nucleus of eukaryotic cells and in the cytoplasm of prokaryotic cells.

Right before the start of transcription, RNA polymerase binds to a specific region in the DNA molecule called the **promoter**. After binding, the enzyme moves along the DNA molecule, joining the complementary nucleotides in the 5' → 3' direction. Guanine pairs with cytosine, and uracil pairs with adenine in RNA. The enzyme stops transcribing the gene when a **termination region** in the DNA strand is reached.

The strand of mRNA made by RNA polymerase is complementary to the strand of DNA which runs in the 3' → 5' direction. In the cytoplasm, the mRNA binds to the ribosomes, which are composed of ribosomal RNA (**rRNA**) and proteins. Transfer RNA (**tRNA**) molecules carry specific amino acids to the ribosomes in the form of complexes named "aminoacyl-tRNAs" . At the ribosome, base triplets in the mRNA (**codons**) pair with base triplets (**anticodons**) of tRNAs. Consequently, the amino acids become properly arranged and are bonded together by enzymes in the ribosome.

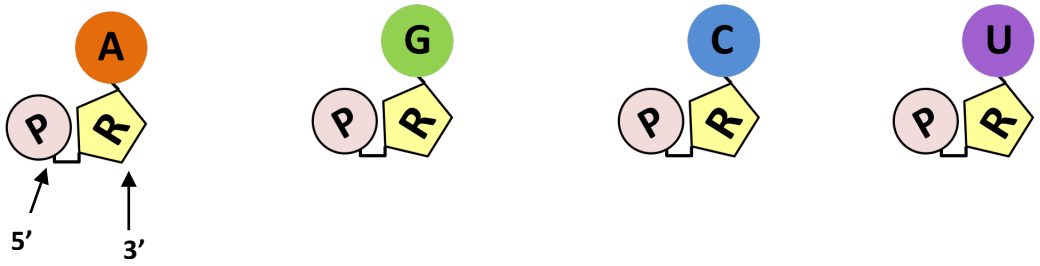
The process is repeated as the ribosome moves along the mRNA molecule. As a result, peptide bonds join amino acids together forming a polypeptide chain. This polypeptide becomes a functional protein. The general flow of genetic information, "The Central Dogma", is shown below:



Activity 13-1: Gene Expression - Transcription

First construct a short chain of RNA (the messenger RNA, or mRNA) complementary to the "gene" on your DNA molecule. To do this, you will need to the RNA nucleotides that will be used to make the chain. A nucleotide of RNA is composed of one ribose (5-carbon sugar), one phosphate group and a nitrogenous base (which can be adenine, guanine, cytosine or uracil).

Notice the laminated nucleotides in your "bag of goodies" and recall the positions 5' and 3' in each.



1. Spread out the strip that represents a section of a gene (DNA) to be transcribed.

2. Using Fig. 13-1 as a guide, place the laminated "RNA polymerase" on the far-left side of the slit between the DNA strands.

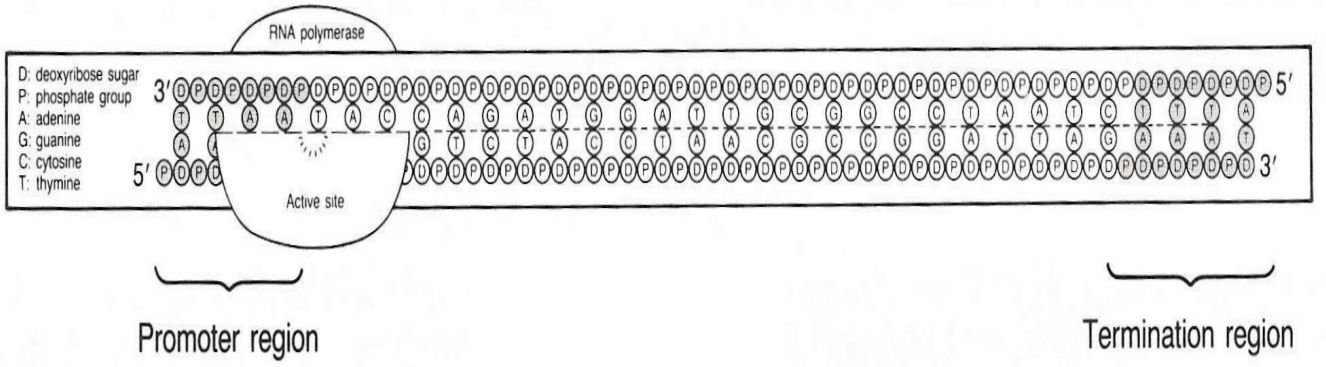


Fig. 13-1 RNA polymerase attached to the DNA fragment.

RNA polymerase binds to a promoter region on the DNA, which marks the beginning of **transcription**. When transcription starts, RNA polymerase moves along the DNA molecule, separating the two strands as it moves. The enzyme can only add nucleotides in the 5'→ 3' direction, therefore, the strand of DNA to be read must run in the 3' → 5' direction (the upper strand in our DNA molecule; see Fig. 13-1). Incidentally, when looking at a double helix, genes are located on both DNA strands, but each gene is read in a single direction so that only one DNA strand is transcribed for each gene.

3. Make sure that the active site of the RNA polymerase is at the first DNA base after the gray promoter (a T).

4. Take an Adenine RNA nucleotide and pair it with the T at the active site. Position the Adenine nucleotide so that it is antiparallel to the upper DNA strand (the 3' position of the RNA nucleotide should point to the right).

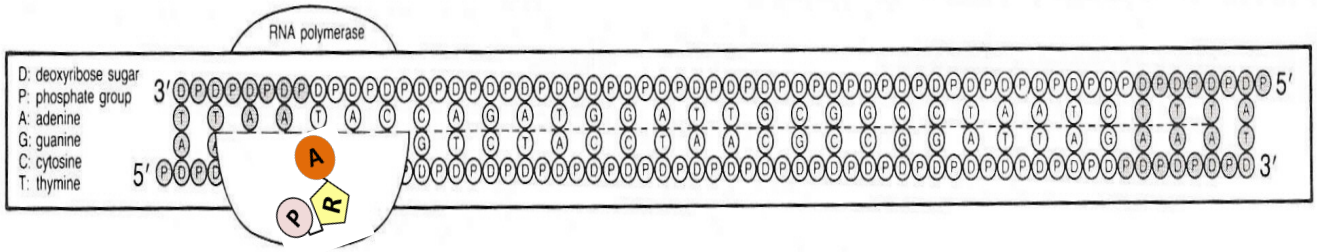


Fig. 13-2 Transcription: the synthesis of mRNA from DNA

5. Holding the RNA nucleotide, move the RNA polymerase forward (right) until the next DNA base (A) is in the active site.

6. Take a Uracil nucleotide and pair it with the next nucleotide in DNA (an A).

7. Use a small piece of tape to hold the two RNA nucleotides together. *NOTE: Make sure to form the "bonds" (tape) between the RNA nucleotides - do NOT tape them to the DNA or you won't be able to move the finished mRNA to the cytoplasm for translation.*

8. Again hold the RNA nucleotides, and move the RNA polymerase forward until the next DNA base is in the active site. Pair the proper RNA nucleotide (follow base pairing rule) with the DNA base and connect them.

9. Follow these steps, one base at a time, along the entire DNA strand.

10. When the RNA polymerase reaches the gray termination region in the gene, remove the enzyme from the DNA molecule.

Activity 13-2: Gene Expression – Protein Synthesis

Now you have a strand of mRNA complementary to the template strand of the DNA molecule. In eukaryotic cells, this mRNA is modified before translation can begin, but for simplification, we are keeping our mRNA exactly as transcribed (no cap, tail, or splicing out introns). Transcription is now complete, and you are ready to start the process of **translation**, or protein synthesis.

1. On the space provided in the next page, write down the sequence of nucleotides in your mRNA, starting with the 5' end.

The genetic code tells which triplet of nucleotides in the mRNA (**a codon**) corresponds to which amino acid in a protein. The genetic code is shown in Fig. 13-3. You do not have to memorize this! The AUG codon is the start signal for translation, as well as a code for the amino acid Methionine. Three codons serve as termination (Stop) signals.

2. Use the genetic code to write the corresponding amino acids in your peptide chain.

mRNA: 5'- ___ -3'

amino acids: _____ _____ _____ _____ _____ _____ _____ _____

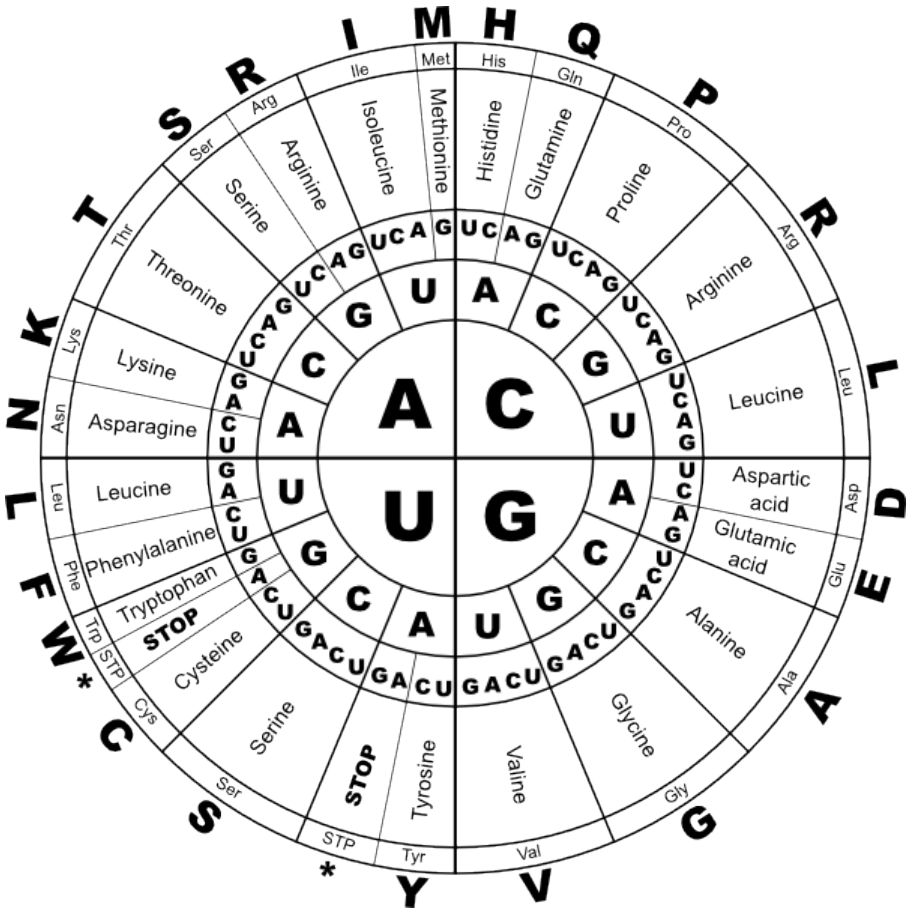


Fig. 13-3 The Genetic Code. Look for the first base of the codon in the center of the circle then move outward as you go to the second and third codons.

3. Position your mRNA strand horizontally on the table so that the 5' end (phosphate) is on the left and the 3' end is at the far right. Arrange all the nitrogenous bases so that they are pointing upwards.

4. Take the laminated "ribosome" and slide it under the mRNA so that the first codon (AUG) is in the P site and the second is in the A site of the ribosome.

5. Take a laminated tRNA molecule and tape its 3' to a yellow circle. Inside this circle write Met with an erasable pen. The tRNA molecules have a triplet of bases called the **anticodon**, which is complementary to the codon on the mRNA.

6. Position this **aminoacyl-tRNA** molecule on the P site of the ribosome, making sure that the nitrogenous bases of the codon pair up with the anticodon bases.

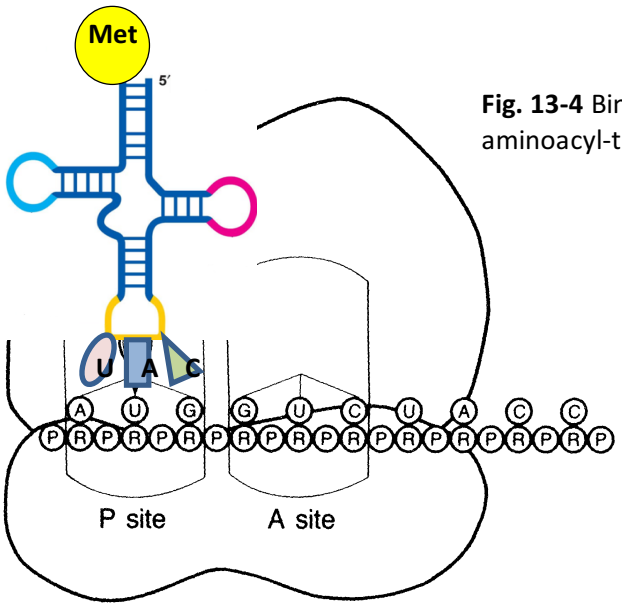


Fig. 13-4 Binding of the initiator aminoacyl-tRNA complex.

7. Prepare the aminoacyl-tRNA complex that corresponds to the next codon (in the A site of the ribosome) and position it so that codon and anticodon pair up. Both P and A sites are now occupied.

8. Now a peptide bond forms between the amino acid in the P site and the amino acid in the A site. Simulate this by removing the amino acid attached to its tRNA in the P site and taping it to the amino acid in the A site (Fig. 13-5).

9. Move the ribosome ahead so that the next codon is exposed in the A site. The two amino acids that you bonded before should be in the P site, and the empty tRNA should be to the left of the ribosome. This tRNA is expelled from the mRNA strand and the ribosome and returns to the cytoplasm, where it can bind to another amino acid of its kind.

10. Now that another codon is exposed, bring the corresponding aminoacyl-tRNA to the A site. Make sure that the codon pairs up with the anticodon in your t-RNA and that the t-RNA has the correct amino acid.

11. With a small piece of tape, form a peptide bond between the last amino acid in the P site with the amino acid in the A site (just like you did before).

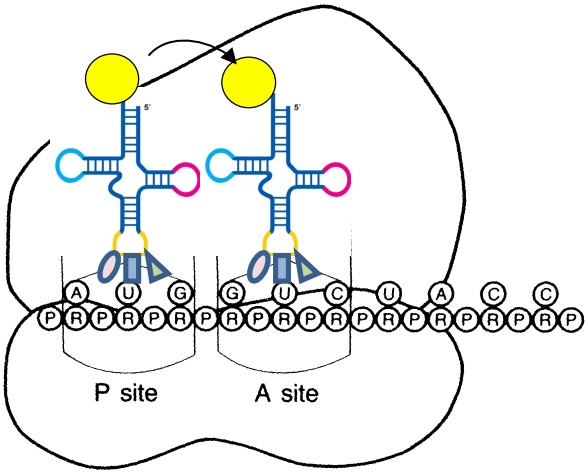


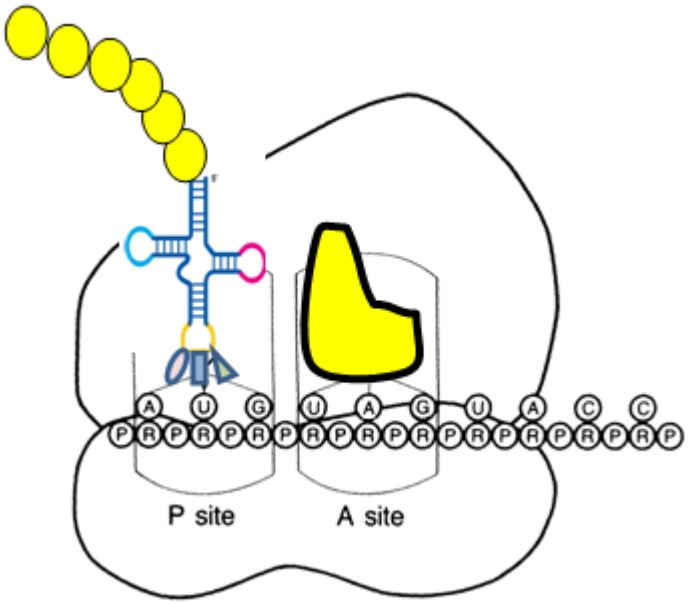
Fig. 13-5 Formation of a peptide bond between two amino acids during translation.

12. Continue doing this until a STOP codon (UAA, UAG, UGA) is exposed in the A-site. A release factor protein binds to the A site and this causes complete transfer of the completed protein from the P site to the A site. Then the protein is released from the ribosome.

13. Insert a release factor protein in the A-site and detach your peptide chain from the ribosome.

14. Translation is now complete.

15. Verify that the sequence of amino acids in your chain of connected yellow circles corresponds to the predicted protein sequence in the lab manual and show your instructor before disassembling the protein.



On-line resources (animations and interactive tutorials) for the processes of transcription and translation:

1. http://sepuplhs.org/high/sgi/teachers/genetics_act16_sim.html
2. http://learn.genetics.utah.edu/content/begin/dna/transcribe/
3. http://highered.mcgraw-Hill.com/sites/0072507470/student_view0/chapter3/animation__how_translation_works.html
4. http://www.youtube.com/watch?v=J3HVVi2k2No

References:

1. RNA Synthesis Simulation BioKit
2. Protein Synthesis1 Simulation BioKit
3. Protein Synthesis2 Simulation Biokit
4. *Carolina Biological Supply Company*, 2700 York Road, Burlington, North Carolina
5. *General Biology Laboratory Manual, Investigations into Life's Phenomena*,
6. Skavaril, Finnen and Lawton, Saunders College Publishing, 1993
7. *Biology in Focus*, 3rd edition, Purcell, Harcourt Brace and Company
8. http://www.clker.com/clipart-genetic-code-rna-black-and-white.html

CONGRATULATIONS!!

YOU HAVE COMPLETED ALL THE LAB EXERCISES FOR BIOLOGY 1406 !!!