BIO MAJORS 4A+4B LAB MANUAL



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Evergreen Valley College

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BIO 4A+4B Lab Manual (2023)

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This text was compiled on 05/03/2026

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Detailed Licensing

About the Book

An Open Educational Resource Initiative at Evergreen Valley College

The Evergreen Valley College Open Educational Resources Initiative (EVC-OER) was funded by President Tammeil Gilkerson beginning in 2022. As part of EVC's focused work advancing opportunity, equity, and social justice, the initiative aims to promote the development of a more robust collection of Open Educational Resources (OER) to support EVC's faculty instruction and student learning. In addition, we hope to build a faculty community of practice engaged in curating, developing, and publishing OER materials to sustainably transform their courses into zero-cost or low-cost courses, eliminating structural barriers that may impede students' success.

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Book Contributors

This book is written for lower-division biology majors for the laboratory portion of EVC courses BIOL 4A and 4B, and C-ID BIOL 190 and 140.

The EVC President's OER initiative funds this book. It is licensed CC BY and is developed and curated by:

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- Bridget Greuel (Chapter 34, Learning Objectives)

Licensing

*A detailed breakdown of this resource's licensing can be found in **Back Matter/Detailed Licensing**.*

Preface

Introduction

Welcome to the lab manual for your introductory biology for majors series. This book provides laboratory experiments and exercises for a two-semester introductory biology majors series, including topics of general principles, cell biology, organismal biology, ecology, and evolution.

Audience

This book is a lab manual for lower division biology majors. Biology majors will typically take a two-semester (one year) introductory series to introduce them to major topics in biology and prepare them for upper division coursework. This book is designed to provide comprehensive laboratory exercises and experiments for this introductory series.

Chapter Outline

The text is organized into 34 chapters, with chapter 1-19 intended for a General Principles and Cell Biology course and 20-34 for an Organismal Biology and Biodiversity course.

General Principles and Cell Biology

- Chapter 1: Microscopy Part I
- Chapter 2: Measurement & Equipment
- Chapter 3: Scientific Investigation
- Chapter 4: Ph & Buffers
- Chapter 5: Organic Macromolecules
- Chapter 6: Microscopy Part II
- Chapter 7: Eukaryotic Cells
- Chapter 8: Bacteriology (Prokaryotic Cells)
- Chapter 9: Diffusion & Osmosis
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- Chapter 28: Plant Growth
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Appendices

Appendix A: Lab Safety

Appendix B: Brief Introduction to Statistics

Appendix C: Sample Lab Report

For the Student

Each chapter in this lab manual begins with several learning objectives (content and skill objectives). Following the objectives is an introduction to the subject, procedures for the lab, and a lab report section. Students should read the introduction before each lab to ensure readiness for the material. The lab report section is the data you will collect and questions you will answer to turn in to your instructor. Your instructor may ask you to print and fill out these pages or answer the questions in a lab notebook.

For the Instructor

Learning objectives can be found at the beginning of each chapter. There are often several exercises in one chapter, and you may omit or modify as time and institution resources permit. The lab report section can be printed, filled out, and turned in, or you can have students complete the exercises and questions in a lab notebook.

A set of instructor resources with formative and summative questions, further lab instruction, and guidelines for lab setup for technicians will be available on Libretext in 2024.

CHAPTER OVERVIEW

1: Microscopy Part I

Learning Objectives

Content Objectives

- Identify the parts of the compound light microscope.
- Comply with Evergreen Valley College's Biology lab safety rules and protocols.

Skill Objectives

- Focus prepared slides of *Amoeba* and *Paramecium* and make labeled sketches of each.
- Calculate total magnification.

1.1: Introduction
1.2: Exercise
1.3: Lab Report

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1.1: Introduction

Introduction

One of the most useful tools in biology is the microscope, which allows you to view organisms, tissues and cells that are too small to be seen with the eyes alone. **Resolving power** is is the ability of a microscope to distinguish two adjacent structures as separate. The higher the resolution, the better the clarity and detail of the image. We will use a **compound microscope** in our laboratory course, with occular eye pieces that magnify objects 10x, and objective lenses that magnify 4x, 10x, 40x, and 100x. Our microscopes also have filters that allow us to view specimens in different ways, most commonly we will us the "BF" filter, which stands for bright-field. The **bright-field** filter focuses simple white light onto the specimen, creating an image where the background will be bright and the sample will be dark. We also have "Ph1" and "Ph2" filters which stands for **phase contrast**, where the background will appear dark against a light sample. Phase contrast is often used for observations of unstained organisms, where as we typically stain our specimens when we use the bright-field filter. Staining techniques almost always will kill the cells, however, it provides great contrast for observations, and can also be used as tool to detect and differentiate particular structures of interest. For bright field and phase contrast image examples, please see the Prokaryotic Cells lecture slides. You will learn about other, more advanced types of microscopy during lecture and during the Microscopes Part II lab.

A. How to Properly Use the Microscope

1. Always hold the arm of the microscope with one hand and support the base with the other
2. **Never drag the microscope across the lab table**
3. Before use and after each use
    A. The stage should be as low as possible and stage controls centered
    B. The lowest objective should be above the center of the stage
    C. No immersion oil should be on the microscope (please check all objective lenses)
        1) Clean lenses with special **lens paper** only
        2) Use designated lens cleaning solvent if necessary (ask instructor before use)
    D. The stage should be free of slides, dimmer at 0, light switch off, cord wrapped neatly
4. **To focus a slide**
    a. Place slide onto stage, right side up, and secure with stage clip
    b. Move stage all the way up, towards the scanning or low objective using the coarse adj. knob
    c. SLOWLY focus the slide by using the coarse adj. knob to move the stage down
    d. Adjust oculars as necessary (width and focus of each ocular lens, if applicable)
    e. Once focused, move to the next, higher, objective lens, but don’t move the stage
        1) Our microscopes are **parfocal** – this means that when one lens is in focus, the others are as well; thus, higher magnifications will only require slight adjustment
    f. Focus using the FINE adj. knob ONLY for the 40x and 100x objective lenses
    g. When finished, rotate the nosepiece until the lowest objective lens clicks into place
    h. Move the stage all the way down, as low as possible

i. Clean off any remaining oil

j. Lower the dimmer, turn off the light and unplug the microscope; please grasp the plug, not the cord when unplugging *(continued on back)*

k. Wrap the cord neatly and replace the microscope cover before storing; be careful not to hit the table or sides of the cabinet when storing the microscope

B. How to Use the Oil Immersion Lens (100x Objective)

At high power (1000X total magnification), the bending of light, or refraction, is magnified and causes the image to be distorted. To remedy this, the space/air between the slide and objective lens is replaced with oil. This will minimize the refraction so we are able to obtain a sharp image at 1000X magnification.

Procedure:

1. Place the prepared slide on the stage and secure with the stage clip
2. Using the 10X objective lens, focus the slide and center the object in the field of view
3. Rotate the nosepiece to the 40X objective lens and focus using the FINE adjustment knob only
4. Rotate the nosepiece until you are at an intermediate position between the 40X and 100X objective lenses (halfway between the 40X and 100X objective lenses)
5. Place one small drop of immersion oil on the center of the viewing area of the slide (use the light from the condenser as a guide). **Do NOT move the stage.**
6. Rotate the nosepiece until the 100X objective lens clicks into place. Look to make sure the tip of the lens is touching the oil
7. Focus SLOWLY using the FINE adjustment knob only
8. When finished, make certain to clean off any oil from the lenses and slides before returning your microscope to the cabinet and your slide to the designated tray.

C. Hints and common errors

1. If the microscope light will not turn on, check the dimmer, and turn it past 0
2. If the light still seems low or dim, try adjusting the iris diaphragm, make sure it is not closed
3. If you cannot see anything through the oculars even though the lamp is on, try rotating the nosepiece to make sure the objective lens is clicked into place
4. If you are having trouble keeping your object in focus, check to make sure the slide is flush with the edges of the slide holder and stage clip
5. If the image appears clear on the 10X objective lens, but blurry on the 40X objective lens, check to make sure the 40X objective lens is clean and free of oil; if this still does not work, check to make sure your slide is **right-side up** (specimen on top, facing the objective lenses)

6. Move the condenser to change the intensity of light focused on your sample: in the up position the light intensity increases, while in a lower position, the light is less intense/more diffuse. We generally move the condenser high for stained samples, but lower for unstained samples to improve contrast
7. A diffuser (which looks like an opaque glass ring) should be resting above your light source. If you do not have one, please notify your instructor.
8. Remember, once you use oil, you can't go back to lower objective unless you clean off all the oil. Only use oil when instructed and be sure your image is clearly in focus on 40x first.

Bring this microscope guide with you to all lab activities.

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1.2: Exercise

Identify and label parts of a microscope

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Exercise 1: Identifying the parts of the microscope

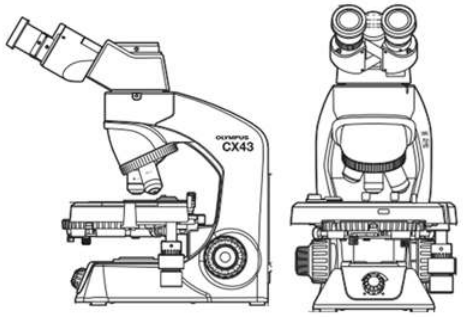


Figure 1.2.1: Side and front view of Olympus CX43 microscope, from user manual.

Instructions: Identify & label the following parts of your microscope onto the image above, and fill-in-the blanks below.

Binocular head, Oculars: _____x

Arm

Revolving nosepiece

Objective lenses: _____x, _____x, _____x, _____x

Mechanical stage and X/Y knobs

Stage/slide clips

Condenser Turret _____, _____, _____, _____

Iris diaphragm and how to adjust it

Coarse adjustment knob & fine adjustment knob

Light source; illuminator

Base

Dimmer; light intensity

Power switch & power cord

Total magnification calculation =

Exercise 2: Using your Microscope

The genus *Amoeba* (sometimes referred to as *Amoebozoa*) are a group of eukaryotic single-cell organisms in the kingdom Protista. Some species can cause serious human diseases, such as *Entamoeba histolytica* which causes amoebic dysentery (bloody diarrhea), and *Naegleria fowleri*, commonly referred to as the "brain-eating" amoeba. **Pseudopodia** are foot-like extensions of the cell membrane and cytoplasm via actin microfilaments and are used for motility and ingestion of food (bacteria and other protists) via phagocytosis. Amoeba are fairly large **multinucleated** cells, meaning they contain more than one nucleus per cell.

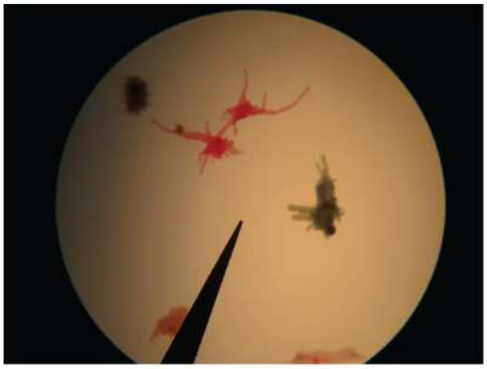


Figure 1.2.2: Amoeba proteus. Amoebae with tubular and lobe-shaped pseudopodia are seen under a microscope. These isolates would be morphologically classified as amoebozoans. OpenStax Figure 23.13 used under the Creative Commons Attribution 4.0 International License.

The genus *Paramecium* are a group of eukaryotic single-cell organisms in the kingdom Protista. They are typically found in freshwater or brackish (slightly salty) environments. *Paramecium* are called ciliates because they have specialized hair-like structures called **cilia**. Their cilia line the outside of the cell and are used for motility. Cilia also line the oral groove (primitive mouth) and are used to ingest of

food such as bacteria. Contractile vacuoles allow the organism to excrete excess water. *Paramecium* have another distinguishing trait - two nuclei; a macronucleus that is used for transcription of genes, and a micronucleus that is used for sexual reproduction.

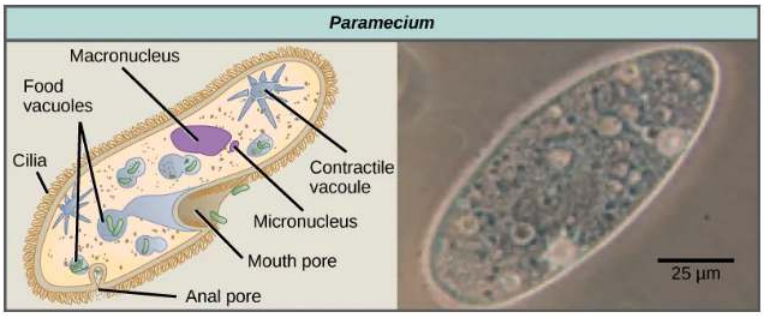


Figure 1.2.3: Paramecium. (credit "paramecium micrograph": modification of work by NIH; scale-bar data from Matt Russell) OpenStax Figure 23.24 used under the Creative Commons Attribution 4.0 International License.

Instructions: View both *Paramecium* and *Amoeba* slides under the 4x, 10x, and 40x objectives, and **sketch and label** each organism in the space below. Do NOT use the oil immersion lens today.

- Pick 1-3 cells of each organism to draw.
- You decide which magnification is best for each specimen. For example, a larger cell/specimen might best be seen with the 10x objective instead of the 40x objective.
- Make sure to label each sketch with the organism's name, the total magnification, the calculation for total magnification, and **label** any visible distinguishing features of the organism.

Organism:	Organism:
Objective:	Objective:
Total magnification:	Total magnification:

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1.3: Lab Report

Exercise 1: Identifying the parts of the microscope

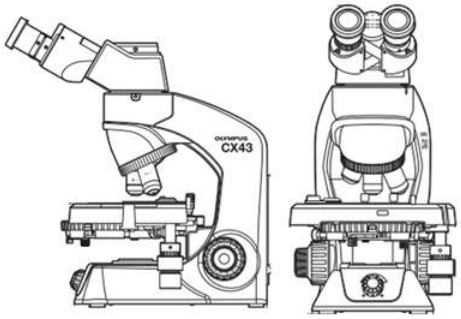


Figure 1.3.1: Side and front view of Olympus CX43 microscope, from user manual.

Identify & label the following parts of your microscope onto the image above, and fill-in-the blanks below.

· Binocular head, Oculars: _____x

· Arm

· Revolving nosepiece

· Objective lenses: _____x, _____x, _____x, _____x

· Mechanical stage and X/Y knobs

· Stage/slide clips

· Condenser Turret _____, _____, _____, _____

· Iris diaphragm and how to adjust it

· Coarse adjustment knob & fine adjustment knob

· Light source; illuminator

· Base

· Dimmer; light intensity

· Power switch & power cord

· Total magnification calculation =

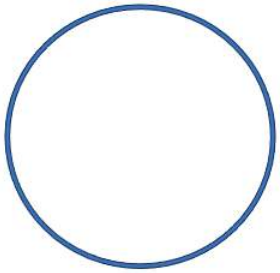
Exercise 2: Using your Microscope

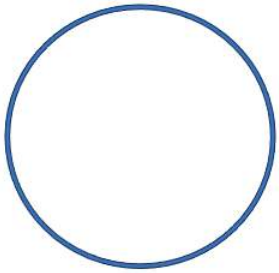
View both *Paramecium* and *Amoeba* slides under the 4x, 10x, and 40x objectives, and **sketch and label** each organism in the space below. Do NOT use the oil immersion lens today.

- Pick 1-3 cells of each organism to draw.
- You decide which magnification is best for each specimen. For example, a larger cell/specimen might best be seen with the 10x objective instead of the 40x objective.
- Make sure to label each sketch with the organism's name, the total magnification, the calculation for total magnification, and **label distinguishing features** of the organism. For example, for *Amoeba* label the nucleus and pseudopod(s).

Organism:
Objective:
Total magnification:

Organism:
Objective:
Total magnification:





Microscopes & Lab Safety Review Questions

After reading the safety guidelines (see appendix) and the microscope introduction, answer the following questions.

1. It is okay to drink bottled water in the lab.
    a. True
    b. False
2. Which type of shoes must students wear in the laboratory?
    a. Sandals
    b. Shoes with non-slip tread/soles
    c. Any type of shoe
    d. Shoes that cover your entire foot
3. The classroom fire extinguishers are located near the
    a. Staff entrance
    b. Student exits
    c. Steel sink
    d. Teacher's desk
4. The classroom phone is located at the
    a. Staff entrance
    b. Student exits
    c. Steel sink
    d. Teacher's desk
5. Guests are allowed in lab if they have prior permission and have agreed to the laboratory safety rules.

a. True

b. False

6. During an emergency evacuation, we should meet at

a. Student Lot 9 (near observatory entrance)

b. The Sequoia / Valle Del Lago roundabout

c. Montgomery Hill Observatory

d. Staff Parking Lot 7

7. While viewing a microscope slide, you find that the light appears too dim for your preference. Adjustment of which of the following might solve your problem?

a. Oculars

b. Course adjustment knob

c. Iris diaphragm

d. Stage clips

8. It is okay to use soft Kleenex to clean your microscope lenses.

a. True

b. False

9. Once you have a slide in focus under one of the lenses, the other lenses will be in focus as well and therefore will only require slight adjustment using the fine focus knob. This is because our microscopes are _____.

a. Expensive

b. Superb

c. Parfocal

d. Refractory

10. We must (always and only) use oil when viewing slides using which objective lens?

a. 4x

b. 10x

c. 40x

d. 100x

11. It is best to always begin viewing a slide using the 40x objective lens.

a. True

b. False

12. When focusing a slide, the stage should be all the way ____ before you look through the oculars.

a. Down

b. Up

13. When storing the microscope, the stage should be ____, and the ____ objective lens should be centered (in front).

    a. Up, 4x

    b. Up, 40x

    c. Down, 4x

    d. Down, 40x

14. True or False: Before and after each use with the microscope, you should clean the objective lenses, starting with the 4x objective.

    a. True

    b. False

15. We never drag the microscope across the bench top because

    a. The sound it makes is irritating

    b. The Professor will have a fit

    c. This loosens and/or damages the parts of the microscope

16. Before and after each lab, you should

    a. Wipe down bench tops and chair

    b. Clear sinks, bench tops, and floors of any trash/debris

    c. Push in your chair

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CHAPTER OVERVIEW

2: Measurement and Equipment

Learning Objectives

Content Objectives

- List the different laboratory equipment used for measuring volumes, mass, and specific gravity.
- Differentiate between **precision** and **accuracy**.
- Explain the relationship between **specific gravity** and concentration of a solution.

Skill Objectives

- Practice using pipettes, graduated cylinders, and volumetric flasks for accurately measuring volumes.
- Practice using a laboratory scale to measure mass.
- Calculate and prepare solutions of differing concentrations.

2.1: Introduction
2.2: Exercise
2.3: Lab Report

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2.1: Introduction

A. Volume

We need to be able to measure volume accurately in order to determine the concentration of dissolved substances in our lab. The most common devices used to measure volume are the **pipette, graduated cylinder,** and **volumetric flask.**

The Pipette

Examine the **pipettes** available in our lab today. Determine how the pipettes are calibrated. Both the largest and smallest volumes that a pipette can measure are marked at the top of the pipette. For example, “10 mL in 1/10” means that the pipette can measure a max of 10 mL, each large line marks 1 mL, and each smaller division marks 1/10 mL. We use **pipette aids** (**propipettes**) to draw liquids into the pipette.

The Micropipette

Micropipettes are used to measure very small volumes accurately and repeatedly. They generally have a continuous range between 1 µL to 100 µL, including fractional volumes. Plastic tips are used to hold the liquid, and the tip can be ejected easily.

The Graduated Cylinder

We use graduated cylinders to measure larger amounts of liquid, typically 5.0 mL or larger. Water sticks more strongly to glass than organic polymers (plastic), so the water will climb up the sides of a glass cylinder to form a bowl-like meniscus. To obtain a precise measurement, we read volume at the bottom of the meniscus.

The Volumetric Flask

Volumetric measuring devices do not have graduations. Instead, they are calibrated to measure a single volume, and should *only* be used to measure that volume. Volumetric equipment is more accurate than graduated equipment. The narrowness of the neck of the volumetric flask extends the solution into the narrow neck, allowing greater accuracy in matching the meniscus of the solution to the line. **Your instructor will demonstrate this activity**.

B. Precision versus Accuracy

Accuracy refers to the closeness of an observed value to the true or known value. Precision is the degree to which measurements are reproducible when repeated. A lack of accuracy and/or precision may be a function of the measuring device or the technique used

Preparing and working with Solutions

We measure mass by using a **balance** or a **scale**. Containers or weighing papers are often used during measurements of mass. The mass of the container must be subtracted from the total mass to obtain the mass of the material of interest. This is completed by **taring** the scale. You will need to measure mass when preparing solutions. Solutions are homogenous mixtures of solute (the dissolved substance) and solvent (the dissolving medium). It is most common to use molar concentrations when working in a lab.

A molar (M) solution contains 1 mole of a solute in one liter of solution. A mole of a substance contains Avogadro’s number (6.02 x 10^{23}) of molecules of that substance. For example, the molecular weight of NaCl is 58.5 g/mol. Thus, one mole of NaCl has a mass of 58.5 grams. To prepare one liter of 1 M solution of NaCl, you would add 58.5 g of NaCl to a 1L (1000mL) volumetric flask and then add water until it reaches the 1000 mL mark, meaning a 1M NaCl solution has a total volume (water plus the solute) that is equal to 1L. Thus, the actual volume of water you add is slightly less than 1 L. It is important to realize that if you were to measure 1000 mL of water and then add 58.5 g of NaCl, you would have more than one liter of the solution.

To calculate the amount of solute required to make a solution of a specific molarity, use the following formula:

Grams of solute = [molecular weight (grams)] x [volume (liters)] x [desired molarity].

You will measure the **specific gravity** of water and different concentrations of NaCl solutions to investigate the relationship between specific gravity and solution concentration. The formula for specific gravity is:

Specific gravity = (the density of a substance) / (the density of water).

A **hand refractometer** is the tool we will use today, however, this is not the only type of tool that can be used to measure specific gravity. You will then prepare a serial dilution of your NaCl solution. A **serial dilution** is a stepwise dilution of a substance in solution. The most common dilution is a 1/10 ratio, where you add 1-part of the stock solution, to 9-parts water/solvent. We use serial dilutions to produce low molarity solutions more accurately.

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2.2: Exercise

A. Volume

The Effect of Glass and Plastic on the Meniscus

1) Obtain a 10 mL pipette and a beaker of colored liquid. Transfer 8 mL of the colored liquid into a 100 mL **glass** graduated cylinder. Repeat 4 times.

   a. What volume of liquid should the cylinder contain? _____

   b. What volume of liquid does your graduated cylinder contain? (Remember that the bottom of the meniscus marks the correct volume) _____

   c. What could contribute to a difference?

2) Repeat step 1 but this time transfer the liquid into a 100 mL **plastic** graduated cylinder instead. Are there any differences? Yes / No **Explain**:

The Micropipette

1) Use a micropipette (p200) to transfer 25.5 µL of red dye solution onto a small piece of Parafilm or wax paper. After dispensing the dye, use the same pipette to pick up the drop and transfer it to a different location. Repeat the step with a new micropipette (p20) and 13.7 µL of red dye solution.

2) Are you able to pick up the entire drop of liquid each time? Yes / No Explain:

The Graduated Cylinder

1) Partially fill a 100 mL **glass** graduated cylinder with tap water and set it on your table. View the cylinder and measure the volume of the water while

(a) looking down at the cylinder _____, (b) at eye level _____, and (c) below and looking up at the cylinder _____.

Is there any difference between the three? Yes / No Explain: _____

2) Transfer the water to a **plastic** graduated cylinder. View the cylinder and measure the volume of the water while (a) looking down at the cylinder _____,

(b) at eye level _____, and (c) below and looking up at the cylinder _____.

Can the volume be read more precisely in the glass or plastic cylinder? Glass / Plastic

3) Add tap water to the 10 mL level in a 100 mL glass graduated cylinder. Then, transfer this water into a 10 mL graduated cylinder.

What measurement do you read on the smaller cylinder? _____ Which cylinder would be more accurate for measuring 5 mL of solution? _____

The Volumetric Flask

Your instructor will demonstrate this activity.

· Instructor: put a few drops of dye into the volumetric flask and add water until it reaches the 100 mL mark on the volumetric flask, use a dropper to be as accurate as possible.

· Pour the water into a graduated cylinder. Is it more, less than, or equal to, the 100 mL mark? _____

· Instructor: Now, pour this water into an Erlenmeyer flask and estimate the volume: _____mL

B. Precision versus Accuracy

1. Which device is more accurate? Volumetric flask / Graduated cylinder / Erlenmeyer flask
2. Which device is more precise? Volumetric flask / Graduated cylinder / Erlenmeyer flask

Preparing Solutions

1. Circle the concentration assigned to your group: 0.4, 0.6, 0.8, 1.0, 1.2, 1.4. Show your entire calculation below, calculate how many grams of NaCl is required to make your assigned molarity. Then, prepare 100 mL of NaCl solution of your assigned molarity.

2. Place a drop of water on the **hand refractometer** to measure the **specific gravity** of water. Record the specific gravity of the water drop: _____, then dry the refractometer with a KimWipe. Next, place a drop of your assigned NaCl solution onto the hand refractometer and record the specific gravity: _____. Clean with water and dry with a KimWipe.

3. Go around the room and measure the specific gravity for each group's solution. Clean with water and dry with a KimWipe after each use. Record the data in the table below, include your group's solution too.

NaCl solution: molarity vs. specific gravity

Molarity of NaCl solution	Specific gravity
0.4 M	
0.6 M	
0.8 M	
1.0 M	
1.2 M	
1.4 M	

Preparing Serial Dilutions

Serial dilutions- a stepwise dilution of a substance in solution. We use serial dilutions to produce low molarity solutions more accurately. Use your original assigned molarity as the stock solution.

- Measure 9 mL of water and transfer into a beaker or test tube. Then, transfer 1 mL of your stock solution into the 9 mL of water and mix well. This is your first dilution. (1/10 dilution)
- Next, measure 9 mL of water and transfer into a new beaker or test tube, then transfer 1 mL of your first dilution into this new 9 mL of water and mix. This is your second dilution. (1/10 * 1/10 = 1/100 dilution)
- Use the hand refractometer to measure the specific gravity of each of the two dilutions and record in the table below.
- Does specific gravity increase or decrease as you dilute a solution? _____

NaCl dilutions: molarity vs. specific gravity

NaCl stock solution	Molarity _____M	Specific gravity:
First dilution	Molarity _____M	Specific gravity:
Second dilution	Molarity _____M	Specific gravity:

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2.3: Lab Report

A. Volume

The Effect of Glass and Plastic on the Meniscus

Glass cylinder

- What volume of liquid should the cylinder contain? _____
- What volume of liquid does your graduated cylinder contain? Remember that the bottom of the meniscus marks the correct volume _____
- What could contribute to a difference between the two?

Plastic cylinder

- You repeated the activity with a plastic graduated cylinder, did you observe any differences? Explain?

The Micropipette

1) Use a micropipette (p200) to transfer 25.5 µL of red dye solution onto a small piece of Parafilm or wax paper. After dispensing the dye, use the same pipette to pick up the drop and transfer it to a different location. Repeat the step with a new micropipette (p20) and 13.7 µL of red dye solution.

2) Are you able to pick up the entire drop of liquid each time? Yes / No Explain:

The Graduated Cylinder

1) Partially fill a 100 mL **glass** graduated cylinder with tap water and set it on your table. View the cylinder and measure the volume of the water while

(a) looking down at the cylinder _____, (b) at eye level _____, and (c) below and looking up at the cylinder _____.

Is there any difference between the three? Yes / No Explain:

2) Transfer the water to a **plastic** graduated cylinder. View the cylinder and measure the volume of the water while (a) looking down at the cylinder _____,

(b) at eye level _____, and (c) below and looking up at the cylinder _____.

Can the volume be read more precisely in the glass or plastic cylinder? Glass / Plastic

3) Add tap water to the 10 mL level in a 100 mL glass graduated cylinder. Then, transfer this water into a 10 mL graduated cylinder.

What measurement do you read on the smaller cylinder? _____

Which cylinder would be more accurate for measuring 5 mL of solution? _____

The Volumetric Flask

Your instructor will demonstrate this activity.

· Put a few drops of dye into the volumetric flask and add water until it reaches the 100 mL mark on the volumetric flask, use a dropper to be as accurate as possible.

· Pour the water into a graduated cylinder. Is it more, less than, or equal to, the 100 mL mark? _____

· Instructor: Now, pour this water into an Erlenmeyer flask and estimate the volume: _____mL

B. Precision versus Accuracy

1. Which device is more accurate? Volumetric flask / Graduated cylinder / Erlenmeyer flask
2. Which device is more precise? Volumetric flask / Graduated cylinder / Erlenmeyer flask

Preparing Solutions

1. Circle the concentration assigned to your group: 0.4, 0.6, 0.8, 1.0, 1.2, 1.4. Show your entire calculation below, calculate how many grams of NaCl is required to make your assigned molarity. Then, prepare 100 mL of NaCl solution of your assigned molarity.

2. Place a drop of water on the **hand refractometer** to measure the **specific gravity** of water. Record the specific gravity of the water drop: _____, then dry the refractometer with a KimWipe. Next, place a drop of your assigned NaCl solution onto the hand refractometer and record the specific gravity: _____. Clean with water and dry with a KimWipe.
3. Go around the room and measure the specific gravity for each group's solution. Clean with water and dry with a KimWipe after each use. Record the data in the table below, include your group's solution too.

NaCl solution: molarity vs. specific gravity

Molarity of NaCl solution	Specific gravity
0.4 M	
0.6 M	
0.8 M	
1.0 M	
1.2 M	
1.4 M	

Preparing Serial Dilutions

Serial dilutions- a stepwise dilution of a substance in solution. We use serial dilutions to produce low molarity solutions more accurately. Use your original assigned molarity as the stock solution.

- Measure 9 mL of water and transfer into a beaker or test tube. Then, transfer 1 mL of your stock solution into the 9 mL of water and mix well. This is your first dilution. (1/10 dilution)
- Next, measure 9 mL of water and transfer into a new beaker or test tube, then transfer 1 mL of your first dilution into this new 9 mL of water and mix. This is your second dilution. (1/10 * 1/10 = 1/100 dilution)
- Use the hand refractometer to measure the specific gravity of each of the two dilutions and record in the table below.
- Does specific gravity increase or decrease as you dilute a solution? _____

NaCl dilutions: molarity vs. specific gravity

NaCl stock solution	Molarity _____M	Specific gravity:
First dilution	Molarity _____M	Specific gravity:
Second dilution	Molarity _____M	Specific gravity:

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CHAPTER OVERVIEW

3: Scientific Investigation

Learning Objectives

Content Objectives

- Explain the relationship between dependent, independent, and control variables.
- Describe the characteristics of a useful hypothesis.
- Identify the characteristics of good experimental design.

Skill Objectives

- Use the scientific method to formulate a hypothesis and prediction.
- Use the scientific method to design an experiment to test a hypothesis and evaluate data.

3.1: Introduction
3.2: Exercises
3.3: Lab Report

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3.1: Introduction

Introduction to the scientific method

The scientific method is central to the study of biology: it is a process of acquiring and verifying information through experimentation. The general steps of the scientific method are depicted in Figure 3.1.1 below. Typically, a scientist begins their research with a question and then will formulate a hypothesis. The **hypothesis** is a tentative answer to their question, or an explanation to an observation. For a hypothesis to be useful it must be testable and falsifiable. Good experimental design is essential to the scientific method. A few keys to good experimental design include effective use of controls, reproducibility, a large sample size, and multiple trials. In an experiment, to be able to determine whether changes that occur are due to the investigator's manipulation, there must be some basis for comparison. A **control group** is necessary to establish this basis of comparison. In the control group, everything is kept the same as the experimental group except for the independent variable. The experimental group is the group that will receive the experimental treatment. For example, in a drug trial there will be a group that receives the drug (the experimental group) and a group that receives a placebo (the control group). The drug itself is considered the **independent variable** and any change(s) that occur because of the drug are considered the **dependent variable(s)**. To ensure that only the drug is causing the changes, all other variables must be tightly controlled (such as diet, exercise, smoking, age, etc.). These are referred to as **controlled variables**.

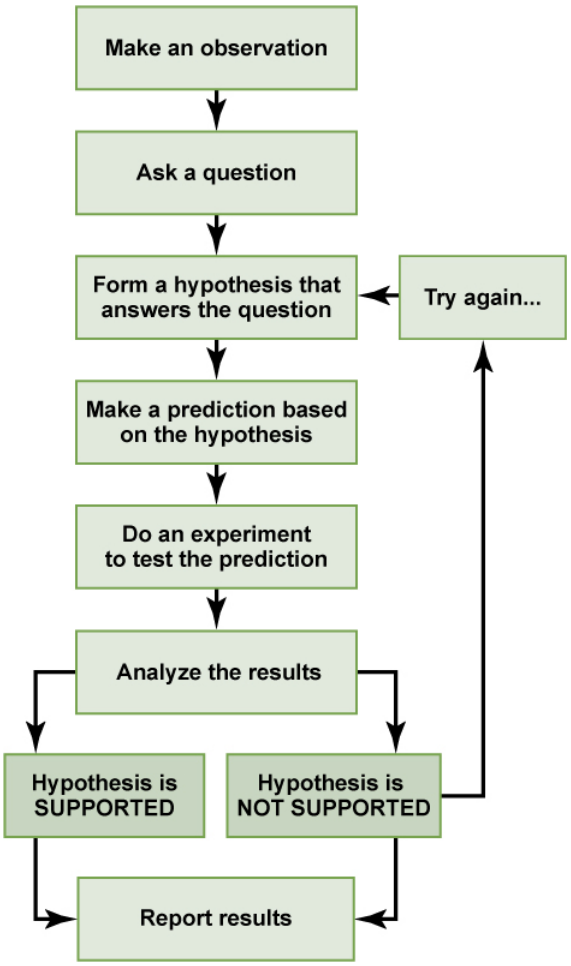


Figure 3.1.1: Scientific Method. The scientific method consists of a series of well-defined steps. If a hypothesis is not supported by experimental data, one can propose a new hypothesis. Figure credit: © Jun 9, 2022 OpenStax Biology 2e. Textbook content produced by OpenStax is licensed under a Creative Commons Attribution License (opens in new window).

Reporting Scientific Work

Whether scientific research is basic science or applied science, scientists must share their findings in order for other researchers to expand and build upon their discoveries. Collaboration with other scientists—when planning, conducting, and analyzing results—is important for scientific research. For this reason, important aspects of a scientist's work are communicating with peers and disseminating results to peers. Scientists can share results by presenting them at a scientific meeting or conference, but this approach only reaches those who are present. Instead, most scientists present their results in peer-reviewed manuscripts that are published in scientific journals. A **peer-reviewed manuscript** is scientific paper of a scientist's research methods, findings and interpretations, which is submitted to a scientific journal for publication. The publisher then forms a committee of reviewers to asses the veracity and merit of the work. Reviewers are qualified individuals that are experts in the same research area. The process of peer review helps to ensure that the research in a scientific paper is original, significant, logical, and thorough. Grant proposals, which are requests for research funding, are also subject to peer review. Scientists publish their work so other scientists can reproduce their experiments and expand on the findings.

A scientific paper is very different from creative writing. Although creativity is required to design experiments, there are fixed guidelines when it comes to presenting scientific results. First, scientific writing must be concise and accurate. A scientific paper needs to be succinct but detailed enough to allow peers to reproduce the experiments.

The scientific paper consists of several specific sections—introduction, materials and methods, results, and discussion. There are usually acknowledgment and reference sections as well as an **abstract** (a summary) at the beginning of the paper. There might be additional sections depending on the type of paper and the journal where it will be published. For example, some review papers require an outline.

The **introduction** starts with brief, but broad, background information about what is known in the field. A good introduction also gives the rationale of the work. It justifies the work carried out and also briefly mentions the end of the paper, where the researcher will present the hypothesis or research question driving the research. The introduction refers to the published scientific work of others and therefore requires citations following the style of the journal. Using the work or ideas of others without proper citation is **plagiarism**.

The **materials and methods** section includes a complete and accurate description of the substances the researchers use, and the method and techniques they use to gather data. The description should be thorough enough to allow another researcher to repeat the experiment and obtain similar results, but it does not have to be verbose. This section will also include information on how the researchers made measurements and the types of calculations and statistical analyses they used to examine raw data. Although the materials and methods section gives an accurate description of the experiments, it does not discuss them.

Some journals require a separate results section followed by a discussion section, but some journals combine both. If the journal does not allow combining both sections, the **results** section simply narrates the findings without any further interpretation. The researchers present results with tables or graphs, but they do not present duplicate information. In the **discussion** section, the researchers will interpret the results, describe how variables may be related, and attempt to explain the observations. It is indispensable to conduct an extensive literature search to put the results in the context of previously published scientific research. Therefore, researchers include proper citations in this section as well.

Finally, the **conclusion** section summarizes the importance of the experimental findings. While the scientific paper almost certainly answers one or more scientific questions that the researchers stated, most research articles will also include a future work statement, which are new questions based from their work. Therefore, a well-done scientific paper allows the researchers and others to continue and expand on the findings.

Review articles do not follow the above format because they do not present original scientific findings, or **primary literature**. Instead, they summarize and comment on findings that were published as primary literature and typically include extensive reference sections. These articles are particularly useful in learning the most current research on a particular topic and for gaining a perceptive on how different studies may used to answer a larger question.

All work adapted from Openstax Biology2e Ch 1.1

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3.2: Exercises

Exercise A: How Penicillin Was Discovered

In 1928, Sir Alexander Fleming was studying *Staphylococcus* bacteria growing in culture dishes. He noticed that a mold called *Penicillium* was also growing in some of the dishes. A clear area formed around the mold, indicating that the bacteria in this area had died. In the culture dishes without the mold, no clear areas were present. Fleming hypothesized that the mold must have produced a chemical that killed the bacteria. He decided to isolate the substance and test it to see if it would kill bacteria. Fleming transferred the mold to a nutrient broth solution. This solution contained all the materials the mold needed to grow. After the mold grew, he removed it from the nutrient broth. Fleming then transferred some of this broth onto a culture of bacteria. He observed that the bacteria died. This finding was later used to develop the first antibiotic, penicillin, which was used to treat a variety of bacterial diseases.

Exercise B: Identify the Controls and Variables

1. Muhammad thinks that a special juice will increase the productivity of workers. He creates two groups of 50 workers each and assigns each group the same task (in this case, they're supposed to staple a set of papers). Group A is given the special juice to drink while they work. Group B is not given the special juice. After an hour, Muhammad counts how many stacks of papers each group has made. Group A made 1,587 stacks; Group B made 2,113 stacks.

2. Sophia notices that her shower is covered in a strange green slime. She reads on social media that coconut juice will get rid of the green slime. Sophia decides to test this claim by spraying half of the shower with coconut juice. She sprays the other half of the shower with water. After three days of "treatment" there is no change in the appearance of the green slime on either side of the shower.

3. Anh was told that a certain anti-itch cream was the newest and best one on the market: it claims to reduce itching 50% longer than the leading brand. Interested in this product, she buys the anti-itch cream and compares it to her usual product. She finds two subjects that have itchy mosquito bites. Test subject A applies the original anti-itch cream, and test subject B applied the new anti-itch cream. Subject A reported their itching returned after two hours. Subject B reported their itching returned after one hour and 45 minutes.

4. Lisa is working on a science project. Her task is to answer the question: "Does Rogooti (which is a commercial hair product) affect the speed of hair growth". Her family is willing to volunteer for the experiment.

The Introduction, Exercise A, and Exercise B are adapted from the following:

Burran, Susan and DesRochers, David, "Principles of Biology I Lab Manual" (2015). Biological Sciences Open Textbooks. https://oer.galileo.usg.edu/biology-textbooks/3(opens in new window)

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Exercise C: Reporting Scientific Work

C.1 What is a primary reference?

C.2 Name two peer-reviewed scientific journals in the field of Biology.

C.2 Use the internet to find a peer-reviewed journal article related to cell and molecular biology. Then, in your own words, write a summary of the research (1-2 paragraphs). Include the full reference citation.

Exercise D: Designing an Experiment

We will split the class data into two groups, those that we consider to be athletic and those that are not. We want to know if athletic individuals have lower resting pulse rates than nonathletic individuals. For

our lab study, we will consider athletic individuals to be anyone that exercises three hours a week, or more. Lastly you will make a graph of the class data and evaluate your hypothesis (supported or falsified).

D.1 Create a testable hypothesis.

D.2 Create a prediction (**if** your hypothesis is true, **then** what results do you expect?).

Data collection: Each student will measure their pulse rate ten times and calculate the average. Be sure to be seated and calm for at least ten minutes prior to taking your pulse. Record your data in the table below and share your average with the class.

D.3 What are the controlled variables in our experiment?

D.4 What is the control group?

D.5 What is the level of treatment?

HR beats per 15 s and per minute

Measurement	1	2	3	4	5	6	7	8	9	10
Beats per 15 seconds										
Beats per minute										

Table 1. Individual Resting Pulse Rates

D.6 What is **your** average resting pulse rate in beats per minute (bpm)? _____ Place your average resting pulse rate onto the white board, make sure you correctly assign yourself as an athlete or non-athlete.

D.7 How many students are in the class? _____ How many students are athletic? _____ How many students are not athletic? _____

D.8 Copy the class information into the table below and calculate the class averages.

BPMs and averages of athletes vs. nonathletes

	Athletic	Nonathletic
List of individual pulse rate averages (bpm)		
Class average (bpm)		

Table 2. Summary of Class Data: Resting Pulse Rates of Athletic and Nonathletic Individuals

D. 9 What was the independent variable for our experiment? On which axis would you graph this?

D.10 What was the dependent variable for our experiment? On which axis would you graph this?

D.11 Design a graph that shows the relationship between the dependent and independent variables in the experiment. Should you use a line graph or a bar graph? Remember to give the graph a proper title, label your x-axis and y-axis, and include their units. Use a separate sheet of paper and staple it to this lab.

D.12 Drawing conclusions: Does the data support your hypothesis?

Exercise E: Design Your Own Experiment

During exercise muscle metabolism increases, resulting in an increase in the production of CO_2. Interestingly, however, our blood levels of CO_2 do not change significantly during exercise because the increased rate in CO_2 production is matched by a greater rate of CO_2 elimination by the body (respiratory rate and depth increase during exercise). The equation below describes how CO_2 is transported through the blood and is important in the study of respiration and acid-base balance.

$CO_2 + H_2O \rightarrow H_2CO_3 \rightarrow H^+ + HCO_3^-$

First, read the procedure. Then, complete the Experimental Design section below. After completing the experiment, record and explain your results.

A. Procedure:

1. Fill a 250 mL beaker with 200 mL of distilled water and add 5.0 mL of 0.10N NaOH and one or two drops of phenolphthalein indicator (turns pink in alkaline solutions; is clear in neutral/acidic solutions). Mix well and pour half of this solution into a 150 mL beaker (save this for later).
2. Select one student in your group to volunteer for this experiment. This student's results will be examined at rest and after 2-5 minutes of vigorous exercise.
3. Student: sitting quietly, exhale through two straws into the solution in the 250 mL beaker. Cover the beaker with a paper towel to avoid splashes. *Be careful NOT to inhale or drink the solution*. Group members: record the time required to turn the solution from pink to clear.
4. Time at rest (pink to clear): _____
5. Discard this first solution. Pour your saved solution from the 150 mL beaker into the 250 mL beaker, replace the two straws and cover with a paper towel.
6. Student: exercise vigorously *with your group members* for 2-5 minutes (find a safe and clear area outside of the lab room to do jumping jacks, etc. Heart rate and breathing rate should be increased.)
7. Student: repeat step 3 immediately after exercise (before your heart rate and breathing rate return to normal). *Be careful NOT to inhale or drink the solution*. Group members: record the time required to turn the solution from pink to clear.
8. Time after exercise (pink to clear): _____
9. Discard the solution and straws. Rinse and replace the glassware.

B. Experimental Design:

Question: _____

Testable hypothesis: _____

Dependent variable: _____

Independent variable: _____

Controlled variables: _____

Control: _____

Level of treatment: _____

Replication: _____

Prediction (if/then): _____

_____

C. Do your results support or refute your hypothesis? Explain your findings below:

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3.3: Lab Report

Exercise A: How Penicillin Was Discovered

A.1. Identify the problem.

A.2. What was Fleming's hypothesis?

A.3. How was the hypothesis tested?

A.4. Do the results indicate that the hypothesis should be rejected?

A.5. This experiment led to the development of what major medical advancement?

Exercise B: Identify the Controls and Variables

B.1.1 Identify the control group.

B.1.2 Identify the independent variable.

B.1.3 Identify the dependent variable.

B.1.4 What should Muhammad's conclusion be?

B.1.5 How could this experiment be improved?

B.2.1 What was the initial observation?

B.2.2 Identify the control group, independent variable and dependent variable.

B.2.3 What can be concluded from Sophia's experiment?

B.3.1 Identify the Control Group, Independent Variable, and Dependent Variable.

B.3.2 Explain whether the data supports the advertisements claims about the new product.

B.3.3 How could this experiment be improved?

B.4.1 Design Lisa's experiment.

The Introduction, Exercise A, and Exercise B are adapted from the following:

Burran, Susan and DesRochers, David, "Principles of Biology I Lab Manual" (2015). Biological Sciences Open Textbooks. https://oer.galileo.usg.edu/biology-textbooks/3

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Exercise D: Designing an Experiment

D.1 Create a testable hypothesis.

D.2 Create a prediction (**if** your hypothesis is true, **then** what results do you expect?).

Data collection: Each student will measure their pulse rate ten times and calculate the average. Be sure to be seated and calm for at least ten minutes prior to taking your pulse. Record your data in the table below and share your average with the class.

D.3 What are the controlled variables in our experiment?

D.4 What is the control group?

D.5 What is the level of treatment?

HR BPM per 15 s and per minute

Measurement	1	2	3	4	5	6	7	8	9	10
Beats per 15 seconds										
Beats per minute										

Table 1. Individual Resting Pulse Rates

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D.8 Copy the class information into the table below and calculate the class averages.

BPM and class average of athletes vs. nonathletes

	Athletic	Nonathletic
List of individual pulse rate averages (bpm)		
Class average (bpm)		

Table 2. Summary of Class Data: Resting Pulse Rates of Athletic and Nonathletic Individuals

D. 9 What was the independent variable for our experiment? On which axis would you graph this?

D.10 What was the dependent variable for our experiment? On which axis would you graph this?

D.11 Design a graph that shows the relationship between the dependent and independent variables in the experiment. Should you use a line graph or a bar graph? Remember to give the graph a proper title, label your x-axis and y-axis, and include their units. Use a separate sheet of paper and staple it to this lab.

D.12 Drawing conclusions: Does the data support your hypothesis?

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CHAPTER OVERVIEW

4: Ph and Buffers

Learning Objectives

Content Objectives

- Describe about pH, and pH indicators.
- Identify how buffers work.

Skill Objectives

- Prepare a pH indicator solution using anthocyanins extracted from red cabbage.
- Determine the pH of various common solutions.
- Visualize how buffers work to maintain a constant pH.

4.1: Introduction
4.2: Exercise
4.3: Lab Report

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4.1: Introduction

Introduction

The **pH** of a solution is a measure of its **acidity** or **alkalinity.** You have probably used litmus paper, paper that has been treated with a natural water-soluble dye so it can be used as a pH indicator, to test how much acid or base (alkalinity) exists in a solution. You might have even used some to make sure the water in an outdoor swimming pool is properly treated. In both cases, this pH test measures the amount of hydrogen ions that exists in a given solution. High concentrations of hydrogen ions yield a low pH, whereas low levels of hydrogen ions result in a high pH. The overall concentration of hydrogen ions is inversely related to its pH and can be measured on the pH scale (Figure 4.1.1). Therefore, the more hydrogen ions present, the lower the pH; conversely, the fewer hydrogen ions, the higher the pH.

The pH scale ranges from 0 to 14. A change of one unit on the pH scale represents a change in the concentration of hydrogen ions by a factor of 10, a change in two units represents a change in the concentration of hydrogen ions by a factor of 100. Thus, small changes in pH represent large changes in the concentrations of hydrogen ions. Pure water is **neutral.** It is neither acidic nor basic, and has a pH of 7.0. Anything below 7.0 (ranging from 0.0 to 6.9) is **acidic,** and anything above 7.0 (from 7.1 to 14.0) is **alkaline (basic).** The blood in your veins is slightly alkaline (pH = 7.4). The environment in your stomach is highly acidic (pH = 1 to 2). Orange juice is mildly acidic (pH = approximately 3.5), whereas baking soda is basic (pH = 9.0).

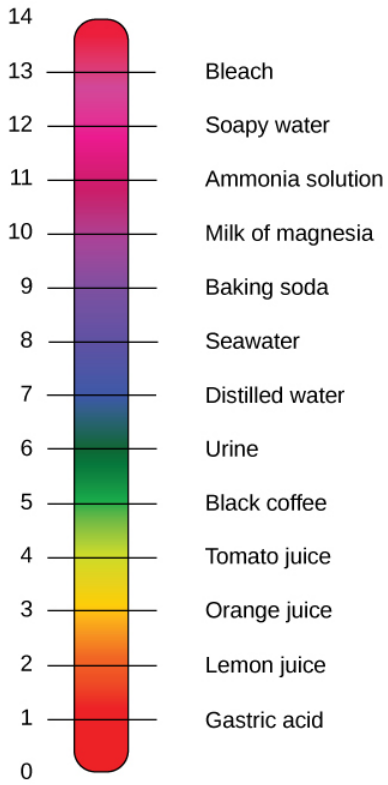


Figure 4.1.1**:** The pH scale measures the amount of hydrogen ions (H^+) in a substance. (credit: modification of work by Edward Stevens)

Acids are substances that provide hydrogen ions (H^+) and lower pH, whereas bases provide hydroxide ions (OH^-) and raise pH. The stronger the acid, the more readily it donates H^+. For example, hydrochloric acid and lemon juice are very acidic and readily give up H^+ when added to water. Conversely, bases are those substances that readily donate OH^-. The OH^- ions combine with H^+ to produce water, which raises a substance's pH. Sodium hydroxide and many household cleaners are very alkaline and give up OH^- rapidly when placed in water, thereby raising the pH.

Most cells in our bodies operate within a very narrow window of the pH scale, typically ranging only from 7.2 to 7.6. If the pH of the body is outside of this range, the respiratory system malfunctions, as do other organs in the body. Cells no longer function properly, and proteins will break down. Deviation outside of the pH range can induce coma or even cause death.

So how is it that we can ingest or inhale acidic or basic substances and not die? Buffers are the key. **Buffers** readily absorb excess H^+ or OH^-, keeping the pH of the body carefully maintained in the aforementioned narrow range. Carbon dioxide is part of a prominent buffer system in the human body; it keeps the pH within the proper range. This buffer system involves carbonic acid (H_2CO_3) and bicarbonate (HCO_3^-) anion. If too much H^+ enters the body, bicarbonate will combine with the H^+ to create carbonic acid and limit the decrease in pH. Likewise, if too much OH^- is introduced into the system, carbonic acid will rapidly dissociate into bicarbonate and H^+ ions. The H^+ ions can combine with the OH^- ions, limiting the increase in pH. While carbonic acid is an important product in this reaction, its presence is fleeting because the carbonic acid is released from the body as carbon dioxide gas each time we breathe. Without this buffer system, the pH in our bodies would fluctuate too much and we would fail to survive.

References

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OpenStax, Concepts of Biology. OpenStax CNX. March 22, 2017 https://cnx.org/contents/s8Hh0oOc@9....0BfSb7@4/Water

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4.2: Exercise

Exercise A: Using pH Indicators to Measure pH

Part 1: Making a pH Indicator

Solutions of anthocyanins (the plant pigments responsible for red, blue, and purple colors in flowers, fruits, autumn leaves, etc.) can be used as pH indicators. In low pH, these solutions turn red, whereas in high pH, they turn blue. In some flowers, however, soil pH impacts the update of certain metals that can complex with anthocyanin and alter its color expression. For example, in hydrangeas, low soil pH stimulates the uptake of aluminum, which then complexes with the anthocyanins and causes the flowers to now appear blue. Raising the pH of soil prevents aluminum uptake and a pink color will now be expressed. When the soil pH values are neutral, the flowers are intermediate in color.

A prepared solution of anthocyanins extracted from red cabbage will be used as a pH indicator in this lab. To use an indicator to determine the pH of unknown solutions, you must first determine the color changes that occur when the cabbage indicator is used with substances whose pH is known (your standards). You can then determine the pH of unknown substances by comparing color changes with your standards.

1. As a group, label eight clean test tubes with the following pH values: 2, 4, 6, 7, 8, 10, 12, and 14. Use the labeled pipettes to measure 5 mL of the appropriate buffer into each tube.
2. Use a pipette to measure 3 mL of cabbage extract and add it to each test tube. Cover each tube with Parafilm and mix well.
3. Record the color of each tube below. Note: record both initial and final colors at pH 12 and 14, since the pigments are not stable at these pH values. **Save these tubes for the entire lab period**.

pH vs. color of solution

pH:	**2**	**4**	**6**	**7**	**8**	**10**	**12**	**14**
Color:								

Part 2: Measuring pH - pH (alkacid) Test Paper versus Cabbage Extract

1. Label 2 clean test tubes: “A” and “B”
2. Transfer 2 mL of unknown solution A into tube A. Hold a test paper with forceps. Use a clean stirring rod to apply a drop of solution A to the test paper. While the paper is still wet, compare its color with the standard pH color scale on the label of the pH paper’s container. Record your pH in the table below.
3. Add 1 mL of cabbage extract into tube A and mix well.
4. Compare the color in tube A with the colors of the standards from part 1. The approximate pH of the unknown solution is the pH of the standard whose color most closely matches the color in tube A. Record your pH below.
5. Repeat the steps above for solution B.

pH and [H+] of unknown solution A and B

Solution	**pH** (pH paper)	**pH** (cabbage extract)	**[H^+]** (use pH from pH paper to calculate)
Unknown solution A			
Unknown solution B			

6. Do the results obtained for solutions A and B using the pH paper match those of the cabbage extract method? Yes / No Explain:

   _____

   _____

7. Use the pH from the pH paper to calculate [H^+]. Record your value in the table.
8. Which solution is more acidic? Unknown solution A / Unknown solution B

Exercise B: Determining the pH of Some Common Solutions

Part 1: pH of Beverages – *test one per group (assigned beverage: _____)*

Many beverages differ in their pH levels due to CO_2 content (if carbonated) or differences in their levels of organic acids.

1. Use the cabbage indicator method (2 mL of assigned beverage + 1 mL cabbage extract) to determine pH levels of Sprite and wine. Use pH paper to determine the pH of apple juice and coffee. Record your values below:

pH and [H+] of household solutions

Beverage	pH	List beverages in order of increasing [H^+]
Apple juice		Lowest:
Coffee (black)		
Sprite		
White wine		Highest:

Part 2: pH and Activity of Common Medicines – *(assigned medication: _____)*

Some medications, such as those used to guard against stomach acidity, are fairly alkaline, whereas others tend to be acidic. Use the cabbage indicator method (2 mL of assigned medication + 1 mL cabbage extract) to determine pH values for the following medications:

pH of household medications

Medication	pH
Aspirin	
Alka-Seltzer / Sodium bicarbonate ($NaHCO_3$)	

1. Health care providers often recommend that aspirin be taken with a large glass of milk or water. Based on your results above, why might this be important? _____
2. Would apple juice be a good replacement for water or milk while taking aspirin? Yes / No Explain: _____
   _____
3. The normal pH of the stomach is around 2-3. How might the Alka-Seltzer affect the stomach and/or digestion? _____ What might happen if someone uses too much Alka-Seltzer? _____ _____

Part 3: pH and the Action of Cleaning Solutions – *(assigned solution: _____)*

Based on your experiences with the cleaning solutions below, predict their pH value. Then, use the cabbage indicator method (2 mL of assigned cleaning solution + 1 mL cabbage extract) to determine the actual pH values of the cleaning solutions:

predicted and actual pH of household solutions

Cleaning solution	Predicted pH	Actual pH
Drano		
Ivory Liquid		
Cascade		
Tide		

Exercise C: The pH Meter - *test one per group (assigned solution: _____)*

In the previous exercises, you learned how the pH of a solution can be estimated by comparing indicator colors with known standards. Sometimes, however, greater accuracy is needed. Electronic methods of pH determination using pH meters provide more precise results. The pH meters provided in our lab will already have been standardized for you using standardization buffers close to the pH of the samples that will be tested.

1. Transfer the estimated pH values from Exercise C to the table below. Then, transfer 20 mL of solution into a 50 mL beaker, and add a small magnetic stirring bar. Turn the stir function on.
2. Remove the pH electrode from the resting solution, gently rinse with distilled water, and then measure the pH of the assigned solution. Make sure that the stir bar does not hit the electrode.
3. Measure the pH by changing the setting from “standby” to “pH” and record your pH.
4. Repeat these steps with the remaining solutions (if applicable). Make sure to switch back to “standby” and rinse the electrode with distilled water between solutions.
5. When you are finished, please rinse the electrode and replace it into the resting solution.

pH, [H+], and [OH-] of household solutions

Solution (20 mL)	Estimated pH	Measured pH	$[H^+]$	$[OH^-]$
Apple juice				
Sprite				
Maalox				
Tide				
Ivory Liquid				

6. How that we have a more accurate measure of pH, we can calculate $[H^+]$ or $[OH^-]$ with our calculators. Since pH is expressed as a negative logarithm of $[H^+]$, you need to determine the antilogarithm of the pH expressed as a negative number. Fill in your calculations above.

7. More practice:

pH and [H+] of household solutions

Solution	$[H^+]$	pH		Solution	pH	$[H^+]$
Urine	5.73×10^{-5}M			Lemon juice	2.7	
Pancreatic juice	7.98×10^{-8}M			Milk	6.5	

8. If the pH of a solution is 6, what is its OH^- concentration? First, find the antilog of -6. This will give you the H+ concentration of the solution. Then, divide 1×10^{-14} by $[H^+]$ to get $[OH^-]$. Calculate this value: _____

- If a solution has an OH^- concentration of 1×10^{-4} M, what is its pH? _____
- If a solution has an $[H^+] = 1 \times 10^{-6}$ M, what is its $[OH^-]$? _____

Exercise D: Buffers

pH levels must remain relatively constant for most physiological processes. For example, the pH of our blood is usually maintained between 7.35-7.45. However, blood returning to the heart contains CO_2 picked up from our tissues, our diets, as well as the normal metabolic reactions in our cells, and may contribute to an excess of hydrogen ions. Buffer systems help maintain constant pH in the body.

A buffer is a solution that resists changes in pH when small amounts of acid or base are added. Bicarbonate, phosphate, and protein buffer systems maintain our blood pH. A buffer is made by mixing a weak acid with its salt in order to have in solution something that can act both as an acid (donate hydrogen ions) and something that can act as a base (accept hydrogen ions). For example,

KH_2PO_4 can be ionized to $K^+ + H^+ + HPO_4^{2-}$. If hydrogen ions are added to the solution, they can be picked up by HPO_4^{2-}, which acts as a base. If hydroxyl ions are added to the solution, they can be picked up by H^+.

1. Find the unknown solutions marked C and D. Pour 40 mL of unknown solution C into a 100 mL beaker. Add a stir bar and place the beaker on the stirring plate.
2. Add 1 drop of Congo red. Record the initial color of the solution in the table below.
3. Measure the initial pH using the pH meter and record this value.
4. Using a pipette, add 2 mL of 0.1 N HCl. Record the final color of the solution and its final pH.
5. Dispose of this solution, and wash and dry the beaker and stir bar thoroughly.
6. Pour another 40 mL of the same unknown solution into the beaker, add the stir bar, and return it to the stirring plate. Add 1 drop of phenolphthalein and again measure the initial color and pH and record in the table below.
7. Now use a pipette to add 2 mL of 0.1 N NaOH. Record the final color and pH.
8. Dispose of this solution, and wash and dry the beaker and stir bar thoroughly.
9. Repeat these steps for unknown solution D.

color and pH of unknown solutions C and D

Unknown solution	Initial color	Initial pH	Solution added	Final color	Final pH
C + Congo red			0.1 *N* HCl		
C + Phenolphthalein			0.1 *N* NaOH		
D + Congo red			0.1 *N* HCl		
D + Phenolphthalein			0.1 *N* NaOH		

10. Which unknown solution contains buffers? C or D How could you tell? _____
    _____
11. Which indicator would be best to use to determine the presence of an acid? _____ What color would it appear at pH 2? _____ pH 7? _____ pH 9? _____
12. Which indicator would be best to use to determine the presence of base? _____ What color would it appear at pH 2? _____ pH 7? _____ pH 9? _____

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4.3: Lab Report

Record the color of each tube below. Note: record both initial and final colors at pH 12 and 14, since the pigments are not stable at these pH values. **Save these tubes for the entire lab period**.

pH vs. color of solution

pH:	**2**	**4**	**6**	**7**	**8**	**10**	**12**	**14**
Color:								

Record your pH values below:

pH and [H+] of unknown solution A and B

Solution	**pH** (pH paper)	**pH** (cabbage extract)	**[H^+]** (use pH from pH paper to calculate)
Unknown solution A			
Unknown solution B			

6. Do the results obtained for solutions A and B using the pH paper match those of the cabbage extract method? Yes / No Explain: _____ _____
7. Use the pH from the pH paper to calculate [H^+]. Record your value in the table.
8. Which solution is more acidic? Unknown solution A / Unknown solution B

Exercise B: Determining the pH of Some Common Solutions

Many beverages differ in their pH levels due to CO_2 content (if carbonated) or differences in their levels of organic acids.

Record your values below:

pH and [H+] of household solutions

Beverage	**pH**	**List beverages in order of increasing [H^+]**
Apple juice		Lowest:
Coffee (black)		
Sprite		
White wine		Highest:

Record your pH values below:

pH of household medications

Medication	**pH**
Aspirin	
Alka-Seltzer / Sodium bicarbonate ($NaHCO_3$)	

1. Health care providers often recommend that aspirin be taken with a large glass of milk or water. Based on your results above, why might this be important? _____
2. Would apple juice be a good replacement for water or milk while taking aspirin? Yes / No Explain: _____ _____

3. The normal pH of the stomach is around 2-3. How might the Alka-Seltzer affect the stomach and/or digestion? _____ What might happen if someone uses too much Alka-Seltzer? _____ _____

Based on your experiences with the cleaning solutions below, predict their pH value. Then, use the cabbage indicator method (2 mL of assigned cleaning solution + 1 mL cabbage extract) to determine the actual pH values of the cleaning solutions:

predicted and actual pH of household solutions

Cleaning solution	Predicted pH	Actual pH
Drano		
Ivory Liquid		
Cascade		
Tide		

Transfer the estimated pH values from Exercise C to the table below. Then, measure the pH and record your values below:

pH, [H+], and [OH-] of household solutions

Solution (20 mL)	Estimated pH	Measured pH	$[H^+]$	$[OH^-]$
Apple juice				
Sprite				
Maalox				
Tide				
Ivory Liquid				

How that we have a more accurate measure of pH, we can calculate $[H^+]$ or $[OH^-]$ with our calculators. Since pH is expressed as a negative logarithm of $[H^+]$, you need to determine the antilogarithm of the pH expressed as a negative number. Fill in your calculations above.

More practice:

pH and [H+] of household solutions

Solution	$[H^+]$	pH		Solution	pH	$[H^+]$
Urine	5.73×10^{-5}M			Lemon juice	2.7	
Pancreatic juice	7.98×10^{-8}M			Milk	6.5	

8. If the pH of a solution is 6, what is its OH^- concentration? First, find the antilog of -6. This will give you the H+ concentration of the solution. Then, divide 1×10^{-14} by $[H^+]$ to get $[OH^-]$. Calculate this value: _____

- If a solution has an OH^- concentration of 1×10^{-4} M, what is its pH? _____
- If a solution has an $[H^+] = 1 \times 10^{-6}$ M, what is its $[OH^-]$? _____

Record the initial color, initial pH, final color, and final pH for each solution below:

color and pH of unknown solutions C and D

Unknown solution	Initial color	Initial pH	Solution added	Final color	Final pH
C + Congo red			0.1 *N* HCl		
C + Phenolphthalein			0.1 *N* NaOH		
D + Congo red			0.1 *N* HCl		
D + Phenolphthalein			0.1 *N* NaOH		

10. Which unknown solution contains buffers? C or D How could you tell? _____
    _____
11. Which indicator would be best to use to determine the presence of an acid? _____ What color would it appear at pH 2? _____ pH 7? _____ pH 9? _____
12. Which indicator would be best to use to determine the presence of base? _____ What color would it appear at pH 2? _____ pH 7? _____ pH 9? _____

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CHAPTER OVERVIEW

5: Organic Macromolecules

Learning Objectives

Content Objectives

- Recognize and discuss three of the four biological macromolecules.
- Learn to identify an unknown macromolecule.

Skill Objectives

- Practice using a variety of tests to identify carbohydrates, lipids, and proteins/amino acids.
- Use your testing skills to identify an unknown macromolecule.

5.1: Introduction
5.2: Exercise
5.3: Lab Report

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5.1: Introduction

Introduction

As you've learned, biological macromolecules are large molecules, necessary for life, that are built from smaller organic molecules. There are four major classes of biological macromolecules (carbohydrates, lipids, proteins, and nucleic acids); each is an important cell component and performs a wide array of functions. Combined, these molecules make up the majority of a cell's dry mass (recall that water makes up the majority of its complete mass). Biological macromolecules are organic, meaning they contain carbon. In addition, they may contain hydrogen, oxygen, nitrogen, and additional minor elements.

In today's lab, we will learn to identify three of the four types of macromolecules: carbohydrates, fats, and proteins. The fourth type, nucleotides, will be studied in detail in future labs.

A. Carbohydrates

Most people are familiar with carbohydrates, one type of macromolecule, especially when it comes to what we eat. To lose weight, some individuals adhere to "low-carb" diets. Athletes, in contrast, often "carb-load" before important competitions to ensure that they have enough energy to compete at a high level. Carbohydrates are, in fact, an essential part of our diet; grains, fruits, and vegetables are all natural sources of carbohydrates. Carbohydrates provide energy to the body, particularly through glucose, a simple sugar that is a component of starch and an ingredient in many staple foods. Carbohydrates also have other important functions in humans, animals, and plants.

Molecular Structures

Carbohydrates can be represented by the stoichiometric formula $(CH_2O)_n$, where n is the number of carbons in the molecule. In other words, the ratio of carbon to hydrogen to oxygen is 1:2:1 in carbohydrate molecules. This formula also explains the origin of the term "carbohydrate": the components are carbon ("carbo") and the components of water (hence, "hydrate"). Carbohydrates are classified into three subtypes: monosaccharides, disaccharides, and polysaccharides.

Monosaccharides

Monosaccharides (mono- = "one"; sacchar- = "sweet") are simple sugars, the most common of which is glucose. In monosaccharides, the number of carbons usually ranges from three to seven. Most monosaccharide names end with the suffix -ose. If the sugar has an aldehyde group (the functional group with the structure R-CHO), it is known as an aldose, and if it has a ketone group (the functional group with the structure RC(=O)R'), it is known as a ketose. Depending on the number of carbons in the sugar, they also may be known as trioses (three carbons), pentoses (five carbons), and or hexoses (six carbons). See Figure 5.1.1 for an illustration of the monosaccharides.

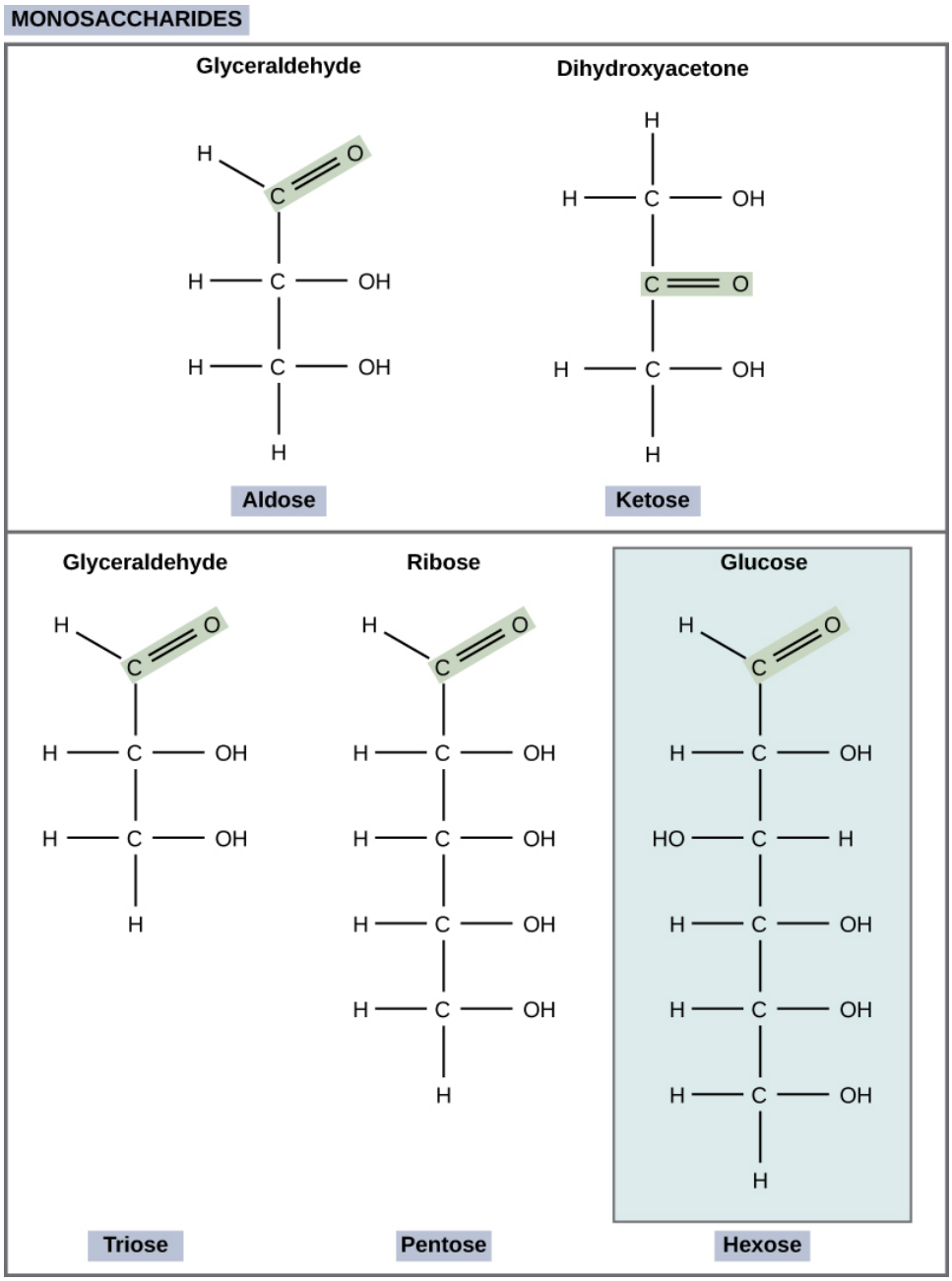


Figure 5.1.1: Monosaccharides are classified based on the position of their carbonyl group and the number of carbons in the backbone. Aldoses have a carbonyl group (indicated in green) at the end of the carbon chain, and ketoses have a carbonyl group in the middle of the carbon chain. Trioses, pentoses, and hexoses have three, five, and six carbon backbones, respectively.

The chemical formula for glucose is $C_6H_{12}O_6$. In humans, glucose is an important source of energy. During cellular respiration, energy is released from glucose, and that energy is used to help make adenosine triphosphate (ATP). Plants synthesize glucose using carbon dioxide and water, and glucose in turn is used for energy requirements for the plant. Excess glucose is often stored as starch that is catabolized (the breakdown of larger molecules by cells) by humans and other animals that feed on plants.

Galactose (part of lactose, or milk sugar) and fructose (found in sucrose, in fruit) are other common monosaccharides. Although glucose, galactose, and fructose all have the same chemical formula ($C_6H_{12}O_6$), they differ structurally and chemically (and are known as isomers) because of the different arrangement of functional groups around the asymmetric carbon; all of these monosaccharides have more than one asymmetric carbon (Figure 5.1.2).

Art Connection

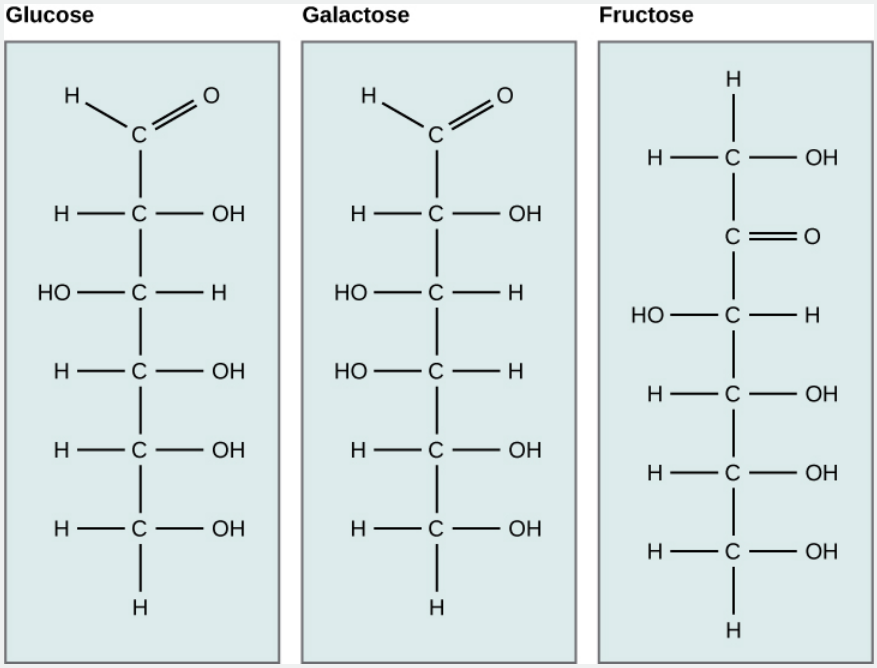


Figure 5.1.2: Glucose, galactose, and fructose are all hexoses. They are structural isomers, meaning they have the same chemical formula ($C_6H_{12}O_6$) but a different arrangement of atoms.

What kind of sugars are these, aldose or ketose?

Glucose, galactose, and fructose are isomeric monosaccharides (hexoses), meaning they have the same chemical formula but have slightly different structures. Glucose and galactose are aldoses, and fructose is a ketose.

Monosaccharides can exist as a linear chain or as ring-shaped molecules; in aqueous solutions they are usually found in ring forms (Figure 5.1.3). Glucose in a ring form can have two different arrangements of the hydroxyl group (OH) around the anomeric carbon (carbon 1 that becomes asymmetric in the process of ring formation). If the hydroxyl group is below carbon number 1 in the sugar, it is said to be in the alpha (α) position, and if it is above the plane, it is said to be in the beta (β) position.

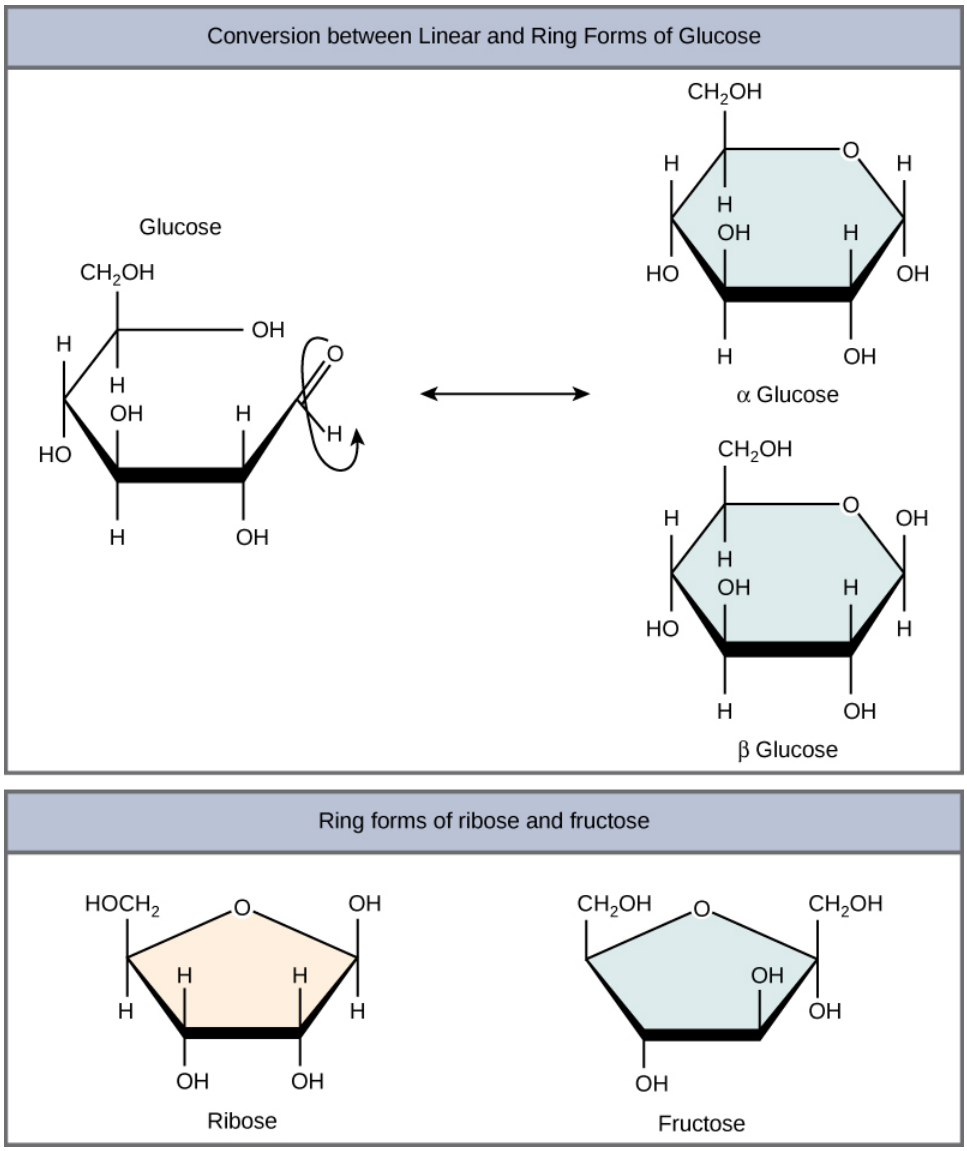


Figure 5.1.3: Five and six carbon monosaccharides exist in equilibrium between linear and ring forms. When the ring forms, the side chain it closes on is locked into an α or β position. Fructose and ribose also form rings, although they form five-membered rings as opposed to the six-membered ring of glucose.

Disaccharides

Disaccharides (di- = “two”) form when two monosaccharides undergo a dehydration reaction (also known as a condensation reaction or dehydration synthesis). During this process, the hydroxyl group of one monosaccharide combines with the hydrogen of another monosaccharide, releasing a molecule of water and forming a covalent bond. A covalent bond formed between a carbohydrate molecule and another molecule (in this case, between two monosaccharides) is known as a glycosidic bond (Figure 5.1.4). Glycosidic bonds (also called glycosidic linkages) can be of the alpha or the beta type.

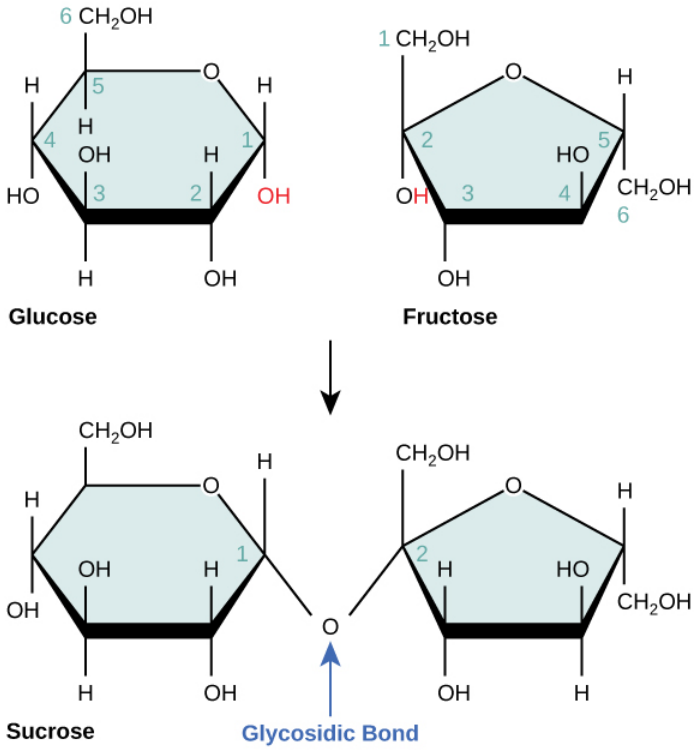


Figure 5.1.4: Sucrose is formed when a monomer of glucose and a monomer of fructose are joined in a dehydration reaction to form a glycosidic bond. In the process, a water molecule is lost. By convention, the carbon atoms in a monosaccharide are numbered from the terminal carbon closest to the carbonyl group. In sucrose, a glycosidic linkage is formed between carbon 1 in glucose and carbon 2 in fructose.

Common disaccharides include lactose, maltose, and sucrose (Figure 5.1.5). Lactose is a disaccharide consisting of the monomers glucose and galactose. It is found naturally in milk. Maltose, or malt sugar, is a disaccharide formed by a dehydration reaction between two glucose molecules. The most common disaccharide is sucrose, or table sugar, which is composed of the monomers glucose and fructose.

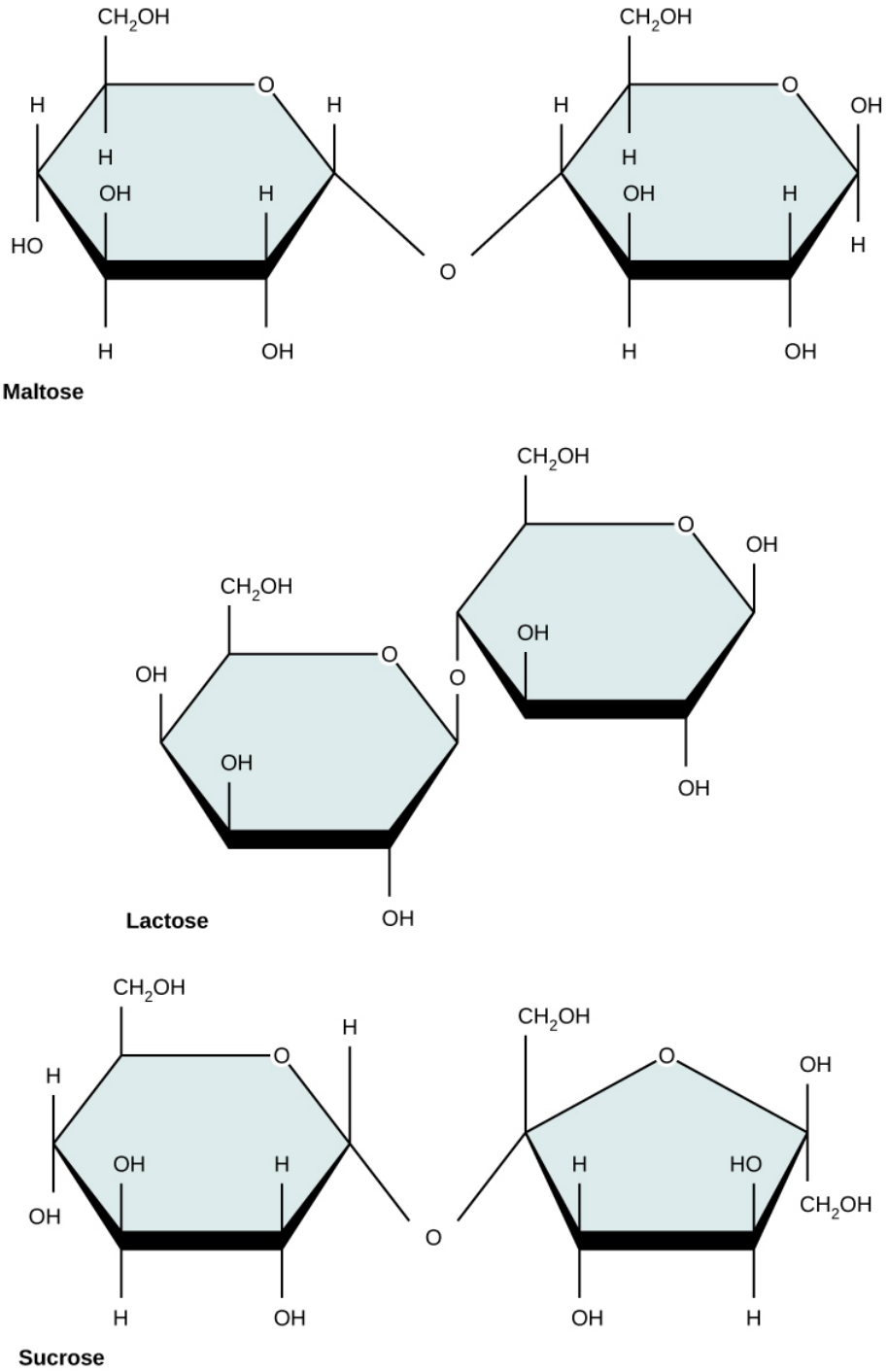


Figure 5.1.5: Common disaccharides include maltose (grain sugar), lactose (milk sugar), and sucrose (table sugar).

Polysaccharides

A long chain of monosaccharides linked by glycosidic bonds is known as a polysaccharide (poly- = “many”). The chain may be branched or unbranched, and it may contain different types of monosaccharides. The molecular weight may be 100,000 daltons or more depending on the number of monomers joined. Starch, glycogen, cellulose, and chitin are primary examples of polysaccharides.

Starch is the stored form of sugars in plants and is made up of a mixture of amylose and amylopectin (both polymers of glucose). Plants are able to synthesize glucose, and the excess glucose, beyond the plant’s immediate energy needs, is stored as starch in different plant parts, including roots and seeds. The starch in the seeds provides food for the embryo as it germinates and can also

act as a source of food for humans and animals. The starch that is consumed by humans is broken down by enzymes, such as salivary amylases, into smaller molecules, such as maltose and glucose. The cells can then absorb the glucose.

Starch is made up of glucose monomers that are joined by α 1-4 or α 1-6 glycosidic bonds. The numbers 1-4 and 1-6 refer to the carbon number of the two residues that have joined to form the bond. As illustrated in Figure 5.1.6, amylose is starch formed by unbranched chains of glucose monomers (only α 1-4 linkages), whereas amylopectin is a branched polysaccharide (α 1-6 linkages at the branch points).

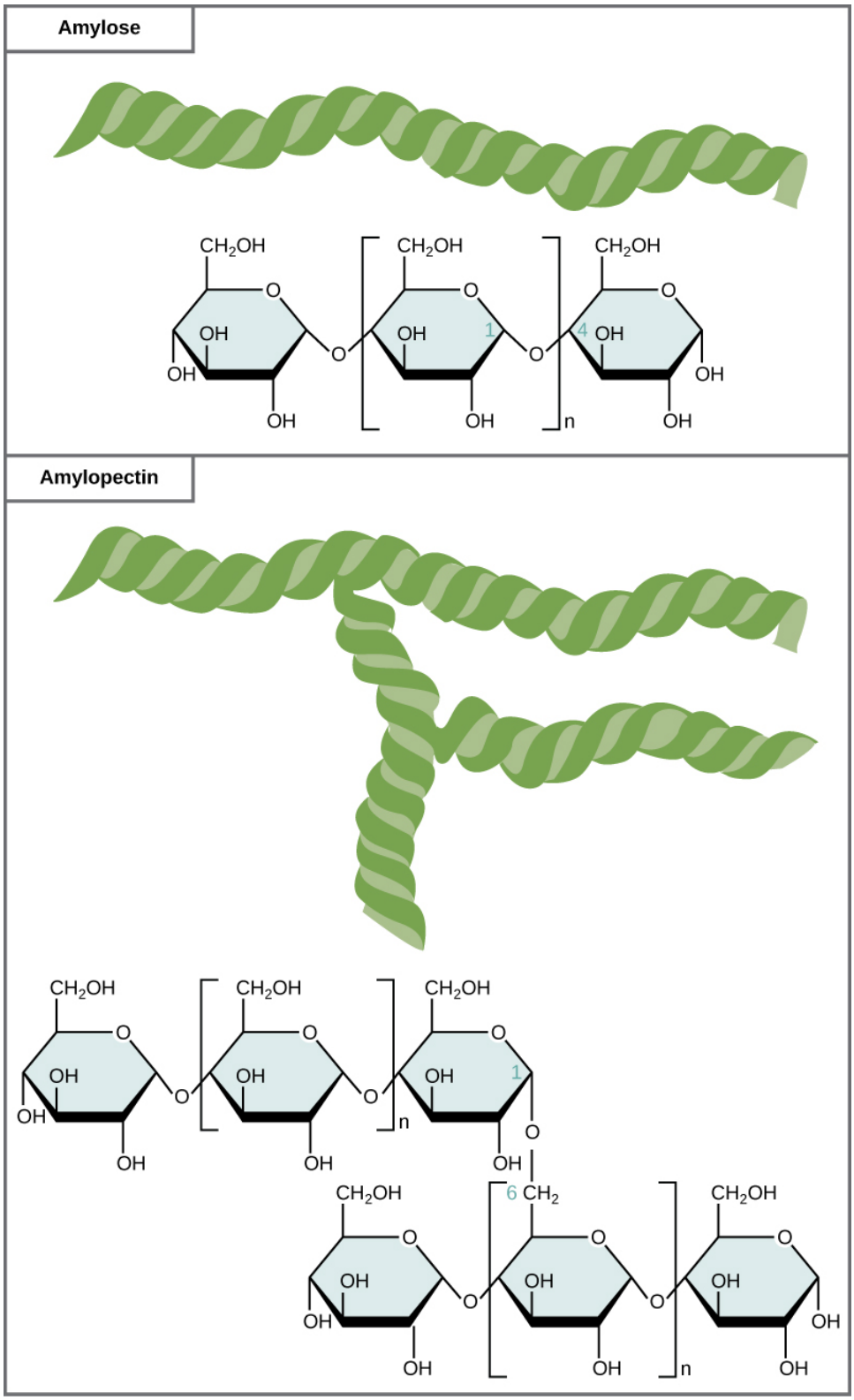


Figure 5.1.6: Amylose and amylopectin are two different forms of starch. Amylose is composed of unbranched chains of glucose monomers connected by α 1,4 glycosidic linkages. Amylopectin is composed of branched chains of glucose monomers connected by α 1,4 and α 1,6 glycosidic linkages. Because of the way the subunits are joined, the glucose chains have a helical structure. Glycogen (not shown) is similar in structure to amylopectin but more highly branched.

Glycogen is the storage form of glucose in humans and other vertebrates and is made up of monomers of glucose. Glycogen is the animal equivalent of starch and is a highly branched molecule usually stored in liver and muscle cells. Whenever blood glucose

levels decrease, glycogen is broken down to release glucose in a process known as glycogenolysis.

Cellulose is the most abundant natural biopolymer. The cell wall of plants is mostly made of cellulose; this provides structural support to the cell. Wood and paper are mostly cellulosic in nature. Cellulose is made up of glucose monomers that are linked by β 1-4 glycosidic bonds (Figure 5.1.7).

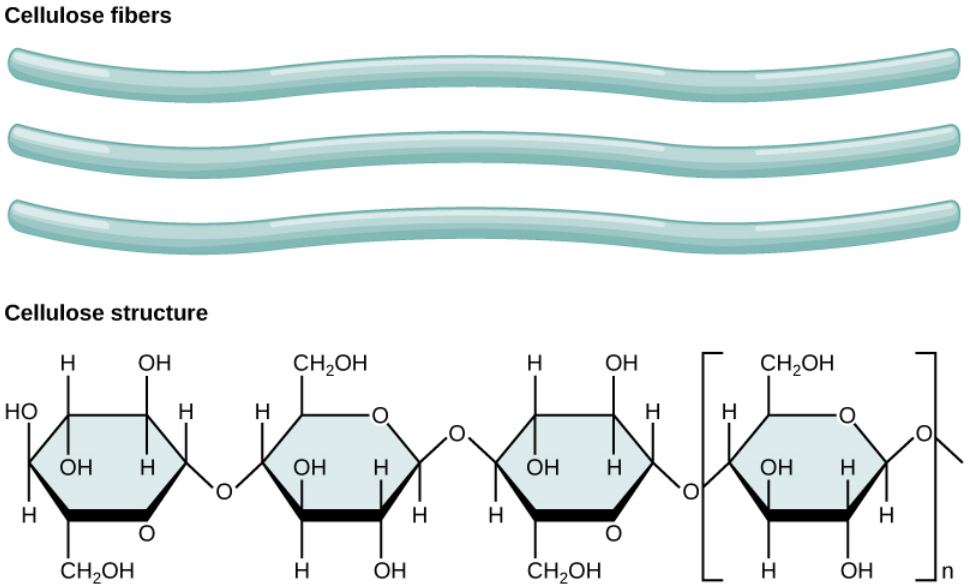


Figure 5.1.7: In cellulose, glucose monomers are linked in unbranched chains by β 1-4 glycosidic linkages. Because of the way the glucose subunits are joined, every glucose monomer is flipped relative to the next one resulting in a linear, fibrous structure.

B. Lipids

Lipids include a diverse group of compounds that are largely nonpolar in nature. This is because they are hydrocarbons that include mostly nonpolar carbon–carbon or carbon–hydrogen bonds. Non-polar molecules are hydrophobic (“water fearing”), or insoluble in water. Lipids perform many different functions in a cell. Cells store energy for long-term use in the form of fats. Lipids also provide insulation from the environment for plants and animals. For example, they help keep aquatic birds and mammals dry when forming a protective layer over fur or feathers because of their water-repellant hydrophobic nature. Lipids are also the building blocks of many hormones and are an important constituent of all cellular membranes. Lipids include fats, oils, waxes, phospholipids, and steroids.

Fats and Oils

A fat molecule consists of two main components—glycerol and fatty acids. Glycerol is an organic compound (alcohol) with three carbons, five hydrogens, and three hydroxyl (OH) groups. Fatty acids have a long chain of hydrocarbons to which a carboxyl group is attached, hence the name “fatty acid.” The number of carbons in the fatty acid may range from 4 to 36; most common are those containing 12–18 carbons. In a fat molecule, the fatty acids are attached to each of the three carbons of the glycerol molecule with an ester bond through an oxygen atom (Figure 5.1.2).

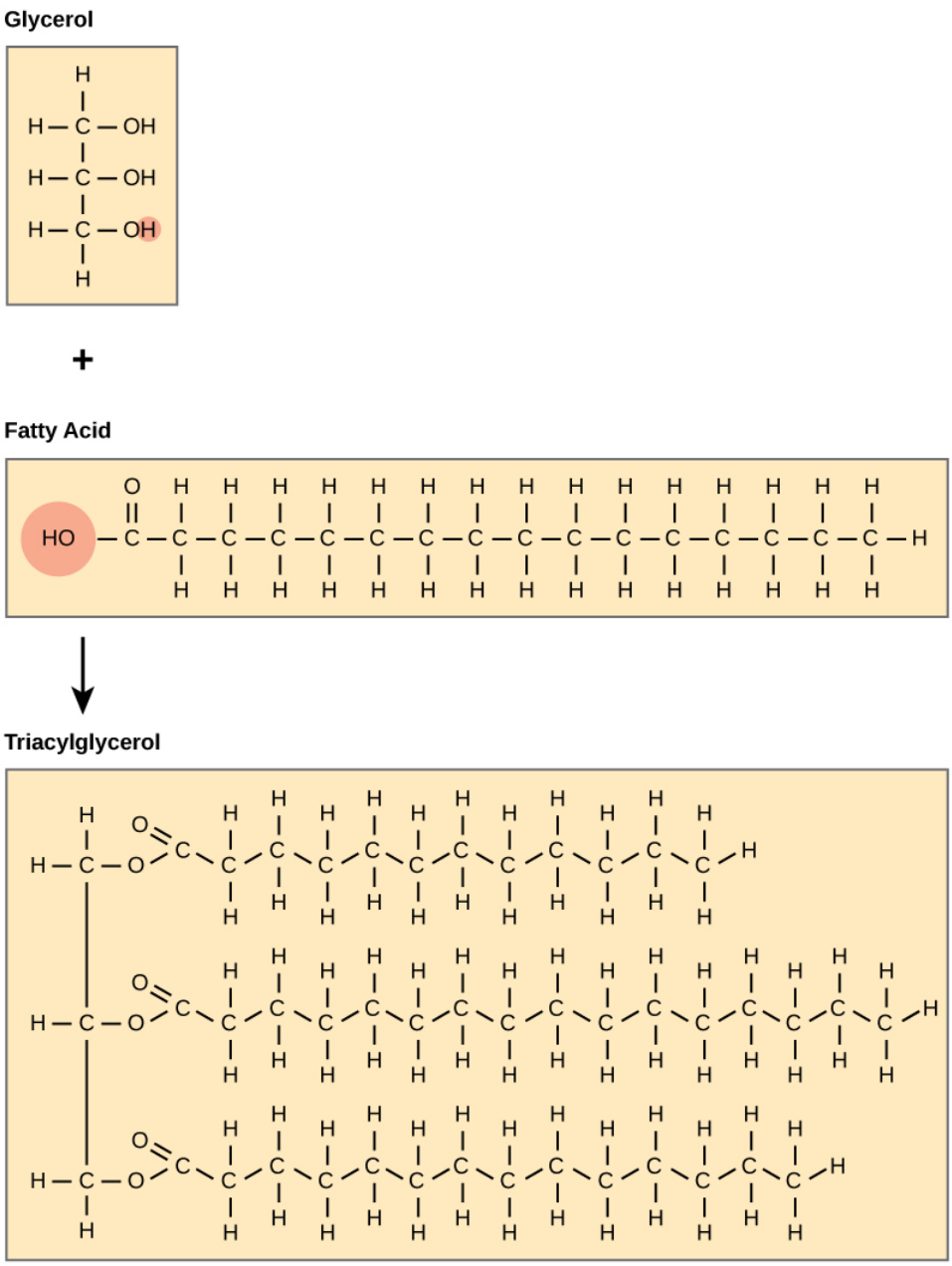


Figure 5.1.8: Triacylglycerol is formed by the joining of three fatty acids to a glycerol backbone in a dehydration reaction. Three molecules of water are released in the process.

During this ester bond formation, three water molecules are released. The three fatty acids in the triacylglycerol may be similar or dissimilar. Fats are also called triacylglycerols or triglycerides because of their chemical structure. Some fatty acids have common names that specify their origin. For example, palmitic acid, a saturated fatty acid, is derived from the palm tree. Arachidic acid is derived from *Arachis hypogea*, the scientific name for groundnuts or peanuts.

Fatty acids may be saturated or unsaturated. In a fatty acid chain, if there are only single bonds between neighboring carbons in the hydrocarbon chain, the fatty acid is said to be saturated. Saturated fatty acids are saturated with hydrogen; in other words, the number of hydrogen atoms attached to the carbon skeleton is maximized. Stearic acid is an example of a saturated fatty acid (Figure 5.1.3)

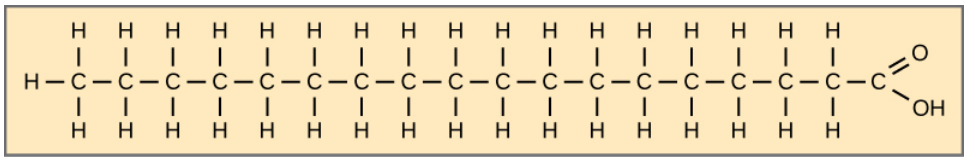


Figure 5.1.3: Stearic acid is a common saturated fatty acid.

When the hydrocarbon chain contains a double bond, the fatty acid is said to be unsaturated. Oleic acid is an example of an unsaturated fatty acid (Figure 5.1.4).

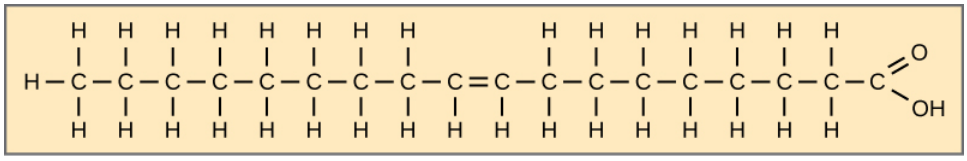


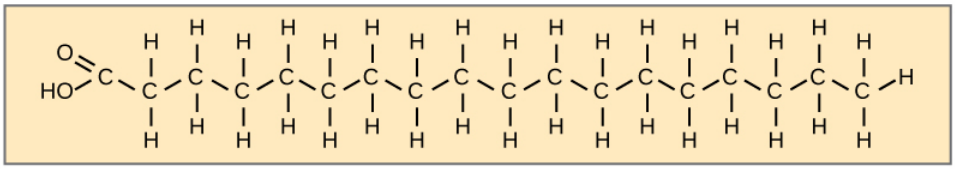
Figure 5.1.9: Oleic acid is a common unsaturated fatty acid.

Most unsaturated fats are liquid at room temperature and are called oils. If there is one double bond in the molecule, then it is known as a monounsaturated fat (e.g., olive oil), and if there is more than one double bond, then it is known as a polyunsaturated fat (e.g., canola oil).

When a fatty acid has no double bonds, it is known as a saturated fatty acid because no more hydrogen may be added to the carbon atoms of the chain. A fat may contain similar or different fatty acids attached to glycerol. Long straight fatty acids with single bonds tend to get packed tightly and are solid at room temperature. Animal fats with stearic acid and palmitic acid (common in meat) and the fat with butyric acid (common in butter) are examples of saturated fats. Mammals store fats in specialized cells called adipocytes, where globules of fat occupy most of the cell's volume. In plants, fat or oil is stored in many seeds and is used as a source of energy during seedling development. Unsaturated fats or oils are usually of plant origin and contain *cis* unsaturated fatty acids. *Cis* and *trans* indicate the configuration of the molecule around the double bond. If hydrogens are present in the same plane, it is referred to as a cis fat; if the hydrogen atoms are on two different planes, it is referred to as a trans fat. The *cis* double bond causes a bend or a "kink" that prevents the fatty acids from packing tightly, keeping them liquid at room temperature (Figure 5.1.5). Olive oil, corn oil, canola oil, and cod liver oil are examples of unsaturated fats. Unsaturated fats help to lower blood cholesterol levels whereas saturated fats contribute to plaque formation in the arteries.

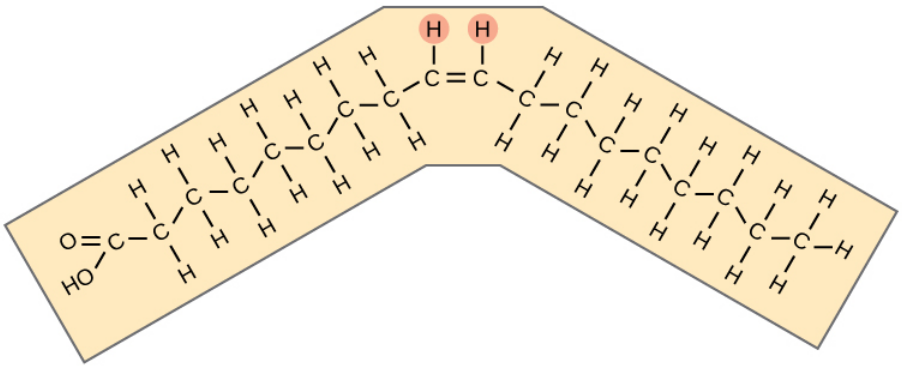
Saturated fatty acid

Stearic acid



Unsaturated fatty acids

Cis oleic acid



Trans oleic acid

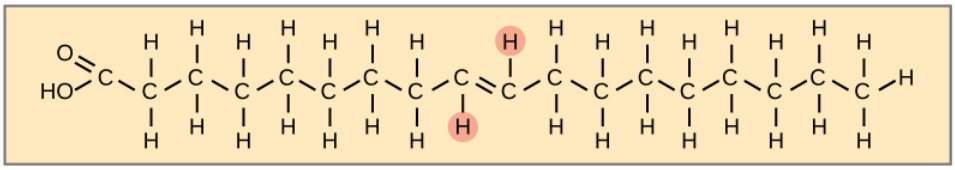


Figure 5.1.10: Saturated fatty acids have hydrocarbon chains connected by single bonds only. Unsaturated fatty acids have one or more double bonds. Each double bond may be in a *cis* or *trans* configuration. In the *cis* configuration, both hydrogens are on the same side of the hydrocarbon chain. In the *trans* configuration, the hydrogens are on opposite sides. A *cis* double bond causes a kink in the chain.

Trans Fats

In the food industry, oils are artificially hydrogenated to make them semi-solid and of a consistency desirable for many processed food products. Simply speaking, hydrogen gas is bubbled through oils to solidify them. During this hydrogenation process, double bonds of the *cis*- conformation in the hydrocarbon chain may be converted to double bonds in the trans- conformation.

Margarine, some types of peanut butter, and shortening are examples of artificially hydrogenated trans fats. Recent studies have shown that an increase in trans fats in the human diet may lead to an increase in levels of low-density lipoproteins (LDL), or “bad” cholesterol, which in turn may lead to plaque deposition in the arteries, resulting in heart disease. Many fast food restaurants have recently banned the use of trans fats, and food labels are required to display the trans fat content.

C. Proteins

Proteins are one of the most abundant organic molecules in living systems and have the most diverse range of functions of all macromolecules. Proteins may be structural, regulatory, contractile, or protective; they may serve in transport, storage, or membranes; or they may be toxins or enzymes. Each cell in a living system may contain thousands of proteins, each with a unique function. Their structures, like their functions, vary greatly. They are all, however, polymers of amino acids, arranged in a linear sequence.

Amino Acids

All proteins are made up of different arrangements of the same 20 types of amino acids. Amino acids are the monomers that make up proteins. Each amino acid has the same fundamental structure, which consists of a central carbon atom, also known as the alpha (α) carbon, bonded to an amino group (NH_2), a carboxyl group (COOH), and to a hydrogen atom. Every amino acid also has another atom or group of atoms bonded to the central atom known as the R group (Figure 5.1.11).

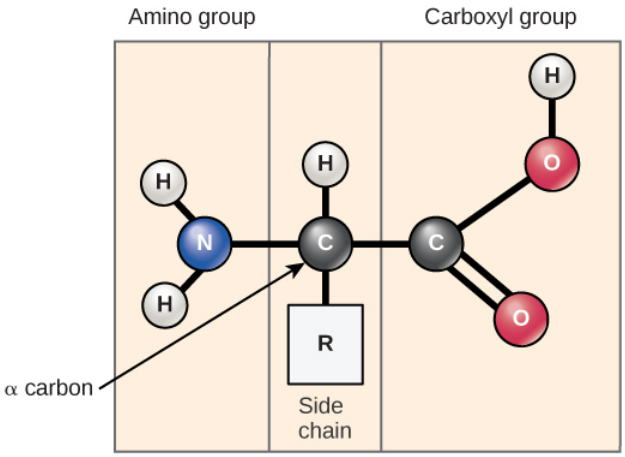


Figure 5.1.11: Amino acids have a central asymmetric carbon to which an amino group, a carboxyl group, a hydrogen atom, and a side chain (R group) are attached.

The name "amino acid" is derived from the fact that they contain both amino group and carboxyl-acid-group in their basic structure. As mentioned, there are 20 amino acids present in proteins. Ten of these are considered essential amino acids in humans because the human body cannot produce them and they are obtained from the diet. For each amino acid, the R group (or side chain) is different (Figure 5.1.12).

Art Connection

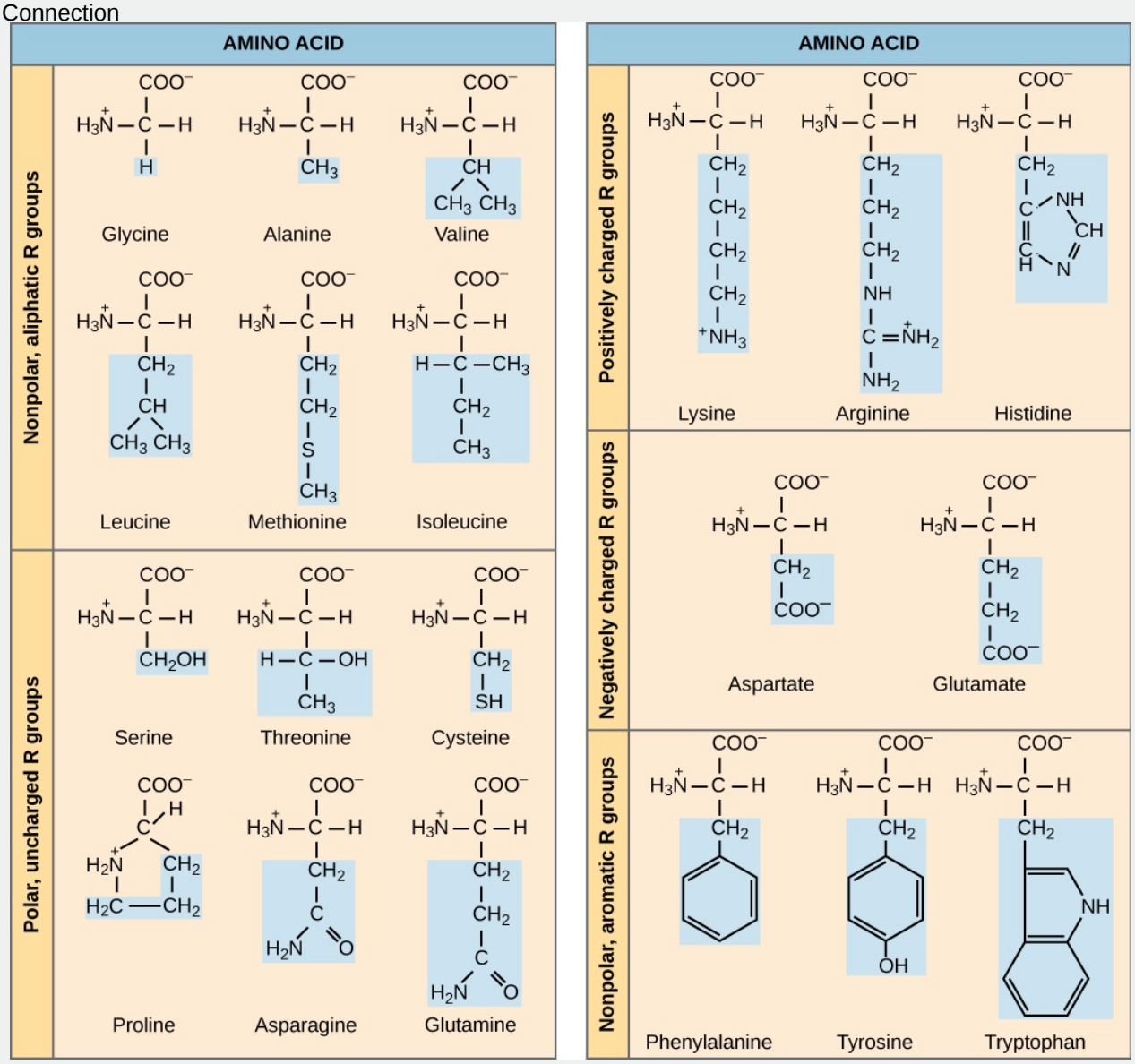


Figure 5.1.12: There are 20 common amino acids commonly found in proteins, each with a different R group (variant group) that determines its chemical nature.

Which categories of amino acid would you expect to find on the surface of a soluble protein, and which would you expect to find in the interior? What distribution of amino acids would you expect to find in a protein embedded in a lipid bilayer?

The sequence and the number of amino acids ultimately determine the protein's shape, size, and function. Each amino acid is attached to another amino acid by a covalent bond, known as a peptide bond, which is formed by a dehydration reaction. The carboxyl group of one amino acid and the amino group of the incoming amino acid combine, releasing a molecule of water. The resulting bond is the peptide bond (Figure 5.1.13).

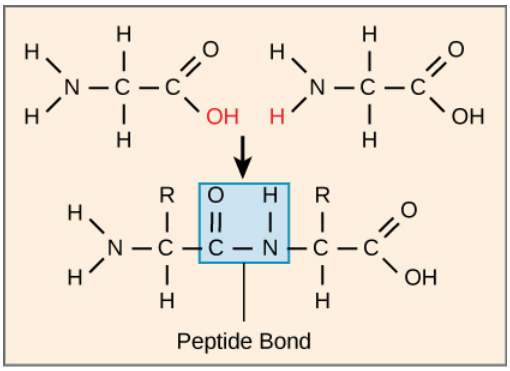


Figure 5.1.13: Peptide bond formation is a dehydration synthesis reaction. The carboxyl group of one amino acid is linked to the amino group of the incoming amino acid. In the process, a molecule of water is released.

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5.2: Exercise

Exercise A: Testing for Carbohydrates

The basic structural unit of carbohydrates is the monosaccharide (single sugar), which contains either a terminal aldehyde group or an internal ketone group. Both of these functional groups contain a double-bonded oxygen atom that reacts with **Benedict's reagent**. When two monosaccharides are joined together, they form a disaccharide. Some disaccharides retain their reactive functional group; others involve these functional groups to form the new bond between the monosaccharides and no longer react with Benedict's reagent. Monosaccharides and disaccharides with free aldehyde or ketone groups are called reducing sugars since they are able to reduce the alkaline solution of cupric ions (Cu^{2+}) found in Benedict's reagent to cuprous ions (Cu^{+}), forming an orange-colored precipitate composed of cuprous oxide (Cu_2O). In this exercise, we will use Benedict's reagent to test for the presence of reducing sugars.

Polysaccharides are formed when many monosaccharides join together to form long chains of carbohydrates. Starch is a polysaccharide composed entirely of glucose molecules. Starch does not react with Benedict's reagent because the number of free functional groups is too low (found only at the ends of the polysaccharide). Thus, we test for the presence of starch with **Lugol's reagent** (iodine/potassium iodide, I_2KI) instead.

Part 1: Benedict's Test for Reducing Sugars

When we heat a mixture of Benedict's reagent with reducing sugars, the color of the reagent will change from blue to green/yellow/reddish-orange, depending on the amount of reducing sugar present in the sample (red = most). Benedict's reagent might show a false-positive reaction for starch if the starch has broken down due to excessive heating.

Data Table for Carbohydrates

Data Table for Carbohydrates	**Benedict's Test** (reducing sugars)		**Lugol's Test** (starch)
Color before boiling	Color after boiling	Color before adding I_2KI	Color after adding I_2KI
1) Distilled Water			
2) Starch			
3) Glucose			
4) Maltose			
5) Sucrose			
6) Onion juice			
7) Potato slice			
8) Milk			

- Examine the carbohydrates data table. Create a hypothesis and a prediction for at least one of the samples you will be testing using Benedict's test:

Hypothesis: _____

Prediction: _____

What is the independent variable? _____

What is the dependent variable? _____

Procedure

1. Set up a row of 8 test tubes using a test tube rack. Label them 1 through 8.
2. Add 2 mL of each solution (from the data table) to each tube, matching each number to the number on the tube.

3. Add 2 mL of Benedict's reagent to each tube and mix gently. Record the initial color of each.
4. Heat the tubes in boiling water bath for 5 minutes. Record your color changes in the data table.

- Which samples tested positive for reducing sugars? _____
- Why did we test water with Benedict's reagent? _____
- Did the results from your test support your hypothesis? Yes / No Explain: _____
  _____

Part 2: Lugol's Test for Starch

Lugol's reagent changes from a brown-yellow color to a blue-black color when starch is present. There is no reaction (no color change) with monosaccharides or disaccharides.

- Examine the previous data table. Create a hypothesis and a prediction for at least one of the samples you will be testing using Lugol's test:

Hypothesis: _____

Prediction: _____

What is the independent variable? _____

What is the dependent variable? _____

Procedure

1. Set up another row of 8 test tubes in your test tube rack. Label them 1 through 8.
2. Add 1 mL of each solution (from the data table) to each tube, matching each number to the number on the tube. Record the initial color of each sample in your data table.
3. Add 2-3 drops of Lugol's reagent to each tube and mix well. Immediately record the final color change in the data table. (We do NOT heat these tubes.)

- Which samples tested positive for starch? _____
- From the results of your tests, do you think that a potato stores its carbohydrates as sugars or as starch? _____
  Explain:
  _____
- How does an onion store its carbohydrates? _____ How can you tell?
  _____
- Did the results from your test support your hypothesis? Yes / No Explain:
  _____

Exercise B: Testing for Lipids

Lipids are not soluble in water, but are soluble in nonpolar solvents. Although fats, steroids, and phospholipids are all types of lipids, this portion of the lab focuses primarily on fats. Triglycerides are the most common form of fat, and are composed of one glycerol molecule attached to three fatty acids. Triglycerides are found predominantly in adipose tissue and store more energy per gram than carbohydrates and proteins. Depending on their fatty acid composition, lipids may be solid or liquid at room temperature. Since both solid and liquid fats are nonpolar, we will test for their presence using **Sudan IV**, a nonpolar dye that dissolves nonpolar substances such as fat.

Part 1: Grease Spot Test for Lipids

Another very simple test for fats is the familiar "grease spot" test. On a piece of unglazed paper (i.e. brown paper grocery bags), place one drop of vegetable oil and a separate drop of water. Allow the drops to dry. Describe the difference between the oil and water drop after drying: _____
_____

Data Table for Lipids

Data Table for Lipids

Substance	**Sudan IV Solubility Reaction** *(note the color and if + or – for lipids)*
1) Distilled Water	
2) Vegetable oil	
3) Onion juice	
4) Cola (regular)	
5) Hamburger juice	

Part 2: Sudan IV Test for Lipids

The Sudan IV test is more useful laboratory test for fats. **Note: Sudan IV is toxic. Use gloves and caution when handling this reagent.**

- Examine the data table for lipids. Create a hypothesis and a prediction for at least one of the samples you will be testing using the Sudan IV test:

Hypothesis: _____

Prediction: _____

What is the independent variable? _____

What is the dependent variable? _____

Procedure

1. Set up a row of 5 test tubes using your test tube rack. Label them 1 through 5.
2. Add 1 mL of each solution (from the data table) to each tube, matching each number to the number on the tube.
3. Add 3 drops of Sudan IV to each tube. Mix and then add 2 mL of DI water to each tube.
4. If fats or oils are present, these will appear as floating red droplets or as a floating red layer colored by Sudan IV. Record the reactions in the lipid data table.

- Which samples tested positive for lipids? _____
- Why do the red droplets float? _____
- Did the results from your test support your hypothesis? Yes / No Explain: _____

_____

Exercise C: Testing for Proteins and Amino Acids

Proteins are composed of one or more polypeptides, which are polymers of smaller subunits known as amino acids. Each amino acid contains an amino group and a carboxyl group. Polypeptides form when amino acids are joined together by peptide bonds between the amino group of one amino acid and the carboxyl group of another.

Biuret reagent reacts with the peptide bonds found within proteins, such as egg albumin, but not with free amino acids like glycine or alanine. A separate reagent, **ninhydrin reagent**, reacts with the amino group of free amino acids, but not with polypeptides.

Part 1: Biuret Reagent Test for Proteins

Biuret reagent is light blue, but in the presence of proteins it turns violet/purple. Other types of molecules may cause other color changes, but only the violet color indicates presence of polypeptides.

- Examine the data table for proteins. Create a hypothesis and a prediction for at least one of the samples you will be testing using biuret reagent:

Hypothesis: _____

Prediction: _____

What is the independent variable? _____

What is the dependent variable? _____

Procedure

1. Set up a row of 5 test tubes in your test tube rack. Label them 1 through 5.
2. Add 2 mL of each solution (from the data table) to each tube, matching each number to the number on the tube.
3. Add 2 mL of biuret reagent to each tube. Mix well and incubate at your desk for 2 minutes.
4. After the 2 minutes, record your results in the data table below and determine if protein is present or absent in each solution. Base your results only on the presence or absence of a violet color.

Data Table for Proteins

Data Table for Proteins

Substance	Color with Biuret Reagent after 2 Minutes	Protein Present (+) or Absent (-)
1) Distilled water		
2) Egg albumin		
3) Potato starch		
4) Glucose		
5) Amino acid		

- Which samples tested positive for proteins? _____
- What does this test tell you about the biochemical composition of starch or glucose? _____

  _____
- Did the results from your test support your hypothesis? Yes / No Explain:

  _____

Part 2: Ninhydrin Test for Amino Acids

Ninhydrin reagent turns purple or violet when free amino groups found in amino acids are present in the solution. When ninhydrin reagent reacts with the amino acid proline, however, it turns yellow. This is because proline's amino group is not free. Rather, its amino group is part of the ring structure of the molecule. **Note: Ninhydrin is poisonous. Use gloves and caution when handling this reagent.**

- Examine the data table for amino acids. Create a hypothesis and a prediction for at least one of the samples you will be testing using the ninhydrin test:

Hypothesis: _____

Prediction: _____

What is the independent variable? _____

What is the dependent variable? _____

Procedure

1. Divide a piece of filter paper into 4 quadrants with a pencil. Label the quadrants A, B, C, and D.
2. Place 1 drop of each solution (from the data table) to each quadrant with the corresponding letter. Allow the spots to dry.
3. Apply 1 drop of ninhydrin reagent to each spot. **Caution: avoid skin contact – wear gloves.**
4. Allow the paper to dry at room temperature for 20-30 minutes. (You can speed up the reaction by passing the filter paper lightly over a warm hotplate.) Record the final color in your data table for amino acids.
5. One of the solutions distilled water, two contain amino acids other than proline (methionine or alanine), and one contains proline. Complete the table, indicating the content of each solution.

Data Table for Amino Acids

Data Table for Amino Acids

Solution	Final Color with Ninhydrin	Type of Molecule in Solution
A		
B		
C		
D		

Exercise D: Testing Unknowns (assigned unknowns: #_____ and #_____)

Use knowledge gained in the previous exercises to identify the organic molecules present in unknown solutions. Your instructor will assign unknowns to each group. Conduct all tests according to the directions found in Exercises A-C. Record your results in the table below. Meet with other groups to exchange data results until your table is complete.

Hints/Tips: Avoid cross-contamination with pipettes between solutions. Wash glassware thoroughly between experiments if needed.

Data Table for Unknowns

Data Table for Unknowns

Unknown Tested	Positive (+) or Negative (-) Results				
Benedict's Test	**Lugol's Test**	**Biuret Test**	**Ninhydrin Test**	**Sudan IV Test**	
1:					
2:					
3:					
4:					
5:					
6:					
7:					
8:					

- Your instructor will provide you with a list of the possible unknown contents. Once you have completed the data table, match the names of the unknowns to the numbers in your table by writing the name of the unknown next to each respective number.

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5.3: Lab Report

Exercise A: Testing for Carbohydrates

Part 1: Benedict's Test for Reducing Sugars

Record your results below:

Data Table for Carbohydrates

Data Table for Carbohydrates	**Benedict's Test** (reducing sugars)		**Lugol's Test** (starch)	
Color before boiling	Color after boiling	Color before adding I_2KI	Color after adding I_2KI	
1) Distilled Water				
2) Starch				
3) Glucose				
4) Maltose				
5) Sucrose				
6) Onion juice				
7) Potato slice				
8) Milk				

- Examine the carbohydrates data table. Create a hypothesis and a prediction for at least one of the samples you will be testing using Benedict's test:

Hypothesis: _____

Prediction: _____

What is the independent variable? _____

What is the dependent variable? _____

Procedure

- Which samples tested positive for reducing sugars? _____
- Why did we test water with Benedict's reagent? _____
- Did the results from your test support your hypothesis? Yes / No Explain: _____

  _____

Part 2: Lugol's Test for Starch

- Examine the previous data table. Create a hypothesis and a prediction for at least one of the samples you will be testing using Lugol's test:

Hypothesis: _____

Prediction: _____

What is the independent variable? _____

What is the dependent variable? _____

- Which samples tested positive for starch? _____
- From the results of your tests, do you think that a potato stores its carbohydrates as sugars or as starch? _____ Explain:

_____

- How does an onion store its carbohydrates? _____ How can you tell?

_____

- Did the results from your test support your hypothesis? Yes / No Explain:

_____

Exercise B: Testing for Lipids

Part 1: Grease Spot Test for Lipids

Describe the difference between the oil and water drop after drying:

_____

_____

Data Table for Lipids

Data Table for Lipids

Substance	**Sudan IV Solubility Reaction** *(note the color and if + or – for lipids)*
1) Distilled Water	
2) Vegetable oil	
3) Onion juice	
4) Cola (regular)	
5) Hamburger juice	

Part 2: Sudan IV Test for Lipids

- Examine the data table for lipids. Create a hypothesis and a prediction for at least one of the samples you will be testing using the Sudan IV test:

Hypothesis: _____

Prediction: _____

What is the independent variable? _____

What is the dependent variable? _____

Procedure

- Which samples tested positive for lipids? _____
- Why do the red droplets float? _____
- Did the results from your test support your hypothesis? Yes / No Explain: _____

_____

Exercise C: Testing for Proteins and Amino Acids

Part 1: Biuret Reagent Test for Proteins

- Examine the data table for proteins. Create a hypothesis and a prediction for at least one of the samples you will be testing using biuret reagent:

Hypothesis: _____

Prediction: _____

What is the independent variable? _____

What is the dependent variable? _____

Data Table for Proteins

Data Table for Proteins

Substance	Color with Biuret Reagent after 2 Minutes	Protein Present (+) or Absent (-)
1) Distilled water		
2) Egg albumin		
3) Potato starch		
4) Glucose		
5) Amino acid		

- Which samples tested positive for proteins? _____
- What does this test tell you about the biochemical composition of starch or glucose? _____
  _____
- Did the results from your test support your hypothesis? Yes / No Explain:
  _____

Part 2: Ninhydrin Test for Amino Acids

- Examine the data table for amino acids. Create a hypothesis and a prediction for at least one of the samples you will be testing using the ninhydrin test:

Hypothesis: _____

Prediction: _____

What is the independent variable? _____

What is the dependent variable? _____

Procedure

Complete the table, indicating the content of each solution.

Data Table for Amino Acids

Data Table for Amino Acids

Solution	Final Color with Ninhydrin	Type of Molecule in Solution
A		
B		
C		
D		

Exercise D: Testing Unknowns (assigned unknowns: #_____ and #_____)

Data Table for Unknowns

Data Table for Unknowns

Unknown Tested	Positive (+) or Negative (-) Results			
Benedict's Test	**Lugol's Test**	**Biuret Test**	**Ninhydrin Test**	**Sudan IV Test**
1:				
2:				
3:				
4:				
5:				
6:				
7:				
8:				

- Your instructor will provide you with a list of the possible unknown contents. Once you have completed the data table, match the names of the unknowns to the numbers in your table by writing the name of the unknown next to each respective

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CHAPTER OVERVIEW

6: Microscopy Part II

Learning Objectives

Content Objectives

- Compare and contrast light and electron microscopy.
- Compare and contrast in vitro and in vivo staining.
- Generalize the process of dark-field microscopy.
- Describe the mechanics, advantages, and disadvantages of phase-contrast microscopy.

Skill Objectives

- Measure organisms using the ocular micrometer.
- Use the total magnification and ocular micrometer to estimate the actual size of an organism.
- Prepare and observe a wet mount using brightfield and phase-contrast microscopy.

6.1: Introduction
6.2: Exercise
6.3: Lab Report

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6.1: Introduction

Introduction

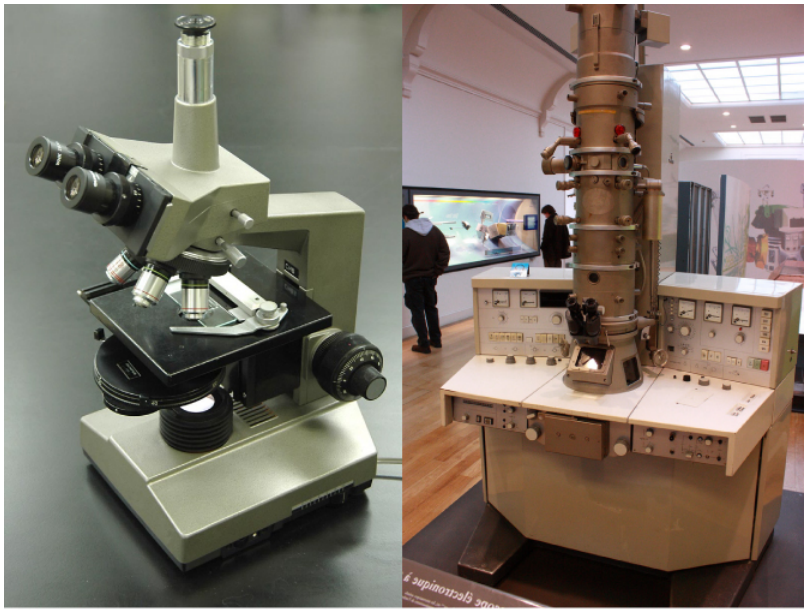
Cells vary in size. With few exceptions, individual cells cannot be seen with the naked eye, so scientists use microscopes (micro- = “small”; -scope = “to look at”) to study them. A microscope is an instrument that magnifies an object. Most photographs of cells are taken with a microscope; these images can also be called micrographs.

The optics of a microscope’s lenses change the orientation of the image that the user sees. A specimen that is right-side up and facing right on the microscope slide will appear upside-down and facing left when viewed through a microscope, and vice versa. Similarly, if the slide is moved left while looking through the microscope, it will appear to move right, and if moved down, it will seem to move up. This occurs because microscopes use two sets of lenses to magnify the image. Because of the manner by which light travels through the lenses, this system of two lenses produces an inverted image (binocular, or dissecting microscopes, work in a similar manner, but they include an additional magnification system that makes the final image appear to be upright).

A. Light Microscopes

To give you a sense of cell size, a typical human red blood cell is about eight millionths of a meter or eight micrometers (abbreviated as eight μm) in diameter; the head of a pin of is about two thousandths of a meter (two mm) in diameter. That means about 250 red blood cells could fit on the head of a pin.

Most student microscopes are classified as light microscopes. Visible light passes and is bent through the lens system to enable the user to see the specimen. Light microscopes are advantageous for viewing living organisms, but since individual cells are generally transparent, their components are not distinguishable unless they are colored with special stains. Staining, however, usually kills the cells.



(a) (b)

Figure: **Light and Electron Microscopes**: (a) Most light microscopes used in a college biology lab can magnify cells up to approximately 400 times and have a resolution of about 200 nanometers. (b) Electron microscopes provide a much higher magnification, 100,000x, and a have a resolution of 50 picometers.

Light microscopes, commonly used in undergraduate college laboratories, magnify up to approximately 400 times. Two parameters that are important in microscopy are magnification and resolving power. Magnification is the process of enlarging an object in appearance. Resolving power is the ability of a microscope to distinguish two adjacent structures as separate: the higher the resolution, the better the clarity and detail of the image. When oil immersion lenses are used for the study of small objects, magnification is usually increased to 1,000 times. In order to gain a better understanding of cellular structure and function, scientists typically use electron microscopes.

B. Electron Microscopes

In contrast to light microscopes, electron microscopes use a beam of electrons instead of a beam of light. Not only does this allow for higher magnification and, thus, more detail, it also provides higher resolving power. The method used to prepare the specimen for viewing with an electron microscope kills the specimen. Electrons have short wavelengths (shorter than photons) that move best in a vacuum, so living cells cannot be viewed with an electron microscope.

In a scanning electron microscope, a beam of electrons moves back and forth across a cell's surface, creating details of cell surface characteristics. In a transmission electron microscope, the electron beam penetrates the cell and provides details of a cell's internal structures. As you might imagine, electron microscopes are significantly more bulky and expensive than light microscopes.

C. Staining

Staining is a technique used in microscopy to enhance contrast in a microscopic image. Stains and dyes are frequently used to highlight structures in microbes for viewing, often with the aid of different microscopes. Stains may be used to define and examine different types of microbes, various stages of cellular life (e.g., the mitotic cycle), and even organelles within individual cells (e.g., mitochondria or chloroplasts).

In-vivo staining is the process of dyeing living tissue — *in vivo* means "in life" (as contrasted to *in-vitro* staining). When a certain cell or structure takes on contrasting color(s), its form (morphology) or position within a cell or tissue can be readily seen and studied. The usual purpose is to reveal cytological details that might otherwise not be apparent; however, staining can also reveal where certain chemicals or specific chemical reactions are taking place within cells. *In-vitro* staining involves coloring cells or structures that have been removed from their biological context. Certain stains are often combined to reveal more details and features than a single stain could reveal alone, and a counterstain is a stain that increases visibility of cells or structures when the principal stain is not sufficient. Scientists and physicians can combine staining with specific protocols for fixation and sample preparation and can use these standard techniques as consistent, repeatable diagnostic tools.

There are an incredible number of stains that can be used in a variety of different methods. What follows here are some common aspects of the process of preparing for *in-vitro* staining.

- Fixation: This can itself consist of several steps. Fixation aims to preserve the shape of the cells (in this case, microbes) as much as possible. Sometimes heat fixation is used to kill, adhere, and alter the cells so they will accept stains. Most chemical fixatives generate chemical bonds between proteins and other substances within the sample, increasing their rigidity. Common fixatives include formaldehyde, ethanol, methanol, and picric acid.
- Permeabilization: This involves treatment of the cells with (usually) a mild surfactant. This treatment dissolves cell membranes, allowing larger dye molecules to enter the cell's interior.
- Mounting: This step usually involves attaching the samples to a glass microscope slide for observation and analysis. In some cases, cells may be grown directly on a slide. For samples of loose cells the sample can be directly applied to a slide.

At its simplest, the actual staining process may involve immersing the sample (before or after fixation and mounting) in dye solution, followed by rinsing and observation. Many dyes, however, require the use of a mordant — a chemical compound that reacts with the stain to form an insoluble colored precipitate. When the excess dye solution is washed away, the mordanted stain remains. There is an incredible array of stains that can be used at this step, from those that stain specific microbial types (see the figure below) to those that highlight sub-compartments or organelles of a cell, such as the nucleus or endoplasmic reticulum. Alternatively, negative staining can be employed. This is a simple staining method for bacteria, performed by smearing the cells onto the slide and then applying nigrosin (a black synthetic dye) or Indian ink (an aqueous suspension of carbon particles). After drying, the microorganisms may be viewed in bright field microscopy as lighter inclusions contrast well against the dark environment surrounding them

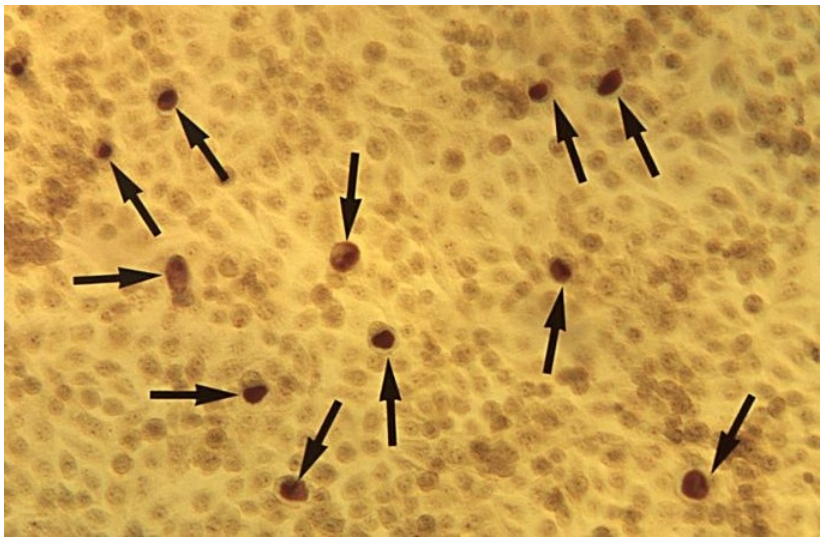


Figure: **Chlamydia Stain**: Cells of the bacterial pathogen chlamydia (indicated by arrows) are highlighted by a stain called "Giemsa."

Live, *in-vivo* staining microscopy shares many of these steps, with the exception of fixation, which invariably kills the microbe to be examined.

D. Dark-Field Microscopy

Dark-field microscopy is ideally used to illuminate unstained samples causing them to appear brightly lit against a dark background. This type of microscope contains a special condenser that scatters light and causes it to reflect off the specimen at an angle. Rather than illuminating the sample with a filled cone of light, the condenser is designed to form a hollow cone of light. The light at the apex of the cone is focused at the plane of the specimen; as this light moves past the specimen plane it spreads again into a hollow cone. The objective lens sits in the dark hollow of this cone; although the light travels around and past the objective lens, no rays enter it.



Figure: **Visualization of live bacteria**: Spirochete bacteria observed under dark field microscopy.

The entire field appears dark when there is no sample on the microscope stage; thus the name dark-field microscopy. When a sample is on the stage, the light at the apex of the cone strikes it. The rays scattered by the sample and captured in the objective lens thus make the image.

Samples observed under dark-field microscopy should be carefully prepared since dust and other particles also scatter the light and are easily detected. Glass slides need to be thoroughly cleaned of extraneous dust and dirt. It may be necessary to filter sample media (agar, water, saline) to exclude confusing contaminants. Sample materials need to be spread thinly; too much material on the slide creates many overlapping layers and edges, making it difficult to interpret structures.

Dark-field microscopy has many applications in microbiology. It allows the visualization of live bacteria, and distinguishes some structure (rods, curved rods, spirals, or cocci) and movement.

E. Phase-Contrast Microscopy

Phase-contrast microscopy visualizes differences in the refractive indexes of different parts of a specimen relative to unaltered light.

Phase-contrast microscopy is a method of manipulating light paths through the use of strategically placed rings in order to illuminate transparent objects. Dutch physicist Fritz Zernike developed the technique in the 1930s; for his efforts he was awarded the Nobel Prize in 1953.

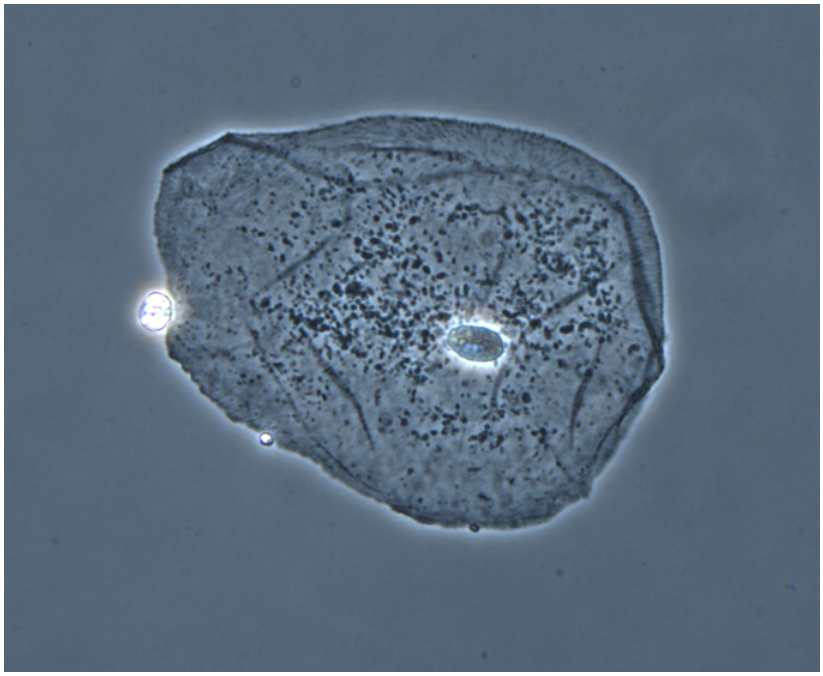


Figure: **Phase-contrast microscopy**: Phase-contrast image of a cheek epithelial cell.

In phase-contrast microscopy, parallel beams of light are passed through objects of different densities. The microscope contains special condensers that throw light “out of phase” causing it to pass through the object at different speeds. Internal details and organelles of live, unstained organisms (e.g. mitochondria, lysosomes, and the Golgi body) can be seen clearly with this microscope.

A phase ring in condenser allows a cylinder of light to pass through it while still in phase. Unaltered light hits the phase ring in the lens and is excluded. Light that is slightly altered by passing through a different refractive index is allowed to pass through. Light passing through cellular structures, such as chromosomes or mitochondria is retarded because they have a higher refractive index than the surrounding medium. Elements of lower refractive index advance the wave. Much of the background light is removed and light that constructively or destructively interfered is let through with enhanced contrast.

Phase-contrast microscopy allows the visualization of living cells in their natural state with high contrast and high resolution. This tool works best with a thin specimen and is not ideal for a thick specimen. Phase-contrast images have a characteristic grey background with light and dark features found across the sample. One disadvantage of phase-contrast microscopy is halo formation called halo-light ring.

F. Key Points

- Light microscopes allow for magnification of an object approximately up to 400-1000 times depending on whether the high power or oil immersion objective is used.
- Light microscopes use visible light which passes and bends through the lens system.
- Electron microscopes use a beam of electrons, opposed to visible light, for magnification.
- Electron microscopes allow for higher magnification in comparison to a light microscope thus, allowing for visualization of cell internal structures.
- In-vivo staining, which visualizes cells that are alive, and in-vitro staining, which visualizes fixed cells, both have important uses.
- There is a vast array of stains that can be used on microbes that can highlight almost any characteristic of a cell, even organelles within a cell.
- Staining protocols can be complex, but they share some basic steps: preparation, fixation, staining, and mounting.
- In dark-field microscopy, the light reaches the specimen from an angle with the help of an opaque disk.
- The specimen appears lit up against a dark background.
- Dark-field microscopy is most useful for extremely small living organisms that are invisible in the light microscope.
- A phase-contrast microscope splits a beam of light into 2 types of light, direct and refracted (reflected) and brings them together to form an image of the specimen.
- Where the lights are “in-phase” the image is brighter, where the lights are “out of phase” the image is darker, and by amplifying these differences in the light, it enhances contrast.
- Phase-contrast microscopy allows for the detailed observation of living organisms, especially the internal structures.

G. Key Terms

- **resolution**: The degree of fineness with which an image can be recorded or produced, often expressed as the number of pixels per unit of length (typically an inch).
- **electron**: The subatomic particle having a negative charge and orbiting the nucleus; the flow of electrons in a conductor constitutes electricity.
- **surfactant**: a surface active agent, or wetting agent, capable of reducing the surface tension of a liquid; typically organic compounds having a hydrophilic “head” and a hydrophobic “tail”
- **organelle**: a specialized structure found inside cells that carries out a specific life process (e.g., ribosomes, vacuoles)
- **condenser**: A lens (or combination of lenses) designed to gather light and focus it onto a specimen or part of a mechanism.
- **refractive index**: the ratio of the speed of light in air or vacuum to that in another medium.

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6.2: Exercise

Microscopy Exercise

Use the slides and cultures provided to learn more advanced uses of the microscope and how to prepare wet mount slides.
We will also be introducing phase contrast microscopy.

A. Brightfield Microscopy - Prepared Slides

1. *Amoeba proteus*

View protozoa (*Amoeba proteus*) using the 4x, 10x, and 40x objective lenses. Select one organism to sketch using the three objective lenses, making sure you are examining the same *Amoeba* each time! (Hint: center the organism in your field of view before moving to the next objective.)

Measuring organisms under the microscope:

The "ruler" that you can see in the eyepiece of your microscope can be used to measure the objects you see through the microscope. This ruler is called the ocular micrometer. The units on the ocular micrometer are arbitrary, and depend on the magnification used to view the sample. As the total magnification increases from 40x to 1000x, each mark on the micrometer measures a smaller and smaller area.

The ocular micrometer on our microscopes is designed so that at 1000x (oil immersion), each mark is equal to 1 micrometer (µm). At this magnification, you can simply read the numbers on the scale as µm.

Each mark on the ocular micrometer measures: (show your work)
At 40x = 25 µm
At 100x = _____
At 400x = _____
At 1000x = 1 µm

Sketch your view of the Amoeba *and* the ocular micrometer for each total magnification. Note: the ocular micrometer does not change; however, your organism will appear larger with each objective.

40X Total Magnification:

100X Total Magnification:

400X Total Magnification:

Choose a sample and measure the same object at three different magnifications. Remember, because the object is the same, its size is not changing during this, and therefore the numbers you get should be the same, or at least close.

Data Table for Microorganism Size

Total Magnification	Number of marks on the ocular micrometer	Multiply by:	Measured size & units:
Amoeba at 40x			
Amoeba at 100x			
Amoeba at 400x			
optional: Bacterium at 1000x			

It is possible that your measured sizes may not be exactly the same, due to increased precision at higher magnification. For example, you may measure an organism as 100 µm at 40x, 110 µm at 100x, and 108 µm at 400x. This can be considered consistent, and within an appropriate range.

2. Bacteria

View one of the bacteria on the "Three Types Bacteria Slide" under the microscope using the 10x, 40x, and 100x objective lenses. Once you have a clear image under the 40x objective lens, rotate the objective lens until there is no objective lens directly above the specimen. DO NOT change the stage height. Place one drop of immersion oil directly on your slide and slowly push the 100x objective (oil immersion) lens into the oil. Use the FINE adjustment knob to focus your specimen.

Sketch your view of the bacteria *and* the ocular micrometer for under the 100x objective lens and complete the last row of the Data Table for Microorganism Size (above).

Bacterium 1000X Total Magnification:

Be sure to use lens paper to remove oil from the 100x objective lens after each use. Remove any oil remaining on the microscope slides before storage as well.

B. Phase Contrast Microscopy - Live Specimens

The purpose of phase contrast microscopy is to see transparent cells; this allows us to see live cells without staining.
Setting up your microscope correctly for phase-contrast depends on the magnification you are using: (refer to the objective lens before turning the phase turret)

- 10x objective (100x total magnification): turn the turret underneath the stage so that the appropriate setting is visible and facing forward.
- 40x objective (400x total): remember to adjust the turret and use the appropriate phase setting.
- 100x objective (1000x total, oil immersion): remember to adjust the turret and use the appropriate phase setting.

Create a wet mount using the live specimens: pond water or hay infusion. If necessary, add 1 or 2 drops of methyl cellulose to slow down the organisms.

View the live organisms under the 10x, 40x, and 100x objective lenses. For each objective lens, view your sample using brightfield microscopy. Then, turn the turret to the appropriate setting and view your organisms under using phase contrast microscopy.

Clean Your Microscope! Please do the following after each lab:

1. Turn the microscope off
2. Wipe off any immersion oil from the lenses. Make sure to check the 40x objective for oil, too!
3. Remove any slides
4. Put the low power (4x) objective in place.

Also, begin using the microscope to measure objects. You can use the Microcopy Part I lab as a reference.

Questions to think about:

1. How do you determine total magnification?
2. How does the size of an organism change as you change magnification? Choose a stationary or very slow organism to focus on as you change magnifications. Use the worksheet on the following page to help you measure the size of the organism.
3. Wet mount: What is the purpose of the phase contrast? Switch back and forth between brightfield and phase contrast. How does your sample look different? Look especially at the smallest organisms on your slide. Describe the differences you see between brightfield and phase contrast under each of the objective lenses.

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6.3: Lab Report

Microscopy Exercise

A. Brightfield Microscopy - Prepared Slides

1. *Amoeba proteus*

Each mark on the ocular micrometer measures: (show your work)
At 40x = 25 µm
At 100x = _____
At 400x = _____
At 1000x = 1 µm

Sketch your view of the Amoeba *and* the ocular micrometer for each total magnification. Note: the ocular micrometer does not change; however, your organism will appear larger with each objective.

40X Total Magnification:

100X Total Magnification:

400X Total Magnification:

Choose a sample and measure the same object at three different magnifications. Remember, because the object is the same, its size is not changing during this, and therefore the numbers you get should be the same, or at least close.

Data Table for Microorganism Size

Total Magnification	Number of marks on the ocular micrometer	Multiply by:	Measured size & units:
Amoeba at 40x			
Amoeba at 100x			
Amoeba at 400x			
optional: Bacterium at 1000x			

2. Bacteria

Sketch your view of the bacteria *and* the ocular micrometer for under the 100x objective lens and complete the last row of the Data Table for Microorganism Size (above).

Bacterium 1000X Total Magnification:

B. Phase Contrast Microscopy - Live Specimens

Questions to think about:

1. How do you determine total magnification?

2. How does the size of an organism change as you change magnification? Choose a stationary or very slow organism to focus on as you change magnifications. Use the worksheet on the following page to help you measure the size of the organism.

3. Wet mount: What is the purpose of the phase contrast? Switch back and forth between brightfield and phase contrast. How does your sample look different? Look especially at the smallest organisms on your slide. Describe the differences you see between brightfield and phase contrast under each of the objective lenses.

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CHAPTER OVERVIEW

7: Eukaryotic Cells

Learning Objectives

Content Objectives

- Describe the major characteristics of eukaryotic cells.
- Compare and contrast eukaryotic and prokaryotic cells.
- List and describe the major organelles and structures that can be used to identify various cell types.

Skill Objectives

- Observe and describe major bacterial cell characteristics using light microscopy.
- Use light microscopy to identify major structures that differentiate animal cells and plant cells.

7.1: Introduction
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7.1: Introduction

Introduction

The cell theory states that all living things are composed of cells, which are the basic units of life, and that all cells arise from existing cells. In this course, we closely study both types of cells: prokaryotic and eukaryotic. Prokaryotes lack a nucleus and true organelles, and are typically significantly smaller than eukaryotic cells. Prokaryotic organisms are found within the domains Bacteria and Archaea. Eukaryotic cells contain nuclei as well as other organelles that work together to support the homeostasis of the whole cell. Though eukaryotes are larger than prokaryotes, we must use a microscope to view all cells, which are typically too small to see with the naked eye.

There are vast differences between cell types, but a few features are common to all cells: plasma membrane, cytoplasm, ribosomes, and cytoskeleton. All cells also use DNA for their genetic material. In eukaryotes, this is within the nucleus while in prokaryotes, it is found in the nucleoid region of the cytoplasm. Prokaryotes generally have a cell wall made of peptidoglycan and some have flagella or fimbriae, which are used for movement or attachment. Eukaryotes have several more organelles and are further differentiated into 2 categories: plant cells and animal cells.

Some organelles common to eukaryotes include mitochondria, peroxisomes, vesicles, lysosomes, smooth and rough endoplasmic reticula, and Golgi bodies. Animal cells tend to lack cell walls and chloroplasts, while plant cells do contain chloroplasts and have cellulose cell walls.

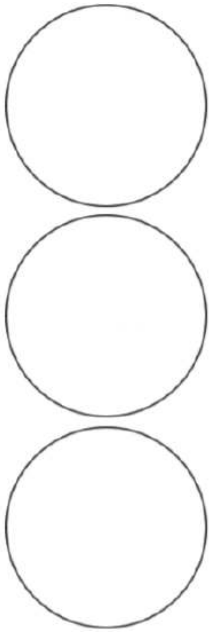
In this lab, bacterial, animal, and plant cells will be observed using the microscope. Students will draw what was visualized to record their observations.

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7.2: Exercise

Part 1: Bacterial Cells



View a prepared slide of common bacterial cell types; prepare a wet mount of cyanobacteria and observe under the microscope.

Materials:

- Compound microscope
- Methylene blue
- Microscope slide
- Cover slip
- Transfer pipette
- Culture of Oscillatoria

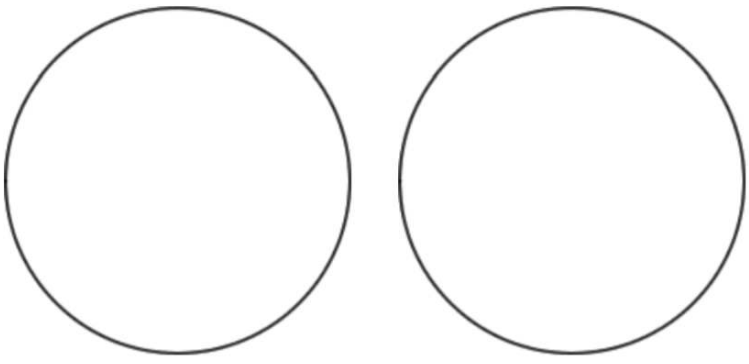
Procedure:

A. Fixed Slide of Bacterial Types

1. Examine the demonstration slide of mixed bacteria. There are 3 common shapes: round (coccus), rod (bacillus), and spiral (spirillum). The slide should have several of each type of bacteria.
2. Draw each of the bacterial shapes in the spaces at right.
3. Use the ocular micrometer to measure the length and width of a single cell for each type of bacteria. Record this using a "l x w" format next to each of your sketches.

B. Wet Mount of *Oscillatoria*

1. Using the transfer pipette, transfer a drop of liquid culture onto a microscope slide.
2. Place coverslip onto the slide.
3. Use the SCANNING (4x) objective to focus. You are looking for very faint green thin filaments.
4. Switch to low power (10x). You may be able to see lines going across the filaments, but the image will likely just look like green floss.
5. Once you have centered and focused the image, switch to high power (40x) and refocus. The individual cells should be visible at this magnification; each filament is composed of cells stacked on top of each other. Remember, do NOT use the coarse adjustment knob at this point!
6. Sketch the bacteria at low and high power. Label the cytoplasm and cell wall of a single cell. Draw your cells to scale.



Oscillatoria at low power (10x)

Oscillatoria at high power (40x)

Questions:

1. Why are nuclei not visible within the cells viewed?

2. The common name for species like Oscillatoria is "blue-green algae" (although they are classified as bacteria, not algae). This group of bacteria is capable of photosynthesizing. Do they contain chloroplasts? Explain.

Part 2: Animal Cells

Prepare a wet mount of a human cheek cell and observe under the microscope.

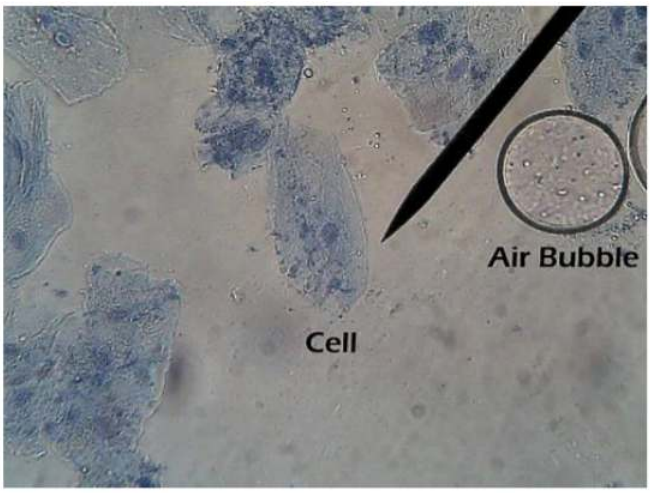


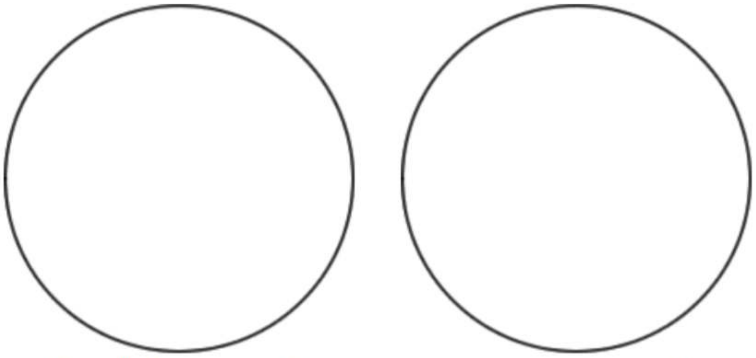
Figure 1: Human cheek cell stain at high power
"Cheek Cells Identified (400x)" by biologycorner, via Flickr

Materials:

- Compound microscope
- Methylene blue
- Microscope slide
- Cover slip
- Toothpick

Procedure:

1. Put a drop of methylene blue on the slide.
2. Gently scrape the inside of your cheek with the flat side of a toothpick. Scrape lightly!
3. Stir the end of the toothpick in the stain and throw the toothpick away.
4. Place a coverslip onto the slide.
5. Use the SCANNING (4x) objective to focus. You probably will not see the cells at this power.
6. Switch to low power (10x). Cells should be visible, but they will be small and look like nearly clear purplish blobs. If you are looking at something very dark purple, it is probably not a cell.
7. Once you think you have located a cell, switch to high power (40x) and refocus. Remember, do NOT use the coarse adjustment knob at this point!
8. Sketch the cell at low and high power. Label the nucleus, cytoplasm, and cell membrane of a single cell. Draw your cells to scale.



Cheek cell at low power (10x)

Cheek cell at high power (40x)

Questions:

1. Why is methylene blue added?

2. The light microscope used in the lab is not powerful enough to view other organelles in the cheek cell.

    a. What parts of the cell were visible?

    b. List 2 organelles that were NOT visible but should have been in the cheek cell.

3. Is the cheek cell a eukaryote or prokaryote? How do you know?

4. Keeping in mind that the mouth is the first site of chemical digestion in a human. Your saliva starts the process of breaking down the food you eat. Keeping this in mind, what organelle do you think would be numerous inside the cells of your mouth? Explain.

Part 3: Plant Cells

Prepare wet mounts of an onion cell and an *Elodea* leaf cell and observe both under the microscope.

Materials:

- Compound microscope
- Microscope slide
- Cover slip
- Dropper bottle with dH_2O
- Forceps
- Pre-cut onion bulb
- Culture of Elodea
- Dissecting needle

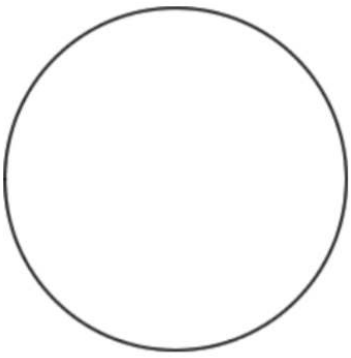


Figure 2: Onion cells at high power
"Onion 100x" by biologycorner, via Flickr

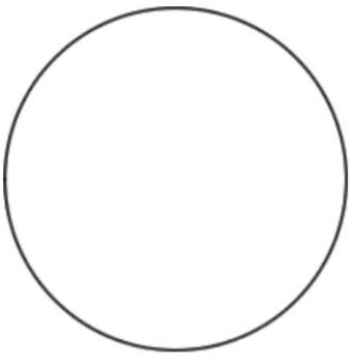
Procedure:

A. Wet Mount of an Onion Cell

1. Put a drop of water onto the microscope slide.
2. Using the forceps, gently peel off a small piece of the "membrane" of the onion (epidermis). It should be very thin and may curl up on itself.
3. Place the onion sample into the drop of water on your slide. Try to unroll/straighten out the sample to view a single layer of cells. You may need to use the dissecting needle to do this.
4. Place a coverslip onto the slide.
5. Use the SCANNING (4x) objective to focus. You probably will not see the cells at this power.
6. Switch to low power (10x). Cells walls should be visible: they will look like semi-clear grid lines.
7. Once you think you have located a cell, switch to high power (40x) and refocus.
8. Sketch the cell at low and high power. Label the nucleus, cytoplasm, and cell wall of a single cell. Draw your cells to scale.



Onion cell at low power (10x)



Onion cell at high power (40x)

B. Wet Mount of an *Elodea* Leaf Cell

1. Put a drop of water onto the microscope slide.
2. Using the forceps, gently tear off a small piece of a leaf from *Elodea*.
3. Place the *Elodea* leaf into the drop of water on your slide.
4. Place a coverslip onto the slide.
5. Use the SCANNING (4x) objective to focus. You probably will not see the cells at this power.
6. Switch to low power (10x). Cells walls should be visible: they will look like dark grid lines.
7. Once you think you have located a cell, switch to high power (40x) and refocus.
8. Sketch the cell at low and high power. Label the chloroplasts, cytoplasm, and cell wall of a single cell. The nucleus may be visible as well—it will be a large, clear figure. Draw your cells to scale.

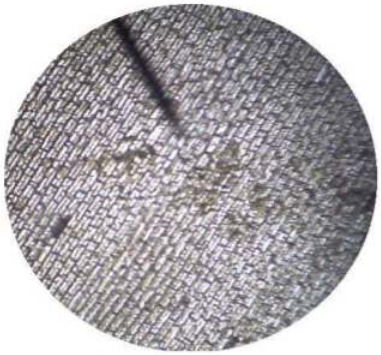
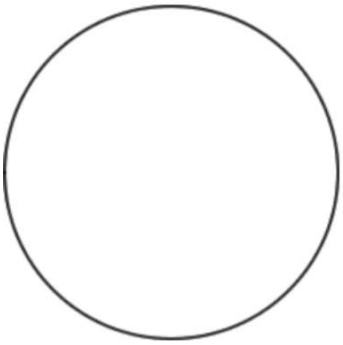
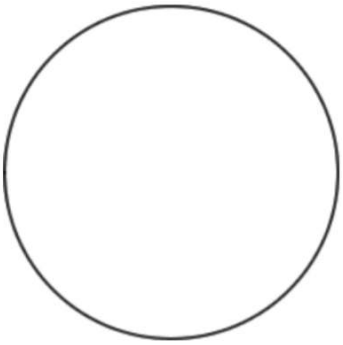


Figure 3: *Elodea* cells at low power
"Anacharis 40x" by biologycorner, via Flickr



Elodea cell at low power (10x)



Elodea cell at high power (40x)

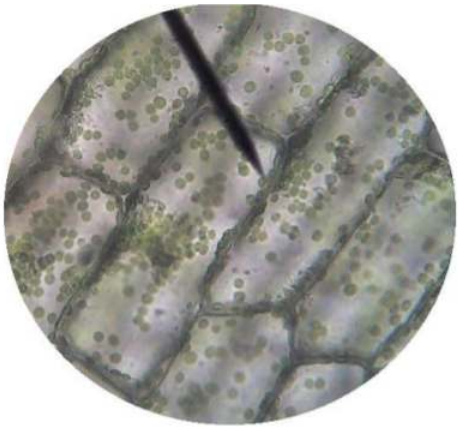


Figure 4: *Elodea* cells at high power
"Anacharis 400x" by biologycorner, via Flickr

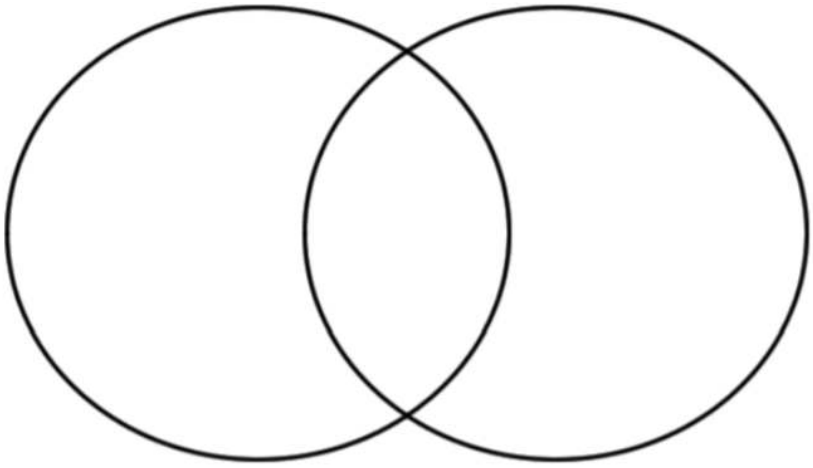
Questions:

1. Describe the shape and the location of chloroplasts.

2. Were chloroplasts observed in the onion cells? Why or why not?

3. "Animal cells have mitochondria; plant cells have chloroplasts." Is this statement true or false? Explain.

4. Fill out the Venn diagram below to show the differences and similarities between the onion cells and the *Elodea* cells.

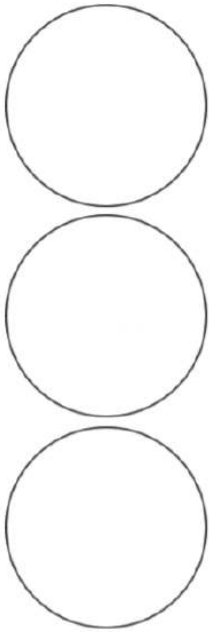


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7.3: Lab Report

Part 1: Bacterial Cells

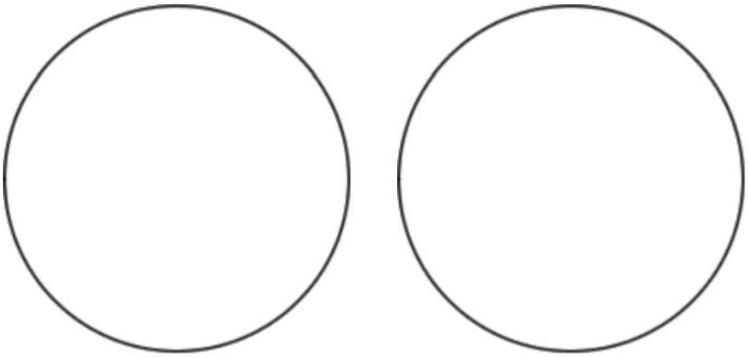


A. Fixed Slide of Bacterial Types

Draw each of the bacterial shapes in the spaces at right. Label each drawing.

B. Wet Mount of *Oscillatora*

Sketch the bacteria at low and high power. Label the cytoplasm and cell wall of a single cell. Draw your cells to scale.



Oscillatoria at low power (10x)

Oscillatoria at high power (40x)

Questions:

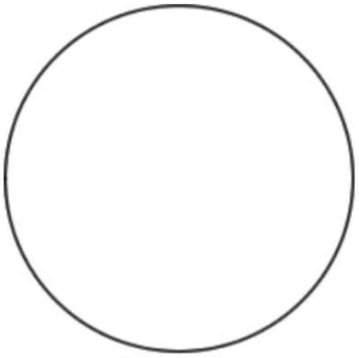
1. Why are nuclei not visible within the cells viewed?

2. The common name for species like Oscillatoria is blue-green algae. This group of bacteria is capable of photosynthesizing. Do they contain chloroplasts? Explain.

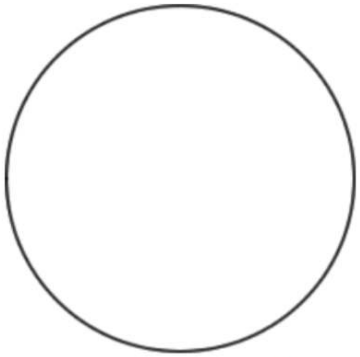
Part 2: Animal Cells

Procedure:

Sketch the cell at low and high power. Label the nucleus, cytoplasm, and cell membrane of a single cell. Draw your cells to scale.



Cheek cell at low power (10x)



Cheek cell at high power (40x)

Questions:

1. Why is methylene blue added?

2. The light microscope used in the lab is not powerful enough to view other organelles in the cheek cell.

    a. What parts of the cell were visible?

    b. List 2 organelles that were NOT visible but should have been in the cheek cell.

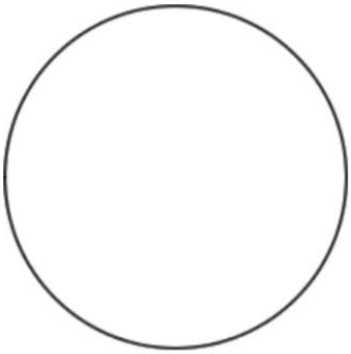
3. Is the cheek cell a eukaryote or prokaryote? How do you know?

4. Keeping in mind that the mouth is the first site of chemical digestion in a human. Your saliva starts the process of breaking down the food you eat. Keeping this in mind, what organelle do you think would be numerous inside the cells of your mouth? Explain.

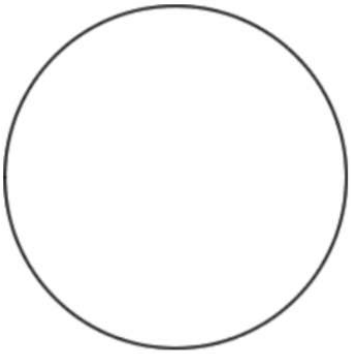
Procedure:

A. Wet Mount of an Onion Cell

Sketch the cell at low and high power. Label the nucleus, cytoplasm, and cell wall of a single cell. Draw your cells to scale.



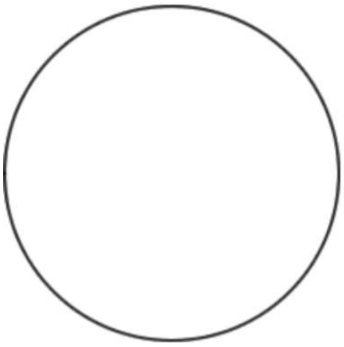
Onion cell at low power (10x)



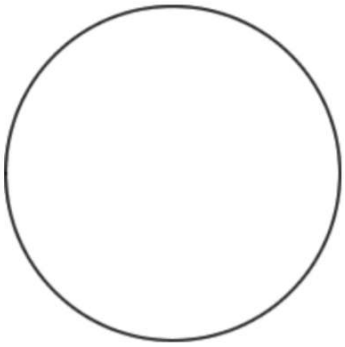
Onion cell at high power (40x)

B. Wet Mount of an *Elodea* Leaf Cell

Sketch the cell at low and high power. Label the chloroplasts, cytoplasm, and cell wall of a single cell. The nucleus may be visible as well—it will be a large, clear figure. Draw your cells to scale.



Elodea cell at low power (10x)



Elodea cell at high power (40x)

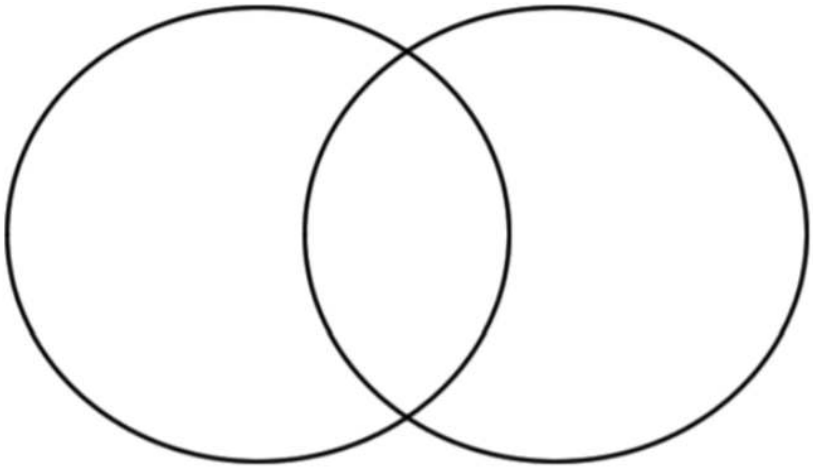
Questions:

1. Describe the shape and the location of chloroplasts.

2. Were chloroplasts observed in the onion cells? Why or why not?

3. “Animal cells have mitochondria; plant cells have chloroplasts.” Is this statement true or false? Explain.

4. Fill out the Venn diagram below to show the differences and similarities between the onion cells and the *Elodea* cells.



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CHAPTER OVERVIEW

8: Bacteriology (Prokaryotic cells)

Learning Objectives

Content Objectives

- Define aseptic technique.
- Describe how to culture bacteria.
- Explain differential staining and why it is useful for viewing bacterial cells.

Skill Objectives

- Observe and describe bacterial colony characteristics on an agar surface.
- Prepare a heat-fixed bacterial smear.
- Perform a Gram stain and interpret the results.

8.1: Introduction
8.2: Exercises
8.3: Lab Report

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8.1: Introduction

The Birth of Bacteriology

While perhaps best known to us as a cause of human disease, bacteria really should be far more famous for their positive contributions than for their negative ones.

Bacteria were first observed by Anton von Leeuwenhoek in the late 17th century, but didn't become the objects of serious scientific study until the 19th century, when it became apparent that some species caused human diseases. The methods devised by Robert Koch, Louis Pasteur, and their associates during the "Golden Age" of microbiology, which spanned from the mid-1800s to early 1900s, are still widely used today. Most of these methods involved isolating single bacteria derived from a natural source (such as a diseased animal or human) and cultivating them in an artificial environment as a pure culture to facilitate additional studies.

During the middle of the twentieth century, when we believed we had defeated them at their disease-causing game, bacteria became popular subjects of empirical study in fields such as genetics, genetic engineering, and biochemistry. With the evolution of antibiotic-resistant strains and our increased knowledge of bacterial stealth attack strategies such as biofilms and intracellular growth, medical researchers have refocused their attention on disease-causing bacteria and are looking for new ways to defeat them.

Growing bacteria in pure culture is still one of the most widely used methods in microbiology. Many bacteria, particularly those that cause diseases and those used in scientific studies, are heterotrophic, which means that they rely on organic compounds as food to provide energy and carbon. Some bacteria also require added nutritional components such as vitamins in their diet. An appropriate physical environment must be created, where important factors such as temperature, pH, and the concentration of atmospheric gases (particularly oxygen) are controlled and maintained.

The nutritional needs of bacteria can be met through specialized microbiological media that typically contain extracts of proteins (as a source of carbon and nitrogen), inorganic salts such as potassium phosphate or sodium sulfate, and in some cases, carbohydrates such as glucose or lactose. For **fastidious** bacteria (meaning, those that are picky eaters) vitamins and/or other growth factors must be added as well.

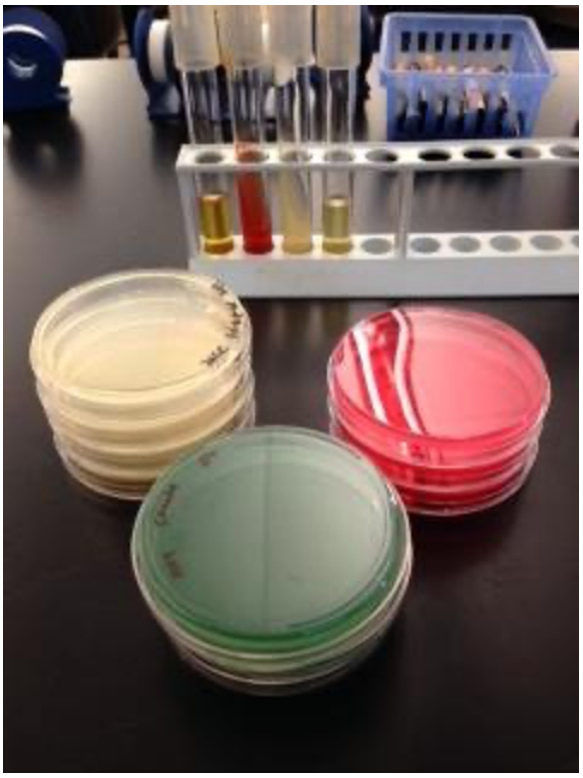


Figure 8.1.1: Different liquid and solid culture media types. Image credit: Microbiology: A Laboratory Experience by Holly Ahern, is licensed under a Creative Commons Attribution-NonCommercial-ShareAlike 4.0 International License.

Bacteriological culture media can be prepared as a liquid (broth), a solid (plate media or slant media), or as a semi-solid (deeps) as illustrated in the figure above. Solid and semi-solid media contain a solidifying agent such as agar or gelatin. Agar, which is a polysaccharide derived from red seaweed (*Rhodophyceae*) is preferred because it is an inert, non-nutritive substance. The agar provides a solid growth surface for the bacteria, upon which bacteria reproduce until the distinctive lumps of cells that we call colonies form.

Koch, Pasteur, and their colleagues in the 19th and early 20th centuries created media formulations that contained cow brains, potatoes, hay, and all sorts of other enticing microbial edibles. Today, bacteriological media formulations can be purchased in powdered form, so that all the preparer has to do is to measure out the correct amount, add the right amount of water, and mix. After the basic formula has been prepared, the medium is sterilized in an autoclave, which produces steam under pressure and achieves temperatures above boiling. Once sterilized media has cooled, it is ready to be used.

Growing Bacteria in Culture

A population of bacteria grown in the laboratory is referred to as a **culture**. A **pure culture** contains only one single type; a **mixed culture** contains two or more different bacteria. If a bacterial culture is left in the same media for too long, the cells use up the available nutrients, excrete toxic metabolites, and eventually the entire population will die. Thus bacterial cultures must be periodically transferred, or **subcultured**, to new media to keep the bacterial population growing.

Microbiologists use subculturing techniques to grow and maintain bacterial cultures, to examine cultures for purity or morphology, or to determine the number of viable organisms. In clinical laboratories, subculturing is used to obtain a pure culture of an infectious agent, and also for studies leading to the identification of the pathogen. Because bacteria can live almost anywhere, subculturing steps must be performed **aseptically**, to ensure that unwanted bacterial or fungal contamination is kept out of an important culture.

In microbiology, aseptic techniques essentially require only common sense and good laboratory skills. First, consider that every surface you touch and the air that you breathe may be contaminated by microorganisms. Then think about the steps you can take to minimize your exposure to unwanted invisible intruders. You should also be thinking about how to prevent contamination of your bacterial cultures with bacteria from the surrounding environment (which includes you).

To maintain an aseptic work environment, everything you work with should be initially free of microbes. Thus, we begin with pre-sterilized pipettes, culture tubes, and glassware. Inoculating loops and needles made of metal wire can be used to transfer bacteria from one medium to another, such as from the surface of an agar plate to a broth. Metal tools may be sterilized by heating them in the flame of a Bunsen burner. Glass tools or metal spreaders or forceps that can't be sterilized by direct heat are dipped in alcohol followed by a brief pass through the flame to speed the evaporation process. Standard aseptic techniques used for culturing bacteria will be demonstrated at the beginning of lab.

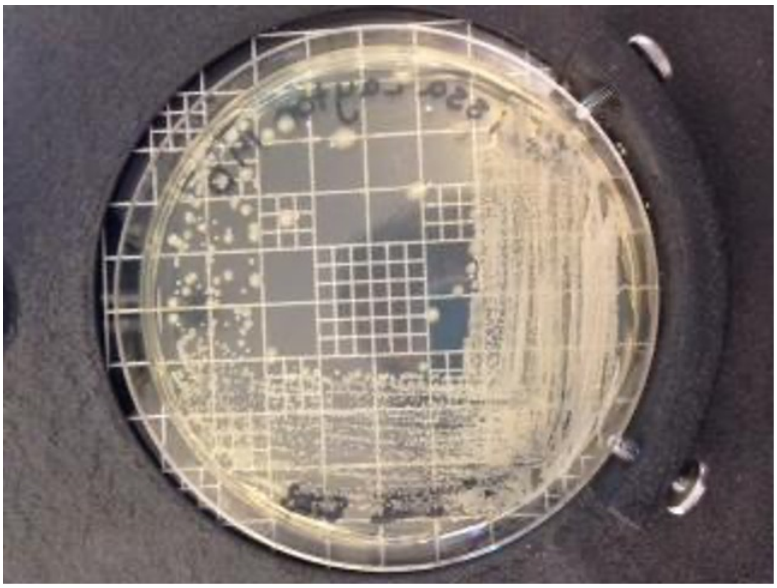


Figure 8.1.2: Colonies of bacteria growing on an agar plate. Image credit: Microbiology: A Laboratory Experience by Holly Ahern, is licensed under a Creative Commons Attribution-NonCommercial-ShareAlike 4.0 International License.

One very important method in microbiology is to isolate a single type of bacteria from a source that contains many. The most effective way to do this is the **streak plate** method, which dilutes the individual cells by spreading them over the surface of an agar plate (see Figure 2). Single cells reproduce and create millions of clones, which all pile up on top of the original cell. The piles of bacterial cells observed after an incubation period are called **colonies**. Each colony represents the descendants of a single bacterial cell, and therefore, all of the cells in the colonies are clones. Therefore, when you transfer a single colony from the streak plate to new media, you have achieved a pure culture with only one type of bacteria.

Different bacteria give rise to colonies that may be quite distinct to the bacterial species that created it. Therefore, a useful preliminary step in identifying bacteria is to examine a characteristic called **colonial morphology**, which is defined as the appearance of the colonies on an agar plate or slant. Ideally, these determinations should be made by looking at a single colony; however, if the colonial growth is more abundant and single colonies are absent, it is still possible to describe some of the colonial characteristics, such as the texture and color of the bacterial growth.

Describing Colonial Morphology of Bacteria

By looking closely at the colonial growth on the surface of a solid medium (such as a petri plate), characteristics such as surface texture, transparency, and the color or hue of the growth can be described. The following three characteristics are readily apparent whether you're looking at a single bacterial colony or more dense growth, without the aid of any type of magnifying device.

Texture—describes how the surface of the colony appears. Common terms used to describe texture may include smooth, glistening, mucoid, slimy, dry, powdery, flaky etc.

Transparency—colonies may be transparent (you can see through them), translucent (light passes through them), or opaque (solid-appearing).

Color or Pigmentation—many bacteria produce intracellular pigments which cause their colonies to appear a distinct color, such as yellow, pink, purple or red. Many bacteria do not produce any pigment and appear white or gray.

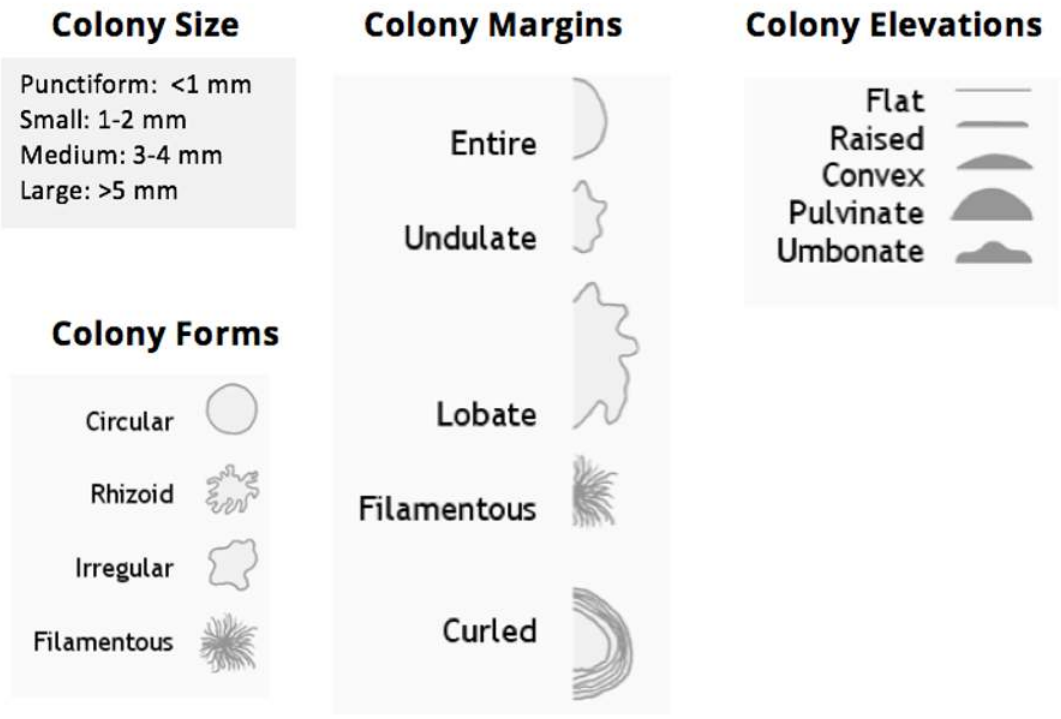


Figure 8.1.3: Bacteriological descriptions of colonial morphology. Image credit: Microbiology: A Laboratory Experience by Holly Ahern, is licensed under a Creative Commons Attribution-NonCommercial-ShareAlike 4.0 International License.

As the bacterial population increases in number, the colonies get larger and begin to take on a shape or form. These can be quite distinctive and provide a good way to tell colonies apart when they are similar in color or texture. The following three characteristics can be described for bacteria when a single, separate colony can be observed. It may be helpful to use a magnifying tool, such as a colony counter or dissecting microscope, to enable a close-up view of the colonies. Colonies should be described as to their overall size, their shape or form, what a close-up of the edges of the colony looks like (edge or margin of the colony), and how the colony appears when you observe it from the side (elevation).

Figure 4 shows a close-up of colonies growing on the surface of an agar plate. In this example, the differences between the two bacteria are obvious, because each has a distinctive colonial morphology.

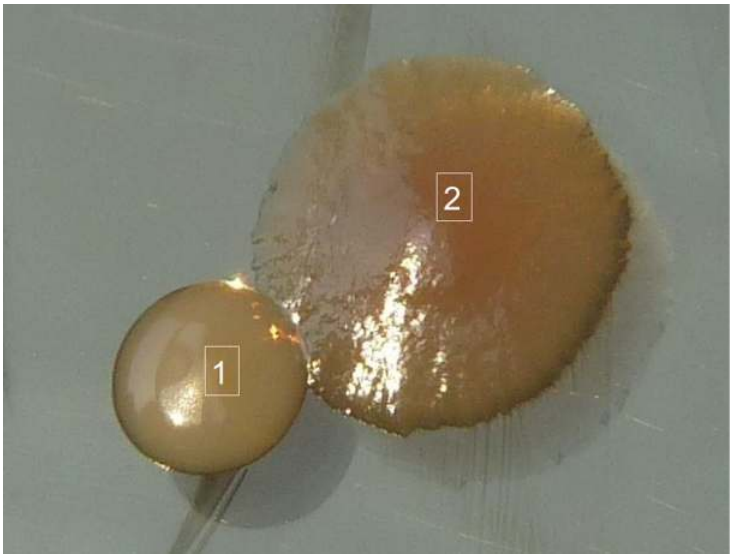


Figure 8.1.4: Two different organisms growing colonies on an agar surface. Image credit: Microbiology: A Laboratory Experience by Holly Ahern, is licensed under a Creative Commons Attribution-NonCommercial-ShareAlike 4.0 International License.

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Viewing Bacterial Cells

The microscope is a very important tool in microbiology, but there are limitations when it comes to using one to observe cells in general and bacterial cells in particular. Two of the most important concerns are resolution and contrast. Resolution is a limitation that we can't do much about, since most bacterial cells are already near the resolution limit of most light microscopes. Contrast, however, can be improved by either using a different type of optical system, such as phase contrast or a differential interference contrast microscope, or by staining the cells (or the background) with a chromogenic dye that not only adds contrast, but gives them a color as well.

There are many different stains and staining procedures used in microbiology. Some involve a single stain and just a few steps, while others use multiple stains and a more complicated procedure. Before you can begin the staining procedure, the cells have to be mounted (smeared) and fixed onto a glass slide.

A **bacterial smear** is simply that—a small amount of culture spread in a very thin film on the surface of the slide. To prevent the bacteria from washing away during the staining steps, the smear may be chemically or physically "fixed" to the surface of the slide. **Heat-fixing** is an easy and efficient method, and is accomplished by passing the slide briefly through the flame of a Bunsen burner, which causes the biological material to become more or less permanently affixed to the glass surface.

Heat fixed smears are ready for staining. In a **simple stain**, dyes that are either attracted by charge (a cationic dye such as methylene blue or crystal violet) or repelled by charge (an anionic dye such as eosin or India ink) are added to the smear. Cationic dyes bind the bacterial cells which can be easily observed against the bright background. Anionic dyes are repelled by the cells, and therefore the cells are bright against the stained background. See Figures 6 and 7 for examples of both.

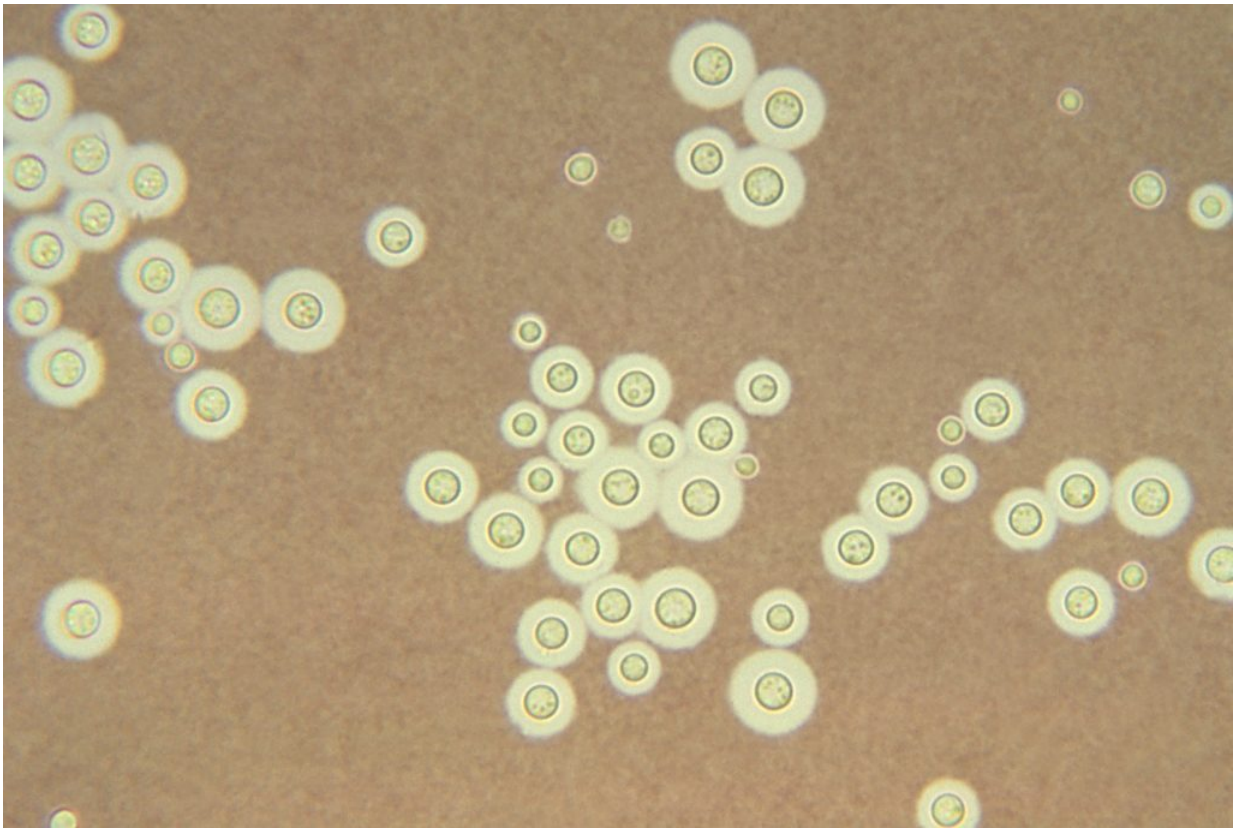


Figure 8.1.5: Negative stain of *Cyptococcus neoformans*, an encapsulated yeast. Image credit: Microbiology: A Laboratory Experience by Holly Ahern, is licensed under a Creative Commons Attribution-NonCommercial-ShareAlike 4.0 International License.

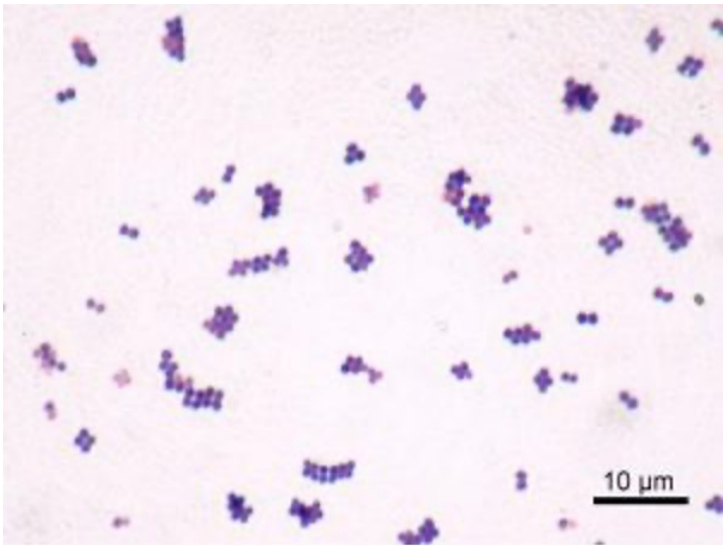


Figure 8.1.6: Positive stain of *Staphylococcus aureus*. Image credit: Microbiology: A Laboratory Experience by Holly Ahern, is licensed under a Creative Commons Attribution-NonCommercial-ShareAlike 4.0 International License.

Probably the most important feature made obvious when you stain bacterial cells is their **cellular morphology** (not to be confused with colonial morphology, which is the appearance of bacterial colonies on an agar plate). Most heterotrophic and culturable bacteria come in a few basic shapes: spherical cells (coccus/cocci), rod-shaped cells (bacillus/bacilli), or rod-shaped cells with bends or twists (vibrios and spirilla, respectively). There is greater diversity of shapes among Archaea and other bacteria found in ecosystems other than the human body.

Often bacteria create specific **arrangements** of cells, which form as a result of binary fission by the bacteria as they reproduce. Arrangements are particularly obvious with non-motile bacteria, because the cells tend to stay together after the fission process is complete. Both the shape and arrangement of cells are characteristics that can be used to distinguish among bacteria. The most commonly encountered bacterial shapes (cocci and bacilli) and their possible arrangements are shown in the Figure below.

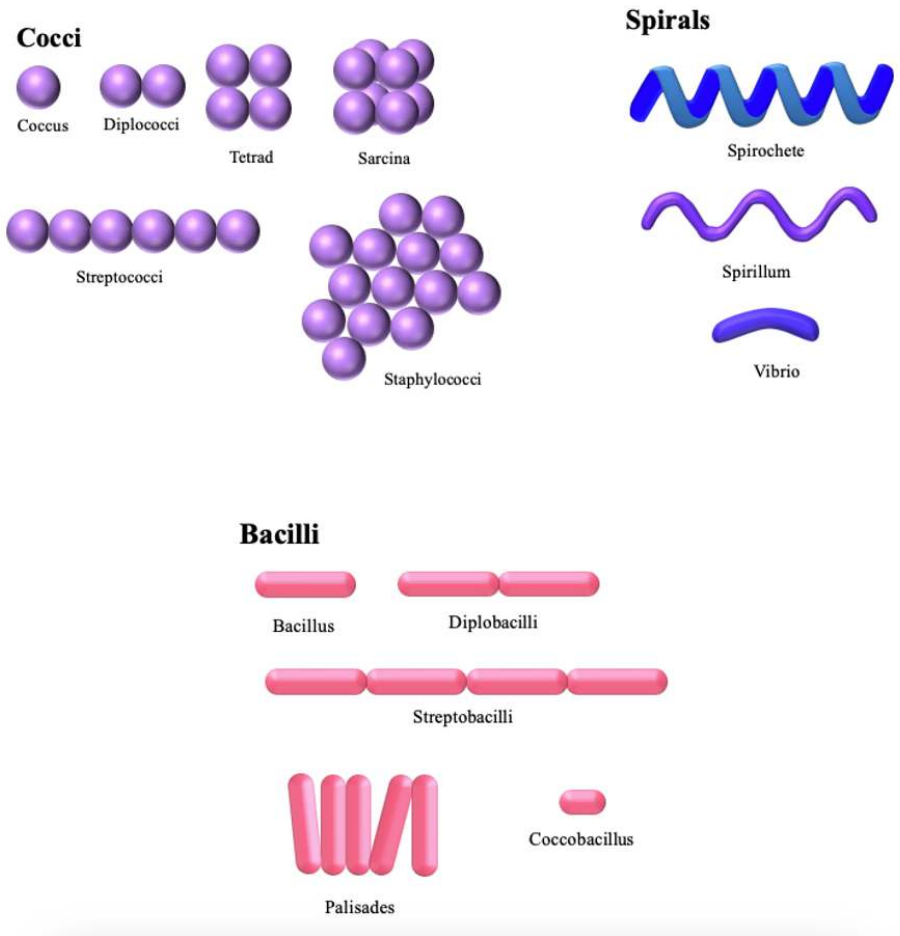


Figure 8.1.7: Bacterial Shapes and Arrangements. Image by McLaughlin and Petersen, work is made publicly available by the City University of New York (CUNY). Contact: AcademicWorks@cuny.edu.

https://academicworks.cuny.edu/cgi/viewcontent.cgi?referer=&httpsredir=1&article=1015&context=qb_oers

Differential Staining Techniques

In microbiology, differential staining techniques are used more often than simple stains as a means of gathering information about bacteria. Differential staining methods, which typically require more than one stain and several steps, are referred to as such because they permit the differentiation of cell types or cell structures. The most important of these is the Gram stain. Other differential staining methods include the endospore stain (to identify endospore-forming bacteria), the acid-fast stain (to discriminate *Mycobacterium* species from other bacteria), a metachromatic stain to identify phosphate storage granules, and the capsule stain (to identify encapsulated bacteria). We will be performing the Gram stain and endospore staining procedures in lab, and view prepared slides that highlight some of the other cellular structures present in some bacteria.

Gram Stain

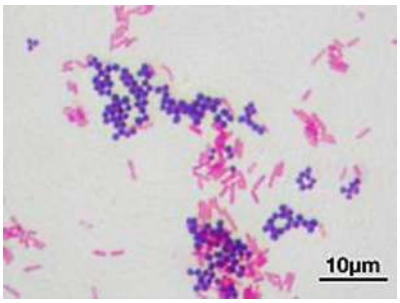


Figure 8.1.8: Gram positive (purple) and Gram negative (pink) stained cells. Image credit: Microbiology: A Laboratory Experience by Holly Ahern, is licensed under a Creative Commons Attribution-NonCommercial-ShareAlike 4.0 International License.

In 1884, physician Hans Christian Gram was studying the etiology (cause) of respiratory diseases such as pneumonia. He developed a staining procedure that allowed him to identify a bacterium in lung tissue taken from deceased patients as the etiologic agent of a fatal type of pneumonia. Although it did little in the way of treatment for the disease, the Gram stain method made it much easier to diagnose the cause of a person's death at autopsy. Today we use Gram's staining techniques to aid in the identification of bacteria, beginning with a preliminary classification into one of two groups: **Gram positive** or **Gram negative**.

The differential nature of the Gram stain is based on the ability of some bacterial cells to retain a primary stain (crystal violet) by resisting a decolorization process. Gram staining involves four steps. First cells are stained with crystal violet, followed by the addition of a setting agent for the stain (iodine). Then alcohol is applied, which selectively removes the stain from only the Gram-negative cells. Finally, a secondary stain, safranin, is added, which counterstains the decolorized cells pink.

Although Gram didn't know it at the time, the main difference between these two types of bacterial cells is their cell walls. Gram negative cell walls have an outer membrane (also called the envelope) that dissolves during the alcohol wash. This permits the crystal violet dye to escape. Only the decolorized cells take up the pink dye safranin, which explains the difference in color between the two types of cells. At the conclusion of the Gram stain procedure, Gram positive cells appear purple, and Gram-negative cells appear pink.

When you interpret a Gram-stained smear, you should also describe the morphology (shape) of the cells, and their arrangement. In Figure 8.1.8, there are two distinct types of bacteria, distinguishable by Gram stain reaction, and also by their shape and arrangement. Can you describe them?

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8.2: Exercises

Exercise A: Colony Characteristics

Work in groups of 2-4 students. Safety: wear gloves, keep petri plates closed at all times, and wipe work benches with disinfectant when finished. Discard gloves in biohazard (red) waste bags. Examine each of the petri plates provided. You may use the stereoscope or your microscope (2x filter with no lens, or BF filter with 4x lens). Record results in Table 8.1 in the lab report (use technical terms).

Data Table for Microbe Identification by Morphology

Name of Bacterium:	**Typically found where?**	**Average colony size (mm):**	**Colony Shape:**	**Margin:**	**Surface:**	**Pigmentation (or none)**
Micrococcus luteus						
Serratia marcescens						
Bacillus						
Escherichia coli						

Table 8.1 Colony Characteristics

Exercise B: Investigating Environmental Microbes

Bacteria, fungi, and viruses can be found just about everywhere on earth! Today, you will collect samples of bacteria and fungi that are either on you or around you. Work with one other student and follow instructions from your instructor. Label your plastic petri dish along the edges, on the agar side, not the lid**,** using a permanent marker. Include your last name, the surface you will swab, the date, your section number and the incubation temperature.

Create a hypothesis and a prediction for your experiment BEFORE you swab, record them in the lab report section. Dip you sterile swab into sterile water and then rub the surface you want to test, several times, back and forth. Rotate your swab and rub your surface again. Now you are ready to rub the swab gently on the surface of your agar plate. Minimize the agar's exposure to air as much as possible. After 5 minutes, flip petri plates upside down to incubate. Incubate at 37 C if your test surface was your skin, otherwise, incubate at room temperature (25 C). Use Parafilm to seal the lid to the bottom before incubation.

B.1. What surfaces will you swab? Examples: you can compare before and after handwashing (don’t dry your hands), or compare two body areas, or environmental samples such as commonly touched areas (phone vs your pencil).

B.2. What is your hypothesis? **Get approval before you swab**.

B.3. What is your prediction (what will your results look like if your hypothesis is true)?

B.4. After incubation: Sketch and describe your results below.

B.5. Do your results support your hypothesis? Explain:

Exercise C: Viewing Prepared Slide of Gram-Stained "Three Bacterial Types"

Obtain a prepared gram-stained slide, labeled “Three Bacterial Types,” from the side counter. Follow the instructions on using oil immersion from our Microscopy Lab I. Each student should focus their own slide for all three bacterial samples in this exercise. Then, fill in Table 8.2 in the lab report. Remember to use color for your sketches.

Data Table for Three Bacterial Types

Type:	Describe the shape and arrangement of cells:	Sketch at 1000x (oil)	Gram +/-:
bacillus (bacilli)			
coccus (cocci)			
spirillum (spirilla)			

Table 8.2 Prepared Slide of Gram-Stained "Three Bacterial Types"

Exercise D: Making a Gram-Stained Slide

Materials:

How to make a heat-fixed bacterial smear

1. Label a clean glass slide as demonstrated by your instructor

2. Add a small drop of water to the center of the slide

3. With an inoculation loop, aseptically pick up a small amount of bacteria

4. Mix it well with into your drop of water and then spread the mixture over a wider area of the slide. Make sure you don't have any clumps and your smear is not thick

5. **Air dry** (completely) (slide warmers may also be used if available).

6. When slides are completely dry, **heat fix** the bacterial specimen by passing the slide slowly over the flame twice (your instructor will demonstrate this) for total of about 3 seconds.

Heat fixing kills cells and adheres them to the slide. Cells will be rinsed off of the slide if they are not heat fixed properly. Be careful not to overheat the slides in this procedure, you can distort the shape of the cells and/or crack your glass slide. After heat-fixing is complete, you are ready to stain your slide.

Gram Stain

For all steps in the gram staining procedure, add enough of the solution to cover the entire smear (usually 2-3 drops). All staining should be done over a staining tray. Be sure to put paper towels under the tray in case of spillage. Gloves should be worn while staining and removed before working with the microscope. Each student will be assigned **one** bacterial culture to stain, you will look at your group member's stains to sketch the remaining three types of bacteria. Use the instructions for oil immersion from our microscopy lab when you view your Gram-stain. Leave your slide, in-focus on 1000x, on your microscope until everyone is finished with it. Then you may place it into the 10% bleach container in the sink.

Staining Steps

1. Add a few drops of crystal violet (primary stain) to the smear and let it sit for 60 seconds.

2. Rinse the slide with water. All cells are purple after this step. Stopping here would be a simple stain. Do not dry the slide.

3. Add a few drops of Gram's iodine (mordant) to the smear and let it sit for 60 seconds. Gram's iodine forms a complex with the crystal violet that is inside the cell wall.

4. Rinse the slide with water. Do not dry the slide. All cells are still purple after this step.

5. Decolorize with 95% ethanol: let the alcohol run over surface of slide until no more crystal violet color comes out of the smear (time varies, 3-10 seconds). Gram-positive cells retain crystal violet and remain purple after this step. Gram-negative cells lose crystal violet and are now colorless after this step.

6. IMMEDIATELY rinse with water. Water rinse stops the decolorization process. If you leave the alcohol on longer than 10 seconds you will damage the cells and they will all look gram-negative, this is called over-decolorization. Do not dry your slide.

7. Add a few drops of safranin (counterstain) and let it sit for 60 seconds. Safranin is a pink/red dye.

8. Rinse with water.

9. Blot dry on bibulous paper. Do not rub, press down gently. Gram-positive cells remain purple; gram-negative cells are now pink/red.

10. Dispose of the staining waste in your tray into the special waste container inside the hood. Rinse and dry staining tray. Disinfect your workspace and remove gloves. (biohazardous waste).

11. Observe your slide under the microscope, view all the way to 1000x (oil). Remember not to use a microscope near a flame.

11. For each organism, determine morphology, arrangement, and Gram reaction. Then, draw (in color) a representative set of cells and include the calculation for total magnification below the table.

Data Table for Gram-positive and Gram-negative Bacteria

Bacterium:	**Color of cells (100x lens)**	**Gram result (+/-)**	**Shape (morphology)**	**Sketch in color, 100x lens**
Micrococcus luteus				
Serratia marcescens				
Bacillus				
Escherichia coli				

Table 8.3 Gram Stain Results

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8.3: Lab Report

Exercise A: Colony Characteristics

Data Table for Microbe Identification by Morphology

Name of Bacterium:	Typically found where?	Average colony size (mm):	Colony Shape:	Margin:	Surface:	Pigmentation (or none)
Micrococcus luteus						
Serratia marcescens						
Bacillus						
Escherichia coli						

Table 8.1 Colony Characteristics

Below, list three ways we benefit from bacteria. You may think of examples from nature, industrial and medical applications, or others. You may reference our lecture material or search the internet (be sure to cite your source).

A.1.

A.2.

A.3.

Exercise B: Investigating Environmental Microbes

B.1. What surfaces will you swab? Examples: you can compare before and after handwashing (don't dry your hands), or compare two body areas, or environmental samples such as commonly touched areas (phone vs your pencil).

B.2. What is your hypothesis? **Get approval before you swab**.

B.3. What is your prediction (what will your results look like if your hypothesis is true)?

B.4. After incubation: Sketch and describe your results below.

B.5. Do your results support your hypothesis? Explain:

Exercise C: Viewing Prepared Slide of Gram-Stained "Three Bacterial Types"

Data Table for Three Bacterial Types

Type:	Describe the shape and arrangement of cells:	Sketch at 1000x (oil)	Gram +/-:
bacillus (bacilli)			
coccus (cocci)			
spirillum (spirilla)			

Table 8.2 Prepared Slide of Gram-Stained "Three Bacterial Types"

Exercise D

Data Table for Gram-positive and Gram-negative Bacteria

Bacterium:	Color of cells (100x lens)	Gram result (+/-)	Shape (morphology)	Sketch in color, 100x lens
Micrococcus luteus				
Serratia marcescens				
Bacillus				
Escherichia coli				

Table 8.3 Gram Stain Results

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CHAPTER OVERVIEW

9: Diffusion and Osmosis

Learning Objectives

Content Objectives

- Describe the processes of diffusion and osmosis.
- Define tonicity and describe its relevance to passive transport.

Skill Objectives

- Determine the relationship between molecular weight and diffusion rate through the analysis of experimental results.
- Employ the scientific method to observe and analyze the effects of osmosis on plant and animal cells.

9.1: Introduction
9.2: Exercise
9.3: Lab Report

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9.1: Introduction

The **cell theory** states that all living things are composed of cells and that cells only arise from other cells. Some cells are fairly simple in structure, while others are extremely complex. For example, some organisms are unicellular—they exist as a single cell, while multicellular organisms are composed of many cells that form tissues and organs. In either case, all cells share some common properties: the presence of DNA, intracellular proteins that enable the cell to perform its functions, and a plasma membrane. Some cells, known as eukaryotic cells, also contain membrane-bound organelles that allow a more complex level of functioning.

Homeostasis is defined as the maintenance of a stable internal environment. In order to maintain homeostasis, cells continually transport substances in and out across their cell (plasma) membrane.

The cell membrane serves as a "gatekeeper" and is the cellular structure that regulates the transport of materials into and out of the cell. The phospholipid bilayer architecture of the cell membrane allows certain molecules to pass through while keeping others out, therefore the cell membrane is **selectively permeable (or semipermeable)**. Things that need to enter a cell for it to function properly include ions, nucleotides, sugars, oxygen, amino acids, water, vitamins, and some hormones. Cells also allow certain molecules like water, ions, and secreted proteins to leave. Additionally, cells must eliminate waste products like urea and carbon dioxide

In the following exercises, you will examine the semipermeable nature of the cell membrane. You will also explore the concept of **tonicity**, which refers to the solute concentration of a solution, and its inherent ability to influence the rate and direction of osmosis.

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9.2: Exercise

PART 1: DIFFUSION & OSMOSIS

Diffusion is the movement of molecules from an area in which they are high in concentration to an area in which they are low in concentration. Molecules move down a **concentration gradient** until they are equally distributed, or **equilibrium** is reached (Fig. 1). At equilibrium, there is no concentration gradient. Molecules still move once equilibrium is reached, but there is no **net** movement in any one direction.



Figure 9.2.1: Diffusion of molecules from an area of high concentration to low concentration in coffee. Equilibrium is reached when the molecules are equally distributed. (Pixabay License; iirliinnaa from Pixabay)

Osmosis is a specific type of diffusion: the diffusion of **water** molecules across a semipermeable membrane. Like other molecules, water molecules diffuse down a concentration gradient, from an area of higher free water concentration to an area of lower free water concentration. This means that water will move across a semipermeable membrane, like the cell membrane, in the direction of the higher solute concentration. (In solution, high solute concentration = low free water concentration; conversely, low solute concentration = high free water concentration.) You will observe this concept in **Part 2: Osmosis & Tonicity**.

In living organisms, most substances are transported as **solutes**, dissolved in water, a **solvent**. For example, if we dissolve salt ($NaCl$) in a beaker of water, salt ($NaCl$) is the solute and water is the solvent. Examples of solutes in the human body include glucose, small proteins, and electrolytes like calcium and sodium ions. Waste products, such as CO_2 and urea are also transported as solutes. Solutes are carried by body fluids, such as blood plasma, and pass into and out of cells through **passive** and **active transport**. In either case, the cell membrane will either inhibit or facilitate the process of diffusion: some molecules can easily diffuse across a plasma membrane and some cannot. For example, small, nonpolar molecules (such as CO_2 and O_2) can cross a membrane by simple diffusion. Large molecules or polar molecules, however, cannot easily diffuse across a membrane. Cells must have specialized membrane-bound proteins that function to transport such substances across the membrane.

In **Part 1: Diffusion & Osmosis**, you will learn about diffusion and osmosis using dialysis membrane, a selectively permeable sheet of cellulose that permits the passage of water and small solutes, but does not allow larger molecules to diffuse across. This is because the membrane has microscopic pores that only allow small molecules through; anything larger than the size of the pores is prevented from crossing. Some of the solutes in this experiment, **sucrose** ($C_{12}H_{22}O_{11}$) and **starch** (Extra close brace or missing open brace are too large to pass through the pores of the dialysis tubing, but the solvent molecules (H_2O) and **glucose** ($C_{12}H_{22}O_{11}$), are small enough to pass easily.

Exercise 1: Molecular Weight and Diffusion Rate

Molecular weight is an indication of the mass and size of a molecule. The purpose of this experiment is to determine the relationship between molecular weight and the rate of diffusion through a semisolid gel. You will investigate two dyes, methylene blue and potassium permanganate.

Molecular Weight and Color of Dyes

Molecule	Molecular Weight	Color
Methylene blue	300 grams/mole	blue
Potassium permanganate	150 grams/mole	purple

Employing Steps in the Scientific Method:

1. Record the **Question** that is being investigated in this experiment.

   _____

2. Record a **Hypothesis** for the question stated above.

   _____

3. Predict the results of the experiment based on your hypothesis (if/then).

   _____

4. Perform the experiment below and collect your data.

Materials:

- Petri dish of agar semi-solid gel (Mueller Hinton agar plates, 150 x 15 mm) - make sure the agar has been allowed to come to room temperature
- Methylene blue solution (0.2% in 25% EtOH)
- Potassium permanganate solution (0.1% KMnO4)
- Small straws
- Small plastic metric ruler

Procedure:

1. Obtain a petri dish of agar

2. Take the plastic straw and gently stick it down into one side of the agar. Lift up the straw, withdrawing a small plug of agar. Repeat on the other side of the dish.

3. Using a 1mL transfer pipet, place a single drop of each dye into the appropriate agar well. (Fig. 2).

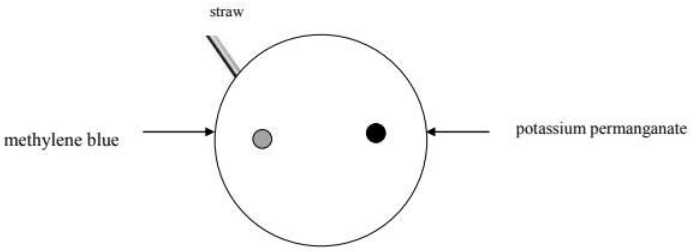


Figure 9.2.2: Placing a drop of dye into a small well on an agar plate.

4. After 20 minutes, place a small, clear metric ruler underneath the Petri dish to measure the distance (diameter) that the dye has moved. Enter the data in Table 1.

5. Repeat step 4 at 40, 60, and 80 minutes.

Table 1 - Diffusion results

	Molecular Weight (g/mole)	Diameter after 20 min. (mm)	Diameter after 40 min. (mm)	Diameter after 60 min. (mm)	Diameter after 80 min. (mm)
Methylene blue					
Potassium permanganate					

Questions:

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

  _____

- Go back and look at your initial hypothesis. Does your data support this hypothesis? Explain.

  _____

Extension Activity: (Optional)

The results of this experiment can be presented graphically. The presentation of your data in a graph will assist you in interpreting your results. Based on your results, you can complete the final step of scientific investigation, in which you must be able to propose a logical argument that either allows you to support or reject your initial hypothesis.

1. Graph your results using the data from Table 1.
2. What is the dependent variable? Which axis is used to graph this data?

   _____

3. What is your independent variable? Which axis is used to graph this data?

   _____

Exercise 2: Diffusion Across a Membrane

Employing Steps in the Scientific Method:

1. Record the **Question** that is being investigated in this experiment.

   _____

2. Record a **Hypothesis** for the question stated above.

   _____

3. Predict the results of the experiment based on your hypothesis (if/then).

   _____

4. Perform the experiment below and collect your data.

Materials:

- Dialysis tubing
- Plastic clips or string
- 5 x 400 mL beakers (or plastic cups)
- Electronic balance
- Weigh boats
- 15% Sucrose solution (MW sucrose = 342 g/mol)
- 30% Sucrose solution (MW sucrose = 342 g/mol)
- 30% Glucose solution (MW glucose = 180 g/mol)
- Graduated cylinders (10 mL and 100 mL)
- Wax pencil or sharpie
- 15% starch solution (MW = variable)
- Iodine solution (MW = 166 g/mol)
- Benedict’s reagent
- Hot plate or heat block

Procedure:

1. Cut 5 pieces of dialysis membrane approximately 10 cm long. Soak the pieces in tap water until they are soft and pliable (3-5 minutes). *This step may be done for you; check with your instructor.
2. Obtain 5 beakers (plastic cups) and label them #1 - 5. Fill each beaker with 150 mL of a solution as follows:

- Beaker #1 – H2O
- Beaker #2 – H2O

- Beaker #3 – H2O
- Beaker #4 – 30% sucrose solution
- Beaker #5 - H2O and 1mL Iodine solution

3. Set beakers aside.
4. Remove one piece of dialysis membrane from the soaking water and open it, forming a tube. Close one end of the tube with a plastic clip, a piece of string, or simply tie it with a knot (Fig. 3)



Figure 9.2.3: Dialysis tube “bag” (By BB)

5. Fill the bag with 10 mL of H2O. Remove excess air, and close the other end of the bag with a plastic clip, a piece of string, or tie with a knot. Set aside on a paper towel.
6. Repeat steps 4 and 5 for the 4 remaining dialysis tubes, filling them with 10 mL of a solution as follows:

• Bag #2 – 15% sucrose

• Bag #3 – 30% sucrose

• Bag #4 – H2O

• Bag #5 - 5 mL 30% glucose solution and 5 mL 15% starch solution

7. Rinse off the outside of the bags with water and carefully blot dry.
8. Weigh bags #1 - 4 to the nearest 0.5g. Record the weights in Table 1 below, in the column labeled “0 min.”
9. Place each bag in the corresponding beaker (Bag #1 in Beaker #1, etc.). Make sure each bag is fully submerged in the solution.
10. Set a timer for 5 minutes.
11. At the end of 5 minutes, remove bags 1 - 4 from their beakers, blot excess fluid, and record the mass (in grams) in Table 1.
12. Return the bags to the appropriate beaker, and wait another 5 minutes.
13. Repeat steps 11 - 13 every 5 minutes and record the weights in Table 1.

Table 1. Osmosis - mass (g) over time for dialysis bags

Time (min.)		0	5	10	15	20
Beaker #1 (water)	**Bag #1 (water)**					
Beaker #2 (water)	**Bag #2 (15% sucrose)**					
Beaker #3 (water)	**Bag #3 (30% sucrose)**					
Beaker #4 (30% sucrose)	**Bag #4 (water)**					

14. Calculate the total weight change (weight change = final weight – initial weight) for each bag. Record the values in Table 2. Calculate the **rate** (g/min) of osmosis for each bag by dividing the weight change by the time change. Since all 4 bags were recorded for a total of 20 minutes, the time change for all 4 bags is 20 minutes. Record the rate of osmosis for all 4 bags in Table 2.

Table 2. Rate of osmosis

	Weight Change (g)	Time (min)	Rate (g/min)
Bag #1 (water)			
Bag #2 (15% sucrose)			
Bag #3 (30% sucrose)			
Bag #4 (water)			

15. Make observations about bag #5 and beaker #5 in Table 3.
16. Remove several mL of liquid from bag #5 and beaker #5 and add each to separate test tubes.
17. Add several drops of Benedict's solution to each of the two test tubes and heat to 100 degrees Celsius in a boiling water bath or heat block for 2 - 5 minutes. Record the test results in Table 3.

Table 3. Selective Permeability

	Appearance of liquid after 20 minutes	Appearance of liquid after heating
Bag #5 (5 mL 30% glucose solution and 5 mL 15% starch solution)		
Beaker #5 (H2O and 1mL Iodine solution)		

Questions

1. Did the weight of each bag (#1 - #4) change significantly over 20 minutes? Explain.
2. In which bag(s) was there a **net movement** of water?
3. Explain what is meant by “net movement”.
4. Which carbohydrate molecules (glucose, sucrose, starch) were not able to move across the membrane? Explain.
5. In terms of **solvent** (water) concentration, water moved from the area of _____ concentration to the area of _____ concentration across a selectively permeable membrane, which is defined as _____.
6. What can you conclude about the movement of Iodine, glucose, and starch across the dialysis membrane based on your results in Table 3? Support your answers for each with the observation from bag #5 and beaker #5.

- Iodine -
- Glucose -
- Starch -

7. We used the dialysis tubing to simulate a cell membrane. How is the dialysis tubing functionally the same as a cell membrane?
8. We used the dialysis tubing to simulate a cell membrane. How is the dialysis tubing functionally different from a cell membrane?

Extension Activity: (Optional)

The results of this experiment can be presented graphically. The presentation of your data in a graph will assist you in interpreting your results. Based on your results, you can complete the final step of scientific investigation, in which you must be able to propose a logical argument that either allows you to support or reject your initial hypothesis.

1. Prepare a line graph using the data from Table 2.
2. What is the dependent variable? Which axis is used to graph this data?

   _____

3. What is your independent variable? Which axis is used to graph this data?

   _____

PART 2: OSMOSIS & TONICITY

Tonicity is the relative concentration of solute (particles), and therefore also a solvent (water), outside the cell compared with inside the cell.

• An **isotonic solution** has the same concentration of solute (and therefore of free water) as the cell. When cells are placed in an isotonic solution, there is no net movement of water.

• A **hypertonic solution** has a higher solute (therefore, lower free water) concentration than the cell. When cells are placed in a hypertonic solution, water moves out of the cell into the lower free water solution.

• A **hypotonic solution** has a lower solute (therefore, higher free water) concentration than the cell. When cells are placed in a hypotonic solution, water moves into the cell from the higher free water solution.

IMPORTANT NOTE: Notice that all of the above definitions have 'solution' as the noun. Sometimes the noun will refer to the cell instead of the solution. For example, a hypotonic cell will experience a net movement of water out of the cell. What this means is that if the 'solution' is hypotonic, the cell is hypertonic and vice versa.

Exercise 1: Observing Osmosis in Potato Strips

Employing Steps in the Scientific Method:

1. Record the **Question** that is being investigated in this experiment.

   _____

2. Record a **Hypothesis** for the question stated above.

   _____

3. Predict the results of the experiment based on your hypothesis (if/then).

   _____

4. Perform the experiment below and collect your data.

Materials:

- Potatoes
- Cork borer
- Petri dish
- Wax pencil or Sharpie
- Metric ruler
- 10% NaCl solution
- 0.9% NaCl solution
- Forceps and scalpel

Procedure:

1. Obtain a potato and use a cork borer to prepare 3 cylinders of potato. Push the borer through the length of the potato. When the borer is filled, use the flat end of a wooden skewer to gently push out the potato cylinder into the petri dish. Use the scalpel to cut each potato cylinder into a length of 5 cm.
2. With a wax pencil or Sharpie, label 3 test tubes (#1, #2, #3).
3. Using the metric ruler, mark each tube at the 10 cm mark level from the bottom of the tube.
4. Set up three test tubes with 10 cm of solutions as follows:

1. Tube #1 - distilled water
2. Tube #2 - 10% sodium chloride (NaCl)
3. Tube #3 - 0.9% NaCl

5. Place one potato cylinder into each test tube and allow them to soak for about 15 minutes in the solutions.
6. You can now move on to **Exercises 2 and 3** while your potatoes soak.
7. After the elapsed time, observe each strip for limpness (water loss, flaccid) or stiffness (water gain,turgor).

Questions

1. Which tube contained the limp (flaccid) potato strip? Explain.
2. Which tube contained the stiff (turgid) potato strip? Explain.
3. Which solution is isotonic to the inside of the potato cell?
4. What happened to the potato strip in the isotonic solution?

Osmoregulation in Living Cells

Some organisms, known as **osmoregulators**, have special adaptations to keep tight control over their internal osmotic conditions while still others, known as **osmoconformers**, are able to live in a variety of osmotic conditions. Most living cells, however, are often at the mercy of their surrounding osmotic environment. Many freshwater plants live in an isotonic or hypotonic environment, so they have no adaptations to protect them from a hypertonic environment. Likewise, mammalian red blood cells live in the isotonic plasma inside your circulatory system so they have no protection from either a hypertonic nor a hypotonic environment.

A plant cell is surrounded by a rigid cell wall, so when the cell is placed in a hypotonic environment, the net flow of water is from the surrounding medium into the cell, and it simply expands to the cell wall and becomes turgid. When the same plant cell is placed in a hypertonic environment, water leaves the central vacuole and the cytoplasm shrinks. This causes the cell membrane to pull away from the cell wall. In this situation, the plant cell will undergo plasmolysis and die. Animal cells have no cell wall so when they are in a hypotonic environment they will expand and fill with water until they burst in a condition known as lysis. **Figures 4 and 5** demonstrate these conditions.

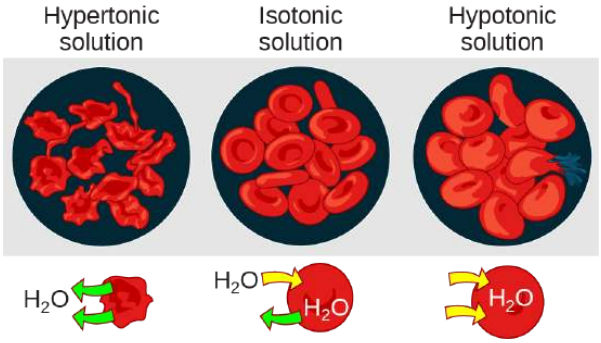


Figure 9.2.4: Red blood cells in varied osmotic environments.(Diffusion and Osmosis. (2021, February 28). Retrieved April 1, 2021, from https://chem.libretexts.org/@go/page/34826 CCBY)

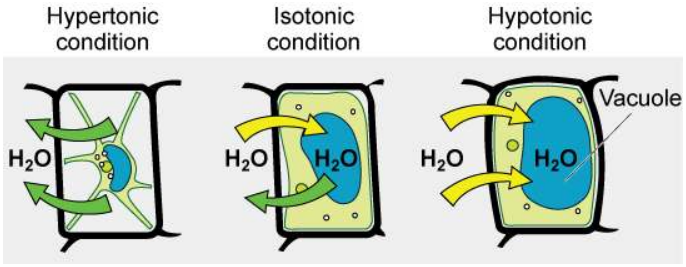


Figure 9.2.5: Plant cell tonicity. The turgor pressure within a plant cell depends on the solution's tonicity in which it is bathed. (credit: modification of work by Mariana Ruiz Villareal CCBY)

Exercise 2: Observing Osmosis in Elodea Cells

Employing Steps in the Scientific Method:

1. Record the **Question** that is being investigated in this experiment.

   _____

2. Record a **Hypothesis** for the question stated above.

   _____

3. Predict the results of the experiment based on your hypothesis (if/then).

   _____

4. Perform the experiment below and collect your data.

Materials

- Microscope
- Blank slides and coverslips
- DI water
- 10% salt solution
- Forceps
- Elodea leaf

Procedure

1. Place a drop of water onto a clean microscope slide.
2. Using the forceps, gently tear off a small leaf from the *Elodea* plant.
3. Prepare a wet mount by placing the *Elodea* leaf into the drop of water on your slide.
4. Place a coverslip onto the slide.
5. Use the **scanning** (4x) objective to bring the *Elodea* cells into focus. You may not be able to observe individual cells at this power.
6. Switch to the **low power** (10x) objective. The *Elodea* cell walls should be visible. They will look like dark green grid lines. Use the fine focus adjustment to focus the specimen.
7. Once you think you have located an *Elodea* cell, switch to the **high power** (40x) objective and refocus using the fine focus adjustment.
8. Next, add several drops of 10% salt (NaCl) solution to the edge of the coverslip to allow the salt to diffuse under the coverslip. Observe what happens to the cells (this may require you to search around along the edges of the leaf). Look for cells that have been visibly altered.
9. Record your observations in the following table. The cells in distilled water should look similar to the figure below.

Data Table for Elodea

Solution	Appearance of *Elodea* Cells
Distilled water (0% NaCl)	
10% NaCl	



Figure 3: *Elodea* cells at low power
"Anacharis 40x" by biologycorner, via Flickr

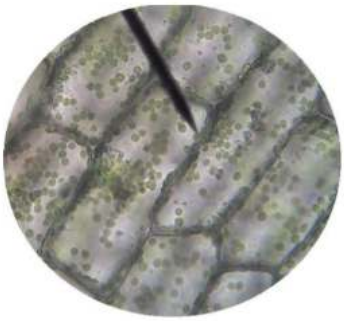


Figure 4: *Elodea* cells at high power
"Anacharis 400x" by biologycorner, via Flickr

Figure 9.2.6: Wet Mount of an Elodea **Leaf Cell (Burran, S., & DesRochers, D. (2021, March 19). Cells. Retrieved April 1, 2021, from https://chem.libretexts.org/@go/page/24084)**

Questions

1. Which solution is hypertonic to an Elodea cell? Use your observations to support your answer.
2. Would you expect pond water to be isotonic, hypotonic, or hypertonic to *Elodea* cells? Explain your answer.
3. Explain what happens to a plant cell that undergoes plasmolysis.

Exercise 3: Observing Osmosis in Red Blood Cells (Erythrocytes)

Employing Steps in the Scientific Method:

1. Record the **Question** that is being investigated in this experiment.

   _____

2. Record a **Hypothesis** for the question stated above.

   _____

3. Predict the results of the experiment based on your hypothesis (if/then).

   _____

4. Perform the experiment below and collect your data.

Materials

- Microscope
- Wax pencil or Sharpie
- Blank slides and coverslips
- DI water
- 10% NaCl solution
- 0.9% NaCl solution
- Sheep red blood cells

Procedure

1. Obtain 3 test tubes and label them (#1, #2, #3) with a wax pencil or Sharpie.
2. Set up the three test tubes with 5 mL of solutions as follows:
    1. Tube #1 - Distilled water
    2. Tube #2 - 10% NaCl
    3. Tube #3 - 0.9% NaCl
3. Using a new transfer pipette, add 2 drops of sheep blood to each tube and swirl gently to mix the contents.
4. Hold each test tube up to a sheet of paper with printed text. Attempt to read the print through each tube and record your results in the following table.

Data Table for Blood

Test Tube / Solution	Appearance of Solution	Can you read print?
#1 - Distilled water		
#2 - 10% NaCl		
#3 - 0.9% NaCl		

5. Label 3 microscope slides (#1, #2, #3) with a wax pencil or Sharpie.
6. Prepare wet mounts of each tube by placing a drop of the solution in each tube (#1 - 3) on the appropriate microscope slide (#1 -3). Add a coverslip to each slide.
7. View slide #1 through the microscope using the scanning (4x) objective first. Focus the image using the coarse adjustment. Then view the blood cells under low power and then high power. Only use the fine focus adjustment to focus the specimen.
8. Observe slide #2 and slide #3 in the same manner.
9. Record the appearance of the red blood cells in each solution in the following table.

Data Table for RBCs

Solution	Appearance of RBCs
#1 - Distilled water	
#2 - 10% NaCl	
#3 - 0.9% NaCl	

Questions

1. Which solution allowed you to read print through the solution? Explain.
2. Which solution is hypertonic to the RBCs? Use your observations to support your answer.
3. Which solution is hypotonic to the RBCs? Use your observations to support your answer.
4. Which solution is isotonic to the RBCs? Use your observations to support your answer.

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9.3: Lab Report

LAB REPORT: Diffusion and Osmosis

Part 1 - Exercise 1: Molecular Weight and Diffusion Rate

Employing Steps in the Scientific Method:

1. Record the **Question** that is being investigated in this experiment.

   _____

2. Record a **Hypothesis** for the question stated above.

   _____

3. Predict the results of the experiment based on your hypothesis (if/then).

   _____

Table 1 - Diffusion results

	Molecular Weight (g/mole)	Diameter after 20 min. (mm)	Diameter after 40 min. (mm)	Diameter after 60 min. (mm)	Diameter after 80 min. (mm)
Methylene blue					
Potassium permanganate					

Questions:

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

  _____

- Go back and look at your initial hypothesis. Does your data support this hypothesis? Explain.

  _____

Extension Activity: (Optional)

The results of this experiment can be presented graphically. The presentation of your data in a graph will assist you in interpreting your results. Based on your results, you can complete the final step of scientific investigation, in which you must be able to propose a logical argument that either allows you to support or reject your initial hypothesis.

1. Graph your results using the data from Table 1.
2. What is the dependent variable? Which axis is used to graph this data?

   _____

3. What is your independent variable? Which axis is used to graph this data?

   _____

Part 1 - Exercise 2: Diffusion Across a Membrane

Employing Steps in the Scientific Method:

1. Record the **Question** that is being investigated in this experiment.

   _____

2. Record a **Hypothesis** for the question stated above.

_____

3. Predict the results of the experiment based on your hypothesis (if/then).

_____

Table 1. Osmosis - mass (g) over time for dialysis bags

Time (min.)		0	5	10	15	20
Beaker #1 (water)	**Bag #1 (water)**					
Beaker #2 (water)	**Bag #2 (15% sucrose)**					
Beaker #3 (water)	**Bag #3 (30% sucrose)**					
Beaker #4 (30% sucrose)	**Bag #4 (water)**					

Table 2. Rate of osmosis

	Weight Change (g)	Time (min)	Rate (g/min)
Bag #1 (water)			
Bag #2 (15% sucrose)			
Bag #3 (30% sucrose)			
Bag #4 (water)			

Table 3. Selective Permeability

	Appearance of liquid after 20 minutes	Appearance of liquid after heating
Bag #5 (5 mL 30% glucose solution and 5 mL 15% starch solution)		
Beaker #5 (H2O and 1mL Iodine solution)		

Questions

1. Did the weight of each bag (#1 - #4) change significantly over 20 minutes? Explain.

2. In which bag(s) was there a **net movement** of water?

3. Explain what is meant by “net movement”.

4. Which carbohydrate molecules (glucose, sucrose, starch) were not able to move across the membrane? Explain.

5. In terms of **solvent** (water) concentration, water moved from the area of _____ concentration to the area of _____ concentration across a selectively permeable membrane, which is defined as _____.
6. What can you conclude about the movement of Iodine, glucose, and starch across the dialysis membrane based on your results in Table 3? Support your answers for each with the observation from bag #5 and beaker #5.

- Iodine -
- Glucose -
- Starch -

7. We used the dialysis tubing to simulate a cell membrane. How is the dialysis tubing functionally the same as a cell membrane?
8. We used the dialysis tubing to simulate a cell membrane. How is the dialysis tubing functionally different from a cell membrane?

Extension Activity: (Optional)

The results of this experiment can be presented graphically. The presentation of your data in a graph will assist you in interpreting your results. Based on your results, you can complete the final step of scientific investigation, in which you must be able to propose a logical argument that either allows you to support or reject your initial hypothesis.

1. Prepare a line graph using the data from Table 2.
2. What is the dependent variable? Which axis is used to graph this data?
   _____
3. What is your independent variable? Which axis is used to graph this data?
   _____

Part 2 - Exercise 1: Observing Osmosis in Potato Strips

Employing Steps in the Scientific Method:

1. Record the **Question** that is being investigated in this experiment.
   _____
2. Record a **Hypothesis** for the question stated above.
   _____
3. Predict the results of the experiment based on your hypothesis (if/then).
   _____

Questions

1. Which tube contained the limp (flaccid) potato strip? Explain.
2. Which tube contained the stiff (turgid) potato strip? Explain.
3. Which solution is isotonic to the inside of the potato cell?
4. What happened to the potato strip in the isotonic solution?

Part 2 - Exercise 2: Observing Osmosis in Elodea Cells

Employing Steps in the Scientific Method:

1. Record the **Question** that is being investigated in this experiment.
   _____

2. Record a **Hypothesis** for the question stated above.

_____

3. Predict the results of the experiment based on your hypothesis (if/then).

_____

Data Table for Elodea

Solution	Appearance of *Elodea* Cells
Distilled water (0% NaCl)	
10% NaCl	

Questions

1. Which solution is hypertonic to an Elodea cell? Use your observations to support your answer.

2. Would you expect pond water to be isotonic, hypotonic, or hypertonic to *Elodea* cells? Explain your answer.

3. Explain what happens to a plant cell that undergoes plasmolysis.

Part 2 - Exercise 3: Observing Osmosis in Red Blood Cells (Erythrocytes)

Employing Steps in the Scientific Method:

1. Record the **Question** that is being investigated in this experiment.

_____

2. Record a **Hypothesis** for the question stated above.

_____

3. Predict the results of the experiment based on your hypothesis (if/then).

_____

Data Table for Blood

Test Tube / Solution	Appearance of Solution	Can you read print?
#1 - Distilled water		
#2 - 10% NaCl		
#3 - 0.9% NaCl		

Data Table for RBCs

Solution	Appearance of RBCs
#1 - Distilled water	
#2 - 10% NaCl	
#3 - 0.9% NaCl	

Questions

1. Which solution allowed you to read print through the solution? Explain.

2. Which solution is hypertonic to the RBCs? Use your observations to support your answer.

3. Which solution is hypotonic to the RBCs? Use your observations to support your answer.

4. Which solution is isotonic to the RBCs? Use your observations to support your answer.

Questions for Review

1. Define diffusion. What is the energy source for diffusion? Is diffusion considered an active or passive process? Explain.

2. Name a molecule that diffused through the artificial membrane (dialysis tubing) that we used in the laboratory. Can diffusion occur without a membrane? Give an example to support your answer.

3. What is osmosis? Is it an active or a passive process? Explain.

4. Fill in the blank in the following statements.
    1. A solution that has a lower solute concentration than another solution is said to be _____ when compared with the second solution.
    2. A solution that has the same solute concentration as another solution is said to be _____ when compared with the second solution.
    3. A solution that has a higher solute concentration than another solution is said to be _____ when compared with the second solution.
5. What does it mean when a membrane is selectively permeable?

Practical Challenge

- Briefly explain what happens to a red blood cell when placed into the following solutions.
    - Isotonic solution –
    - Hypertonic solution –
    - Hypotonic solution -
- The concentration of glucose inside *Elodea* cells is 5 mM. What is the solution in moles (M)? What will happen to an *Elodea* cell if it is placed in a 1 M glucose solution? Explain.

- Apply what you learned in the lab to explain why it is said that marine organisms, which live in saline environments, literally live in a desert environment.

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CHAPTER OVERVIEW

10: Enzymes

Learning Objectives

Content Objectives

- Define the following terms: metabolism, reactant, product, substrate, enzyme, denature.
- Describe the function of an enzyme's active site.
- Describe the specific action of the enzyme catalase, including the substrate and products of the reaction.
- List and describe the factors that can affect the rate of a chemical reaction and enzyme activity.
- If provided with the optimal conditions for a particular enzyme, indicate which experimental conditions would show the greatest and least enzyme activity.

Skill Objectives

- Employ the scientific method to observe and analyze the effects of pH, temperature, and concentration on enzyme activity.
- Generate a laboratory report summarizing the key findings of the environmental factors affecting enzyme activity.

10.1: Introduction
10.2: Exercise
10.3: Lab Report

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10.1: Introduction

Introduction

Hydrogen peroxide is a toxic product of many chemical reactions that occur in living things. Although it is produced in small amounts, living things must detoxify this compound and break down hydrogen peroxide into water and oxygen, two non-harmful molecules. The organelle responsible for destroying hydrogen peroxide is the peroxisome using the enzyme catalase. Both plants and animals have peroxisomes with catalase. The catalase sample for today's lab will be from a potato.

Enzymes

Enzymes speed the rate of chemical reactions. A catalyst is a chemical involved in, but not consumed in, a chemical reaction. Enzymes are proteins that catalyze biochemical reactions by lowering the activation energy necessary to break the chemical bonds in reactants and form new chemical bonds in the products. Catalysts bring reactants closer together in the appropriate orientation and weaken bonds, increasing the reaction rate. Without enzymes, chemical reactions would occur too slowly to sustain life.

The functionality of an enzyme is determined by the shape of the enzyme. The area in which bonds of the reactant(s) are broken is known as the active site. The reactants of enzyme catalyzed reactions are called substrates. The active site of an enzyme recognizes, confines, and orients the substrate in a particular direction.

Enzymes are substrate specific, meaning that they catalyze only specific reactions. For example, proteases (enzymes that break peptide bonds in proteins) will not work on starch (which is broken down by the enzyme amylase). Notice that both of these enzymes end in the suffix -ase. This suffix indicates that a molecule is an enzyme.

Environmental factors may affect the ability of enzymes to function. You will design a set of experiments to examine the effects of temperature, pH, and substrate concentration on the ability of enzymes to catalyze chemical reactions. In particular, you will be examining the effects of these environmental factors on the ability of catalase to convert H_2O_2 into H_2O and O_2.

The Scientific Method

As scientists, biologists apply the scientific method. Science is not simply a list of facts, but is an approach to understanding the world around us. It is use of the scientific method that differentiates science from other fields of study that attempt to improve our understanding of the world.

The scientific method is a systematic approach to problem solving. Although some argue that there is not one single scientific method, but a variety of methods; each of these approaches, whether explicit or not, tend to incorporate a few fundamental steps: observing, questioning, hypothesizing, predicting, testing, and interpreting results of the test. Sometimes the distinction between these steps is not always clear. This is particularly the case with hypotheses and predictions. But for our purposes, we will differentiate each of these steps in our applications of the scientific method.

You are already familiar with the steps of the scientific method from previous lab experiences. You will need to use your scientific method knowledge in today's lab in creating hypotheses for each experiment, devising a protocol to test your hypothesis, and analyzing the results. Within the experimentation process it will be important to identify the independent variable, the dependent variable, and standardized variables for each experiment.

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10.2: Exercise

Part 1: Observe the Effects of Catalase

Procedure

1. Obtain two test tubes and label one as A and one as B.
2. Use your ruler to measure and mark on each test tube 1 cm from the bottom.
3. Fill each of two test tubes with catalase (from the potato) to the 1 cm mark
4. Add 10 drops of hydrogen peroxide to the tube marked A.
5. Add 10 drops of distilled water to the tube marked B.
6. Wait 60 seconds and measure the height of any bubbling you observe.
    1. Bubbling height tube A
    2. Bubbling height tube B
7. What happened when H_2O_2 was added to the potato in test tube A?
8. What caused this to happen?
9. What happened in test tube B?
10. What was the purpose of the water in tube B?

Part 2: Effects of pH, Temperature, and Substrate Concentration

Observations

From the introduction and your reading, you have some background knowledge on enzyme structure and function. You also just observed the effects of catalase on the reaction in which hydrogen peroxide breaks down into water and oxygen.

Questions

From the objectives of this lab, our questions are as follows:

1. How does temperature affect the ability of enzymes to catalyze chemical reactions?
2. How does pH affect the ability of enzymes to catalyze chemical reactions?
3. What is the effect of substrate concentration on the rate of enzyme catalyzed reactions?

Hypotheses

Based on the questions above, come up with some possible hypotheses. These should be general, not specific, statements that are possible answers to your questions.

- Temperature hypothesis
- pH hypothesis
- Substrate concentration hypothesis

Test Your Hypotheses

Based on your hypotheses, design a set of experiments to test your hypotheses. Use your original experiment to shape your ideas. You have the following materials available:

- Test tubes
- Catalase (from potato)
- Hydrogen peroxide
- Distilled water
- Hot plate (for boiling water)
- Ice
- Acidic pH solution
- Basic pH solution
- Thermometer

- Ruler and wax pencil

Write your procedure to test each hypothesis. You should have three procedures, one for each hypothesis. Make sure your instructor checks your procedures before you continue.

- Procedure 1: Temperature
- Procedure 2: pH
- Procedure 3: Concentration

Results

Record your results—you may want to draw tables. Also record any observations you make. Interpret your results to draw conclusions.

1. Do your results match your hypothesis for each experiment?
2. Do the results reject or fail to reject your hypothesis and why?
3. What might explain your results? If your results are different from your hypothesis, why might they differ? If the results matched your predictions, hypothesize some mechanisms behind what you have observed.

Communicating Your Findings

Scientists generally communicate their research findings in written reports. Save the things that you have done above. You will be use them to write a lab report a little later in the course.

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10.3: Lab Report

Data Collection

Part 1: Observe the Effects of Catalase

Procedure

- What happened when H_2O_2 was added to the potato in test tube A?
- What caused this to happen?
- What happened in test tube B?
- What was the purpose of the water in tube B?

Part 2: Effects of pH, Temperature, and Substrate Concentration

Questions

- How does temperature affect the ability of enzymes to catalyze chemical reactions?
- How does pH affect the ability of enzymes to catalyze chemical reactions?
- What is the effect of substrate concentration on the rate of enzyme catalyzed reactions?

Hypotheses

Based on the questions above, come up with some possible hypotheses. These should be general, not specific, statements that are possible answers to your questions.

- Temperature hypothesis
- pH hypothesis
- Substrate concentration hypothesis

Test Your Hypotheses

Based on your hypotheses, design a set of experiments to test your hypotheses. Use your original experiment to shape your ideas. You have the following materials available:

Write your procedure to test each hypothesis. You should have three procedures, one for each hypothesis. Make sure your instructor checks your procedures before you continue.

- Procedure 1: Temperature
- Procedure 2: pH
- Procedure 3: Concentration

Results

Record your results—you may want to draw tables. Also record any observations you make. Interpret your results to draw conclusions.

- Do your results match your hypothesis for each experiment?
- Do the results reject or fail to reject your hypothesis and why?
- What might explain your results? If your results are different from your hypothesis, why might they differ? If the results matched your predictions, hypothesize some mechanisms behind what you have observed.

Communicating Your Findings

Scientists generally communicate their research findings in written reports. Save the data and findings you have recorded above. You will be use them to write a lab report a little later in the course.

Use the guidelines below to complete your lab report. Your report should be typed, use 12-point font, and be double-spaced.

Sections of a Lab Report

- **Title Page:** The title describes the focus of the research. The title page should also include the student's name, the lab instructor's name, and the lab section.
- **Introduction:** The introduction provides the reader with background information about the problem and provides the rationale for conducting the research. The introduction should incorporate and cite outside sources. You should avoid using websites and encyclopedias for this background information. The introduction should start with more broad and general statements that frame the research and become more specific, clearly stating your hypotheses near the end.
- **Methods:** The methods section describes how the study was designed to test your hypotheses. This section should provide enough detail for someone to repeat your study. This section explains what you did. It should not be a bullet list of steps and materials used; nor should it read like a recipe that the reader is to follow. Typically this section is written in first person past tense in paragraph form since you conducted the experiment.
- **Results:** This section provides a written description of the data in paragraph form. What was the most reaction? The least reaction? This section should also include numbered graphs or tables with descriptive titles. The objective is to present the data, not interpret the data. Do not discuss why something occurred, just state what occurred.
- **Discussion:** In this section you interpret and critically evaluate your results. Generally, this section begins by reviewing your hypotheses and whether your data support your hypotheses. In describing conclusions that can be drawn from your research, it is important to include outside studies that help clarify your results. You should cite outside resources. What is most important about the research? What is the take-home message? The discussion section also includes ideas for further research and talks about potential sources of error. What could you improve if you conducted this experiment a second time?

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CHAPTER OVERVIEW

11: Spectrophotometry

Learning Objectives

Content Objectives

- Identify the main features of the spectrophotometer and describe their functions.
- Use the spectrophotometer to obtain an absorbance spectrum.

Skill Objectives

- Identify the parts of a spectrophotometer and their related functions.
- “Blank” a spectrophotometer.
- Obtain an absorbance spectrum for a molecule.
- Use the wavelength absorption scans to determine the dyes in colored skittles.

11.1: Introduction
11.2: Exercise
11.3: Lab Report

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11.1: Introduction

Introduction

Spectrophotometers are one of the most frequently used tools by scientists to determine both the presence and concentration of dissolved chemicals. As radiant energy (visible light) strikes matter, molecules will absorb certain wavelengths of light and transmit or reflect others based on the nature of their chemical bonds. For example, proteins and nucleic acids absorb wavelengths in the visible light range of 240-300 nanometers (nm), pigments and dyes absorb light in the 400-770-nm range, and other organic molecules absorb wavelengths above 770-nm. Each chemical has a distinctive atomic arrangement and bonding pattern, and thus absorbs or transmits different wavelengths of visible light in a pattern that is unique for that chemical. This unique pattern of light absorption and transmittance creates a “fingerprint” for that chemical. In this exercise you will determine the unique “fingerprint” for a colored molecule and use a spectrophotometer to measure the concentration of a chemical in a given sample.

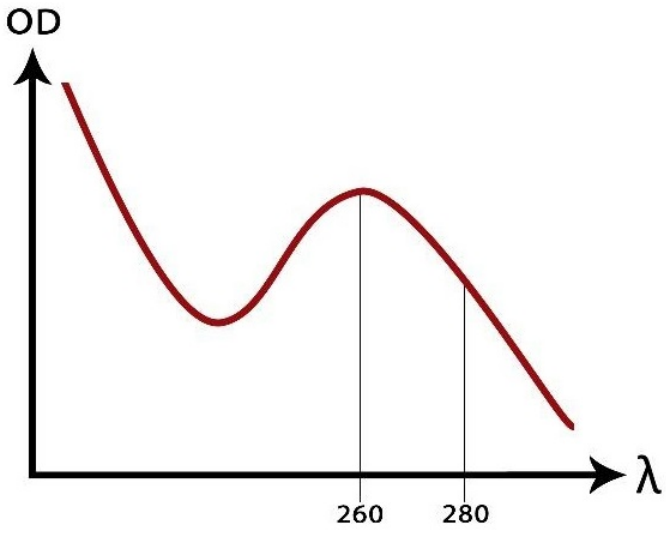


Figure 1: Curve of light absorbance and transmission

Spectrophotometers are instruments designed to detect the amount of light energy that is absorbed or transmitted by molecules dissolved in a solution. Since molecules have wavelengths unique to their structure, different chemicals and their concentrations can be identified based on their absorbance or transmittance.

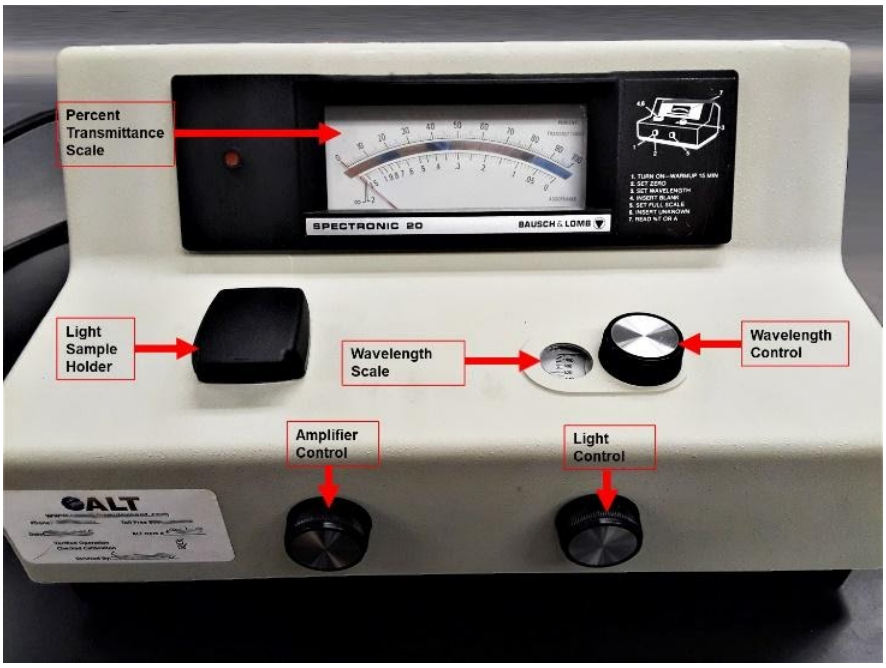


Figure 2. Labeled parts of a spectrophotometer

A spectrophotometer is an instrument used for detecting the presence of any light-absorbing particles dissolved in a solution and for measuring the concentration of those particles. A light source inside the spectrophotometer emits a full spectrum of white light towards a compartment where a sample liquid is placed. The samples are prepared in cuvettes that are made using specialized plastics or quartz so that they do not absorb any light and will not affect our measurements. Before the light passes through the sample in the cuvette, an adjustable prism and diffraction grating filters the light so that only a single wavelength of light can be selected and allowed to pass through the sample. All molecules differ in how strongly they absorb each wavelength of light in the visible spectrum because of differences in their molecular structure and composition. This allows us to use a specific wavelength of light to detect the presence of, and quantify, one molecular compound in a simple or complex liquid mixture. Spectrophotometers are also calibrated by using a "blank" solution that we prepare containing all of the components of the solution to be analyzed except for the one compound we are testing for so that the instrument can zero out these background readings and only report values for the compound of interest.

Light passing through a sample solution will partially be absorbed by molecules present in the sample. The amount of light unable to pass through a sample is measured as the absorbance value. Absorbance is directly proportional to the concentration of the molecules and is measured on a logarithmic scale from 0 to infinity. The amount of light that is not absorbed is transmitted or passed through the sample. Compared to the amount of light entering the sample, the amount that exits is measured as a percentage of the light transmitted. Percent transmittance is inversely proportional to the concentration of the molecules in the sample and is measured on a linear scale from 0% to 100%.

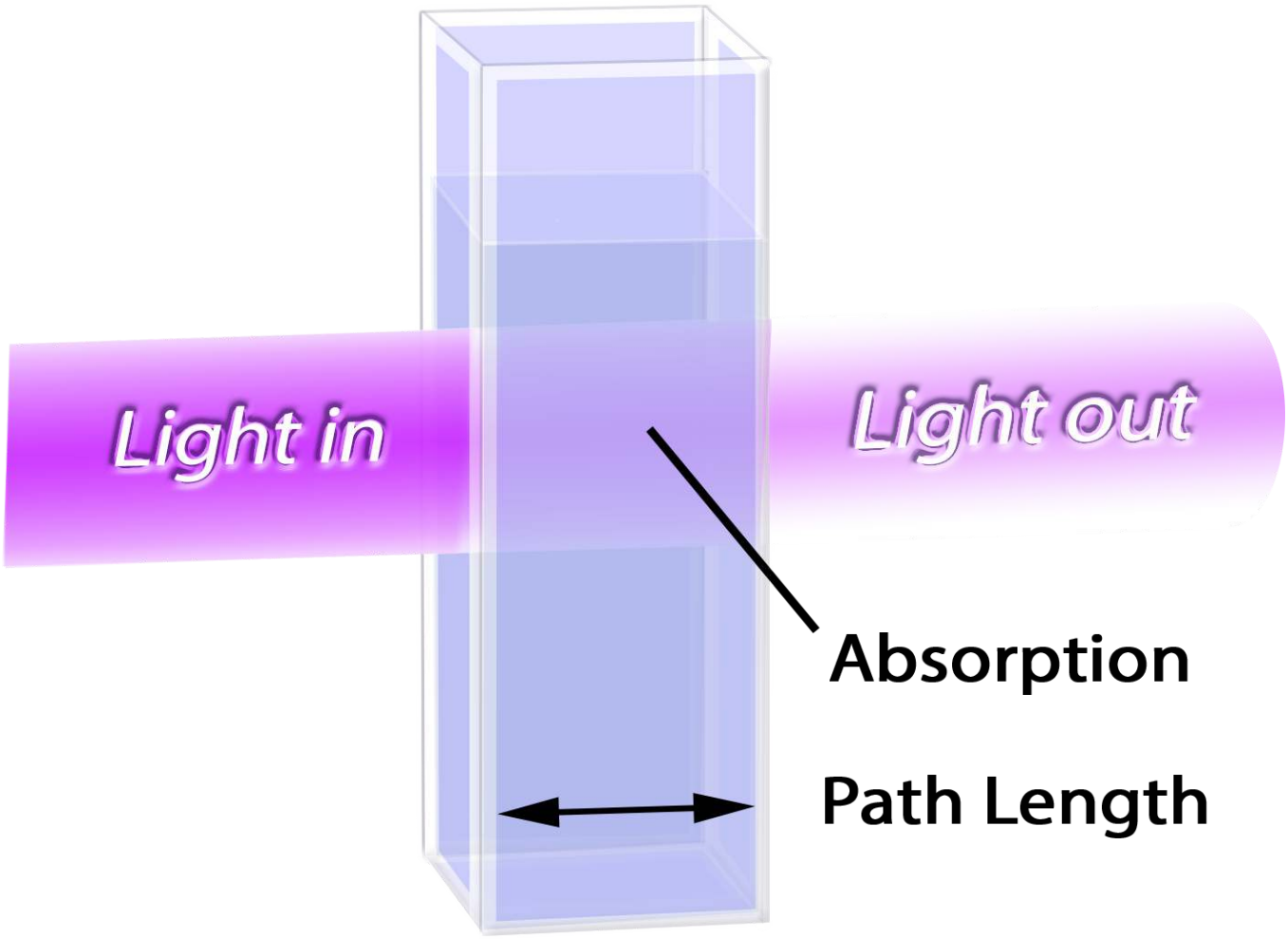


Figure 3: Light is absorbed and transmitted through the spectrophotometer cuvette and solution

A photodetector on the other side of the sample compartment converts the intensity of the light it receives into an electrical signal. The instrument can then calculate and display the absorbance and % transmittance values by measuring the difference between the intensity of light of the selected wavelength entering and exiting the sample.

The absorbance scale reflects the measurement of the amount of light absorbed and converted into absorbance (A) units by the spectrophotometer. Absorbance units are calculated by using the following equation:

Equation 1

$$\text{Absorbance}(A) = \log_{10}\left(\frac{1}{T}\right)$$

where "T" is the decimal form of "$\%T$"

$$T = \frac{\%T}{100}$$

Example

If a solution containing a given dye is found to transmit 10% of the light when placed in a spectrophotometer, its absorbance then would be calculated as follows:

Transmittance (T) = 10% = 10%/100 = 0.10

Absorbance (A) = log10 (1 / 0.10) = 1.0

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11.2: Exercise

Part I: Identifying Food Dyes in Candies

Many foods, drugs and cosmetics are artificially colored with federally approved food dyes (FD & C dyes). These dyes include Red 40, Red 3, Yellow 5, Yellow 6, Blue 1, and Blue 2. Since each dye has an identifiable absorption spectrum and peak, a spectrophotometer may be used to identify the types of FD & C dye used in a product.

Pigments may be extracted from foods and drinks that contain one or more of these dyes. An absorption spectrum of that extract can then determine what dyes are in that food or drink by comparing the peaks of maximum absorbance with information in the table below. If the absorption spectrum of a food extract has a peak at 630 nm and one at 428 nm, you can assume the food contains both Blue #1 and Yellow #5. The following table gives the wavelength of peak absorbance for each of these dyes.

Table 1. Wavelength of Maximum Absorbance of Commonly Used FD & C Dyes

FD & C Dye	Name	Wavelength (nm) of Maximum Absorbance
Blue #1	Brilliant Blue FCF	630
Green #3	Solid Green FCF	625
Blue #2	Indigo Carmine	610
Red #3	Erythrosine	527
Red #40	Allura Red AC	502
Yellow #6	Sunset Yellow FCF	484
Yellow #5	Tartrazine	428

Materials

- Spectrophotometer
- Cuvette
- Skittles
- KimWipes
- Test tubes

Procedure

A. Extracting Dye from Candy

You will need one test tube and one cuvette for each color to be tested. Measure 4 mL water into one tube. Place 2-4 candies of the same color in a test tube with the water. Gently swirl, and wait one minute. After, pour approximately 1 mL of liquid into a microcentrifuge tube. Spin the microcentrifuge tube at max speed for 60 seconds. Make sure the centrifuge is balanced before spinning. Transfer the clear liquid (supernatant) into a cuvette. Make sure to leave behind the particulates (pellet).

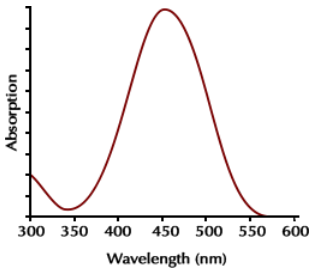


Figure 4. Curve of absorbance

B. Measuring Absorbance with Spectrophotometer

1. Turn on the spectrophotometer. Let it warm up for 15 minutes.
2. Select wavelength scan.
3. Fill a cuvette 2/3 full with DI water to serve as the “BLANK” cuvette.
4. Calibrate the Spectrometer

1. 1. Wipe the outside of the BLANK cuvette with a KimWipe
   2. Place cuvette into the machine so that the clear portions of the cuvette are oriented left to right. (The light needs to pass through clear area on cuvette).
   3. Press "Zero"
   4. Remove the blank.

5. Determine optimum wavelength absorbance and set up data collection mode.

1. 1. Place a cuvette with a sample into the spectrophotometer
   2. Press "read"
   3. Set cursor mode to peak and valley under "Options" > "More" > "Cursor Mode"
   4. Press the left and right arrows to the right of the graph to select the highest peak.
   5. Record the wavelength and absorbance in Table 6.2.

6. Decide which dyes were used to make each color. Enter the Wavelength of that dye in the last column of table 6.2. Explain your reasoning for each choice in your lab notebook.

Results

Table 2.

Color skittle	Maximum Absorbance	Wavelength (nm) at Maximum Absorption	Proposed Dye	Wavelength (nm) of Maximum Absorption for Proposed Dye
Red				
Orange				
Yellow				
Green				
Purple				

Instructions for Cleaning Cuvettes

1. Discard solutions to sink
2. Rinse with tap water once
3. Rinse with DI water two times
4. Place in tube rack, allow to air dry

Study Questions

1. Name the parts of the spectrophotometer and identify their function.
2. What is the difference between % transmittance and absorbance?
3. How did you determine which wavelength was absorbed at the highest level? How is this process useful in determining the identity of a molecule?
4. How do you clean a cuvette?

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11.3: Lab Report

Part I: Identifying Food Dyes in Candies

Results

Table 2.

Color skittle	Maximum Absorbance	Wavelength (nm) at Maximum Absorption	Proposed Dye	Wavelength (nm) of Maximum Absorption for Proposed Dye
Red				
Orange				
Yellow				
Green				
Purple				

Study Questions

1. Name the parts of the spectrophotometer and identify their function.

2. What is the difference between % transmittance and absorbance?

3. How did you determine which wavelength was absorbed at the highest level? How is this process useful in determining the identity of a molecule?

4. How do you clean a cuvette?

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CHAPTER OVERVIEW

12: Cellular Respiration and Fermentation

Learning Objectives

Content Objectives

- Observe the various ways organisms carry out **cellular respiration** and **fermentation**.
- Use the steps of the scientific method to plan and carry out your experiments.

Skill Objectives

- Observe the process of cellular respiration in plants by measuring the production of carbon dioxide (CO_2).
- Observe aerobic respiration and alcoholic fermentation in yeast.
- Investigate different concentrations of yeast.
- Explore fermentation with different carbohydrates.

12.1: Introduction
12.2: Exercise
12.3: Lab Report

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12.1: Introduction

Introduction

All organisms must break down organic molecules to release chemical energy to synthesize adenosine triphosphate (ATP). The energy stored in ATP can be released to perform cellular work. Organisms break down organic molecules, such as glucose, through the common processes of **cellular respiration** and **fermentation** (Figure 2). Cellular respiration is generally described as an aerobic process, requiring oxygen, which yields the most possible ATP generated from one molecule of glucose. But, technically, cellular respiration can occur in an anaerobic environment in some microorganisms. Anaerobic cellular respiration yields variable amounts of ATP, but much less than is generated in aerobic cellular respiration. In this laboratory, our discussion of cellular respiration will focus on **aerobic cellular respiration**.

Both aerobic cellular respiration and fermentation involve many chemical reactions that release high energy electrons from organic molecules and transfer the electrons to other molecules, often referred to as electron carriers (or coenzymes). These chemical reactions involving the transfer of electrons are called reduction-oxidation reactions, or **redox reactions**. In a redox reaction, one of the molecules gains electrons and becomes reduced (rig, **r**eduction **i**s **g**ain of electrons) and one of the molecules loses electrons and becomes oxidized (oil, **o**xidation **i**s **l**oss of electrons). In cellular respiration, electrons are often transferred to the electron carrier nicotinamide adenine dinucleotide (NAD+). When this redox reaction occurs, the organic molecule that loses the electrons has been oxidized. When NAD+ gains the electrons it forms NADH. NADH is the reduced form of NAD.

$$\mathrm{NAD^+ + 2\,e^- + H^+ \xrightarrow{Redox\ Reaction} NADH} \tag{12.1.1}$$

Aerobic cellular respiration involves a series of three processes of enzymatic chemical reactions: **glycolysis**, the **citric acid cycle** (also known as the Kreb's cycle), and the **electron transport chain**. Aerobic cellular respiration begins in the cytoplasm with glycolysis and ends in the mitochondria with the citric acid cycle and the electron transport chain. Aerobic cellular respiration results in fully oxidizing glucose, and can yield a maximum of 32 ATP per glucose molecule. At the culmination of the electron transport chain, the electrons are passed to oxygen, a highly electronegative element, to form water. Therefore, at the end of this process, the high energy electrons that were previously a part of glucose are now at a lower energy state, as they are held very closely by the electronegative oxygen.

Fermentation is an anaerobic process of breaking down organic molecules. It occurs in the absence of oxygen. Fermentation breaks down organic molecules, such as glucose, into smaller organic molecule end products. Fermentation begins with the process of glycolysis to produce pyruvic acid and 2 net ATP. Enzymes then carry out chemical reactions to convert pyruvic acid into various fermentation end products. Two common types of fermentation are named for their end products, **alcoholic fermentation** and **lactic acid fermentation**. Fermentation produces organic end products that still contain high-energy electrons. Fermentation does not fully oxidize glucose, and yields only 2 net molecules of ATP, along with organic end products.

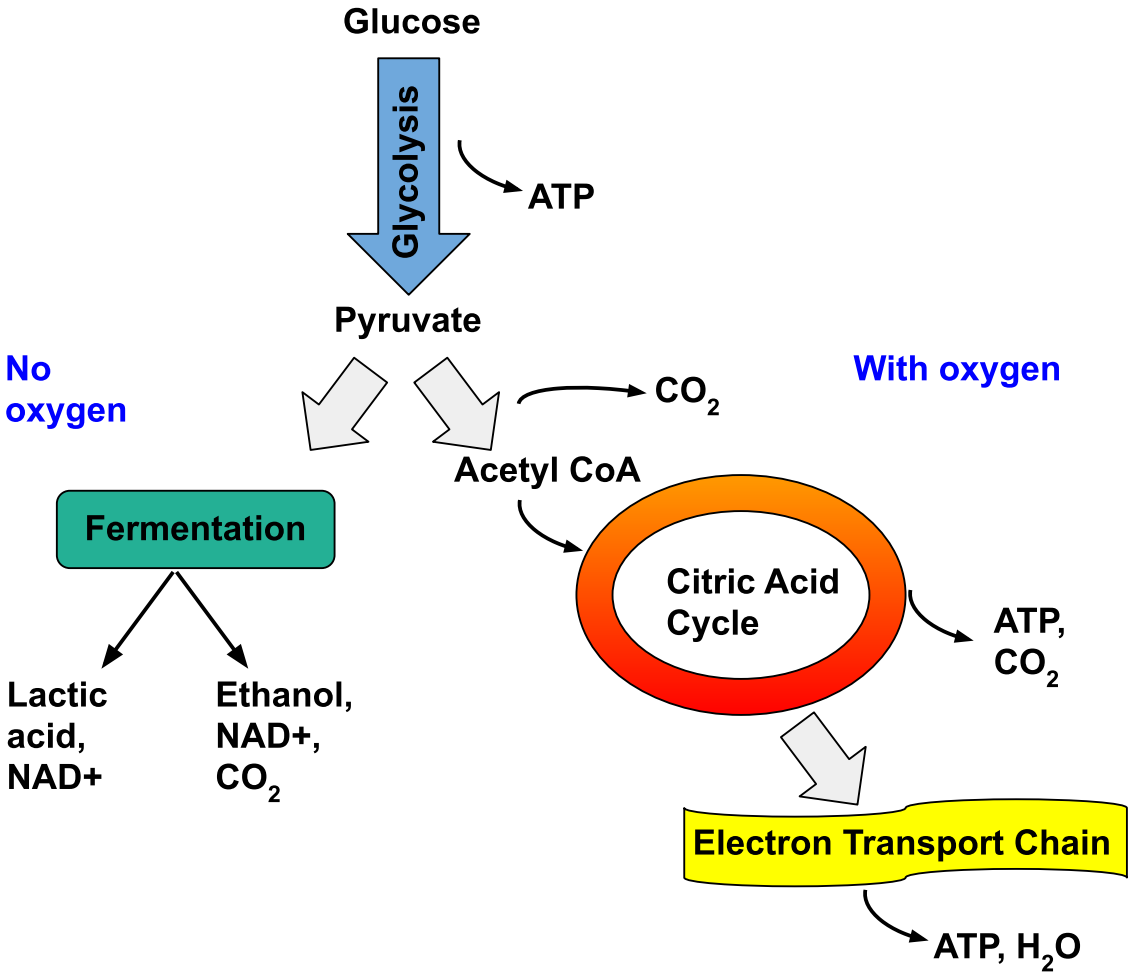


Figure 12.1.2: Cellular respiration and fermentation phases (original figure)

$$NAD^+ + 2e^- + H^+ \xrightarrow[\text{reaction}]{\text{redox}} NADH$$

(Figure 12.1.1: Redox reaction that occurs during cellular respiration)

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12.2: Exercise

PART 1: CELLULAR RESPIRATION

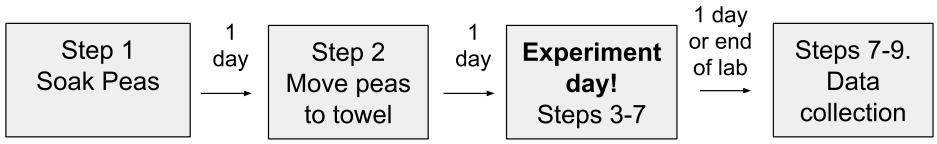
Exercise A : Investigating Cellular Respiration in Plants

This part of the lab investigates cellular respiration in pea seeds. Seeds of plants are stuffed full of sugars like starch. Cellular respiration involves breaking down sugars to generate ATP. Therefore, this process allows plants to harvest energy necessary to produce roots, shoots, and leaves. The process of cellular respiration also results in the release of carbon dioxide gas. Carbon dioxide will react with water to form carbonic acid. The formation of carbonic acid will affect the pH of an aqueous solution. Since carbon dioxide is colorless, odorless, and very hard to detect, we are going to use a pH indicator to detect the presence of carbonic acid and thus carbon dioxide. pH indicators, like red cabbage juice, bromothymol blue, and phenol red are chemicals that change color when pH is altered. In this experiment, we will observe cellular respiration in germinating pea seeds by detecting the production of carbon dioxide and monitoring the changes in the pH of the solution.

Materials:

- Pea seeds (20 germinating/ lab group and 20 dormant / lab group)
- Large sealable bag
- Test tube rack (that can accommodate wide mouth test tubes)
- Wide mouth test tubes with rubber stoppers (3/ lab group)
- Distilled water or spring water (non-chlorinated water)
- Paper towels
- Nonabsorbent cotton plugs
- Glass beads (20 / group)
- Glass rods
- Gloves and safety goggles
- Sharpie or red wax pencil
- Indicator reagent [Choose 1: red cabbage juice, or bromothymol blue (0.04% solution), or phenol red (0.04% solution)] (need 15 ml of indicator solution / lab group)

Overall Timeline:



https://bio.libretexts.org/@go/page/105842

Figure 12.2.1: Overall timeline for experiment.

Employing Steps in the Scientific Method:

1. Record the **Question** that is being investigated in this experiment.
2. Record a **Hypothesis** for the question stated above.
3. Predict the results of the experiment based on your hypothesis (if/then).
4. Perform the experiment below and collect your data.

Procedure:

1. Two days before beginning the experiment, pour half of the peas into a glass container and cover with several inches of non-chlorinated water to compensate for the expansion of the seeds as they swell. Allow the seeds to soak overnight.
2. The next day, pour the water off of the seeds. Place the seeds onto a wet paper towel, place in a plastic sealable bag, seal the bag, and store the seeds overnight in the dark.
3. On the day of the experiment carefully remove the rehydrated (germinating) seeds from the paper towel.
4. Label 3 wide mouth tubes #1 - #3.
5. Wear gloves when handling the indicator solution. Place 5 ml of the indicator solution into each test tube.
6. Using the glass rod, push a plug of non-absrobent cotton into each test tube until it sits right above the indicator solution.
7. Add the following to tubes #1 - #3.
    1. **Tube 1:** add 20 glass beads
    2. **Tube 2:** add 20 germinating peas
    3. **Tube 3:** add 20 dry dormant peas
8. Tightly cap the tubes with rubber stoppers. (If the rubber stoppers have a hole, cover the stoppers with cling wrap, and then place each stopper into a tube.)
9. Observe the color of the indicator reagent at the beginning of the experiment and record your results in Table 1.
10. Observe the color of the indicator reagent after the 2-hr incubation and record your results in Table 1.
11. Observe the color of the indicator reagent after the 24-hr incubation and record your results in Table 1.
12. Once the experiment has been completed, carefully pour the indicator reagent into the appropriate location as indicated by your instructor, being sure to collect the glass beads by pouring through a wire mesh filter.
13. Rinse and wash the test tubes thoroughly.

Table 1. Cellular Respiration of Germinating Peas

Indicator Used:				
Tube contents	**Initial color of indicator**	**Color after 2 hrs**	**Color after 24 hrs**	**Acidic or basic?**
1. Control				
2. Germinating peas				
3. Dormant Peas				

Questions for Review

1. What is the color of the indicator at at

- Neutral pH?
- Basic pH?
- Acidic pH?

2. What was the purpose of **Tube #1**?

3. What **specifically** was produced as a result of cellular respiration that changed the color of the indicator?
4. How is carbon dioxide an indicator that cellular respiration is taking place in these peas?
5. Germination is the process by which a dormant seed begins to sprout and grow into a seedling. What are some possible metabolic processes that are required for seed germination?
6. During respiration, a seed metabolizes sugars. What is the source of the sugar metabolized by the seed?
7. What variables do you think may affect the respiration rate of the seeds?
8. The equation for cellular respiration is:

$C_6H_{12}O_6 + 6O_2 \rightarrow 6\ CO_2 + 6\ H_2O + 32\ ATP$

The energy released from the complete oxidation of glucose under standard conditions is 686 kcal/mol. The energy released from the hydrolysis of ATP to ADP and inorganic phosphate under standard conditions is 7.3 kcal/mol. Using the equation for cellular respiration above, **calculate** the efficiency of respiration (i.e. the percentage of chemical energy in glucose that is transferred to ATP). For help with answering this question, refer to course textbook.

9. How might the process of photosynthesis affect pH? Form a hypothesis.

PART 2: AEROBIC RESPIRATION IN YEAST

Exercise B

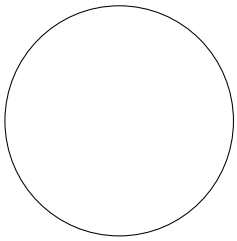
This part of the lab investigates aerobic cellular respiration by *Saccharomyces cerevisiae*, also referred to as “baker’s yeast” and “brewer’s yeast.” Yeast is a unicellular fungus that can convert glucose into carbon dioxide and ATP when oxygen is present. Methylene blue dye can be used as an indicator for aerobic respiration in yeast. Aerobic respiration releases hydrogen ions and electrons that are picked up by the methylene blue dye, gradually turning the dye colorless. This redox reaction can be observed when viewing a wet mount of yeast and methylene blue under the compound light microscope. The mitochondria of yeast cells undergoing aerobic respiration will appear as a clear area surrounded by a ring of light blue cytoplasm. If cellular respiration is not taking place, the mitochondria will absorb the blue dye and will not turn colorless.

Materials:

- Yeast (not quick rise)
- Distilled water
- Transfer pipette
- Methylene blue dye (in dropper bottle)
- Compound light microscope
- Microscope slide and cover slip
- Electronic balance, spatula, and weigh paper

Procedure:

1. Prepare yeast suspension: Add 7 grams yeast to 50 ml warm tap water. Stir to mix. Save the yeast suspension for Part 3.
2. Place a drop of yeast suspension on a clean microscope slide with a transfer pipette.
3. Add one drop of methylene blue dye and place a cover slip on the microscope slide over the yeast suspension.
4. Observe the yeast using the scanning objective lens. Use the coarse adjustment knob to focus on the yeast cells. Switch to the low power objective lens and then to the high power objective lens.
5. In the circle below, draw several yeast cells undergoing aerobic respiration and several yeast cells not undergoing aerobic respiration. Label the cytoplasm and nucleus if visible.



PART 3: ALCOHOLIC FERMENTATION IN YEAST

This part of the lab investigates alcoholic fermentation by *Saccharomyces cerevisiae*, also referred to as “baker’s yeast” and “brewer’s yeast.” Yeast converts pyruvate from glycolysis into acetaldehyde, releasing carbon dioxide gas. Acetaldehyde is then enzymatically converted by the enzyme alcohol dehydrogenase into ethanol (Figure 2). In this lab, we will measure the accumulation of carbon dioxide released in the first enzymatic reaction as an indicator of the progression of fermentation.

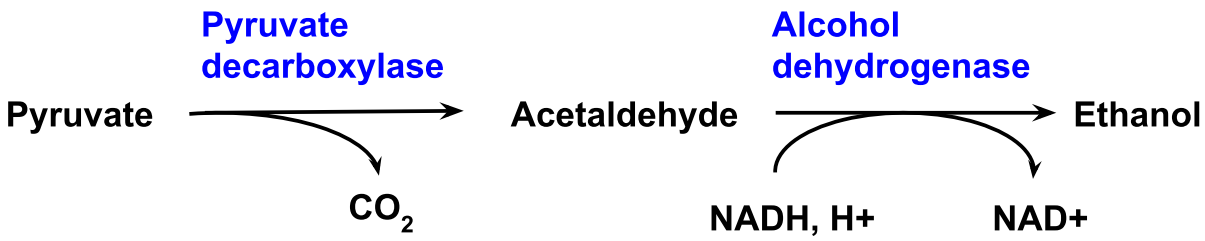


Figure 12.2.2: Alcoholic Fermentation (original figure)

Exercise C: Investigating Different Concentrations of Yeast

Materials:

- 4 identical saccharometers (glass fermentation hydrometer with either a 10-cm or a 15-cm vertical tube, Figure 3) / lab group
- Yeast (not quick rise)
- Wax pencil or Sharpie
- Distilled water
- 10% glucose solution
- Transfer pipettes
- Test tube rack
- 4 large (20 ml) test tubes or small Erlenmeyer flasks for larger volumes
- Large plastic tray
- Masking tape or lab tape
- Large weigh boat (4/group)
- Metric ruler
- Electronic balance
- Spatula
- Weigh paper
- Red food coloring (optional)



Figure 12.2.3: Saccharometer

Table 2. Contents of Saccharometers when testing fermentation with various yeast concentrations.

Saccharometer	DI Water	Glucose Solution	Yeast Suspension
1	*8 ml	*6 ml	0 ml
2	*12 ml	0 ml	*2 ml
3	*6 ml	*6 ml	*2 ml
4	*2 ml	*6 ml	*6 ml

***Double these amounts if using saccharometers that have a 15-cm vertical tube. See table below**

Data Table for Saccharometer Results

Saccharometer	DI Water	Glucose Solution	Yeast Suspension
1	16 ml	12 ml	0 ml
2	24 ml	0 ml	4 ml
3	12 ml	12 ml	4 ml
4	4 ml	12 ml	12 ml

Employing Steps in the Scientific Method:

1. Record the **Question** that is being investigated in this experiment.

   _____

2. Record a **Hypothesis** for the question stated above.

   _____

3. Predict the results of the experiment based on your hypothesis (if/then).

   _____

4. Perform the experiment below and collect your data.

Procedure:

1. Prepare yeast suspension: Add 7 grams yeast to 50 ml warm tap water. Stir to mix. Alternatively, you can use the yeast suspension from Part 2. Optional: Add a few drops of red food coloring to the yeast to increase contrast, allowing easier measuring of the height of yeast in saccharometers.
2. Label 4 test tubes and 4 saccharometers # 1- 4. Use a transfer pipette to add the appropriate amount of glucose and distilled water listed in Table 2 to the corresponding labeled test tubes.
3. Use a transfer pipette to add the appropriate amount of yeast solution listed in Table 1 to the corresponding labeled test tubes. It is important to work carefully and quickly after adding the yeast solution to the glucose and water.

4. Carefully pour the contents of the test tubes into the correspondingly labeled saccharometer, ensuring that the solutions are well mixed.
5. Carefully tilt the saccharometers to allow any air bubbles that are trapped in the arms of the vertical tube to escape.
6. Begin the timer for the experiment and measure the size of any bubbles (in mm) that are trapped in the vertical arms of the saccharometers. Record this measurement as the 0 time point.
7. Position the saccharometers on the large plastic tray, positioning them around a plastic weigh boat to catch any fermentation overflow that may occur.
8. Carefully tape the saccharometers to the large plastic tray to prevent them from falling and breaking.
9. Every 2 minutes measure and record the total amount of bubbles that accumulate in the top of the vertical arm of the saccharometer. Record the mm of carbon dioxide (bubble) measurements in Table 3.
10. Continue recording the total amount of carbon dioxide released every 2 minutes for 20 minutes.
11. After completing the experiment carefully carry the saccharometers to a sink for washing. Carry only one saccharometer at a time. Spill the yeast mixture into the sink and wash the saccharometer carefully and thoroughly. Return the saccharometer to the plastic tray, laying it down on its side when not in use.

Table 3. Carbon dioxide evolved during fermentation by different concentrations of yeast. Carbon dioxide levels measured in millimeters (mm).

Time (min.)	Sacch. 1	Sacch. 2	Sacch. 3	Sacch. 4
0 (initial)				
2				
4				
6				
8				
10				
12				
14				
16				
18				
20				

Extension Activity: (Optional)

The results of this experiment can be presented graphically. The presentation of your data in a graph will assist you in interpreting your results. Based on your results, you can complete the final step of scientific investigation, in which you must be able to propose a logical argument that either allows you to support or reject your initial hypothesis.

1. Graph your results using the data from Table 3.
2. What is the dependent variable? Which axis is used to graph this data?

   _____

3. What is your independent variable? Which axis is used to graph this data?

   _____

Exercise D: Investigating the fermentation of different carbohydrates

Materials:

- 4 identical saccharometers (glass fermentation hydrometer with either a 10-cm or a 15-cm vertical tube) / lab group
- Yeast (not quick rise)
- Wax pencil or Sharpie
- Distilled water
- 10% glucose solution
- 1% starch solution
- 10% sucrose solution
- Metric ruler
- Transfer pipettes
- Test tube rack
- 4 large (20 ml) test tubes or small Erlenmeyer flasks for larger volumes
- Large plastic tray
- Masking or lab tape
- Large weigh boat
- Electronic balance
- Spatula
- Weigh paper
- Red food coloring (optional)

Table 4. Contents of Saccharometers when testing fermentation of different carbohydrates.

Saccharometer	DI Water	Yeast Suspension	Carbohydrate
1	*2 ml	*6 ml	*6 ml glucose
2	*2 ml	*6 ml	*6 ml sucrose
3	*2 ml	*6 ml	*6 ml starch

*Double these amounts if using saccharometers that have a 15-cm vertical tube. See table below

Data Table for Saccharometer Contents

Saccharometer	DI Water	Yeast Suspension	Carbohydrate
1	4 ml	12 ml	12 ml glucose
2	4 ml	12 ml	12 ml sucrose
3	4 ml	12 ml	12 ml starch

Employing Steps in the Scientific Method:

1. Record the **Question** that is being investigated in this experiment.

   _____

2. Record a **Hypothesis** for the question stated above.

   _____

3. Predict the results of the experiment based on your hypothesis (if/then).

   _____

4. Perform the experiment below and collect your data.

Procedure:

1. Prepare yeast suspension: Add 7 grams yeast to 50 ml warm tap water. Stir to mix. Optional: Add a few drops of red food coloring to the yeast to increase contrast, allowing easier measuring of the height of yeast in saccharometers.
2. Label 3 test tubes and 3 saccharometers # 1- 3. Use a transfer pipette to add the appropriate amounts of carbohydrates and distilled water listed in Table 4 to the corresponding labeled test tubes.
3. Use a transfer pipette to add 6 ml yeast solution to each of the test tubes. It is important to work carefully and quickly after adding the yeast solution to the carbohydrate.
4. Carefully pour the contents of the test tubes into the correspondingly labeled saccharometer, ensuring that the solutions are well mixed.
5. Carefully tilt the saccharometers to allow any air bubbles that are trapped in the arms of the vertical tube to escape.
6. Begin the timer for the experiment and measure the size of any bubbles (in mm) that are trapped in the vertical arms of the saccharometers. Record this measurement as the 0 time point.
7. Position the saccharometers on the large plastic tray, positioning them around a plastic weigh boat to catch any fermentation overflow that may occur.
8. Carefully tape the saccharometers to the large plastic tray to prevent them from falling and breaking.
9. Every 2 minutes measure and record the total amount of bubbles that accumulate in the top of the vertical arm of the saccharometer. Record the mm of carbon dioxide (bubble) measurements in Table 5.
10. Continue recording the total amount of carbon dioxide released every 2 minutes for 20 minutes.
11. After completing the experiment carefully carry the saccharometers to a sink for washing. Carry only one saccharometer at a time. Spill the yeast mixture into the sink and wash the saccharometer carefully and thoroughly. Return the saccharometer to the plastic tray, laying it down on its side when not in use.

Table 5. Carbon dioxide evolved during the fermentation of various carbohydrates. Carbon dioxide levels measured in millimeters (mm).

Time (min.)	Sacch. 1	Sacch. 2	Sacch. 3
0 (initial)			
2			
4			
6			
8			
10			
12			
14			
16			
18			
20			

Extension Activity: (Optional)

The results of this experiment can be presented graphically. The presentation of your data in a graph will assist you in interpreting your results. Based on your results, you can complete the final step of scientific investigation, in which you must be able to propose a logical argument that either allows you to support or reject your initial hypothesis.

1. Graph your results using the data from Table 5.

2. What is the dependent variable? Which axis is used to graph this data?

   _____

3. What is your independent variable? Which axis is used to graph this data?

   _____

Questions for Review

1. Fermentation involves **redox reactions**. Explain what happens to electrons during a redox reaction and how this changes a molecule's potential energy.
2. Why did we add the *Saccharomyces cerevisiae* (baker's yeast) to the fermentation tubes? Specifically, what did the yeast provide to the fermentation mixture?
3. What is the purpose of *Saccharomyces cerevisiae* ("baker's yeast) in the bread-making process?
4. We measured the formation of what end product to determine the fermentation rate? Name the end product that we measured.
5. List two specific factors (as they relate to the experiment performed in our lab) that affect the rate of fermentation.

Practical Challenge Questions:

1. What other variables could be investigated that might affect the rate of alcoholic fermentation by yeast?

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12.3: Lab Report

Exercise A

Employing Steps in the Scientific Method:

1. Record the **Question** that is being investigated in this experiment.

2. Record a **Hypothesis** for the question stated above.

3. Predict the results of the experiment based on your hypothesis (if/then).

4. Fill in your data below.

Table 1. Cellular Respiration of Germinating Peas

Indicator Used:				
Tube contents	**Initial color of indicator**	**Color after 2 hrs**	**Color after 24 hrs**	**Acidic or basic?**
1. Control				
2. Germinating peas				
3. Dormant Peas				

5. What is the color of the indicator at at A) neutral pH, B) Basic pH and C) Acidic pH?

6. What was the purpose of **Tube #1**?

7. What **specifically** was produced as a result of cellular respiration that changed the color of the indicator?

8. How is carbon dioxide an indicator that cellular respiration is taking place in these peas?

9. Germination is the process by which a dormant seed begins to sprout and grow into a seedling. What are some possible metabolic processes that are required for seed germination?

10. During respiration, a seed metabolizes sugars. What is the source of the sugar metabolized by the seed?

11. What variables do you think may affect the respiration rate of the seeds?

12. The equation for cellular respiration is: $C6H12O6 + 6O2 \rightarrow 6CO2 + 6H2O + 32ATP$ (1.8.2). The energy released from the complete oxidation of glucose under standard conditions is 686 kcal/mol. The energy released from the hydrolysis of ATP to ADP and inorganic phosphate under standard conditions is 7.3 kcal/mol. Using the equation for cellular respiration above, **calculate** the efficiency of respiration (i.e. the percentage of chemical energy in glucose that is transferred to ATP). For help with answering this question, refer to the course textbook.

13. How might the process of photosynthesis affect pH? Form a hypothesis.

Exercise B

1. In the space below, draw and label several yeast cells undergoing aerobic respiration and several yeast cells not undergoing aerobic respiration. Label the cytoplasm and nucleus if visible.

Exercise C

Employing Steps in the Scientific Method:

1. Record the **Question** that is being investigated in this experiment.

2. Record a **Hypothesis** for the question stated above.

3. Predict the results of the experiment based on your hypothesis (if/then).

4. Record your data below.

Table 3. Carbon dioxide evolved during fermentation by different concentrations of yeast. Carbon dioxide levels measured in millimeters (mm).

Time (min.)	Sacch. 1	Sacch. 2	Sacch. 3	Sacch. 4
0 (initial)				
2				
4				
6				
8				
10				
12				
14				
16				
18				
20				

The results of this experiment can be presented graphically. The presentation of your data in a graph will assist you in interpreting your results. Based on your results, you can complete the final step of scientific investigation, in which you must be able to propose

a logical argument that either allows you to support or reject your initial hypothesis.

5. Graph your results using the data from Table 3.

6. What is the dependent variable? Which axis is used to graph this data?

7. What is your independent variable? Which axis is used to graph this data?

Exercise D

Employing Steps in the Scientific Method:

1. Record the **Question** that is being investigated in this experiment.

2. Record a **Hypothesis** for the question stated above.

3. Predict the results of the experiment based on your hypothesis (if/then).

4. Record your data below.

Table 5. Carbon dioxide evolved during the fermentation of various carbohydrates. Carbon dioxide levels measured in millimeters (mm).

Time (min.)	Sacch. 1	Sacch. 2	Sacch. 3
0 (initial)			
2			
4			
6			
8			
10			
12			
14			
16			
18			
20			

The results of this experiment can be presented graphically. The presentation of your data in a graph will assist you in interpreting your results. Based on your results, you can complete the final step of scientific investigation, in which you must be able to propose a logical argument that either allows you to support or reject your initial hypothesis.

5. Graph your results using the data from Table 5.

6. What is the dependent variable? Which axis is used to graph this data?

7. What is your independent variable? Which axis is used to graph this data?

8. Fermentation involves **redox reactions**. Explain what happens to electrons during a redox reaction and how this changes a molecule's potential energy.

9. Why did we add the *Saccharomyces cerevisiae* (baker's yeast) to the fermentation tubes? Specifically, what did the yeast provide to the fermentation mixture?

10. What is the purpose of *Saccharomyces cerevisiae* (“baker’s yeast) in the bread-making process?

11. We measured the formation of what end product to determine the fermentation rate? Name the end product that we measured.

12. List two specific factors (as they relate to the experiment performed in our lab) that affect the rate of fermentation.

13. What other variables could be investigated that might affect the rate of alcoholic fermentation by yeast?

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CHAPTER OVERVIEW

13: Photosynthesis

Learning Objectives

Content Objectives

- Describe the process of photosynthesis.
- Describe how plant pigments are separated through paper chromatography.
- Predict and describe the absorption spectrum of a plant pigment based on its color.

Skill Objectives

- Extract plant pigments using polar and nonpolar solutions.
- Separate and identify plant pigments using paper chromatography.
- Analyze the absorption spectrum of various plant pigments.
- Measure the rate of photosynthesis using gas production as an indirect method.

13.1: Introduction
13.2: Exercise
13.3: Lab Report

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13.1: Introduction

Introduction

Have you ever wondered why leaves change colors in the fall but remain green during other seasons of the year? Leaves contain multiple types of pigments that are involved in many different functions in the plant, including photosynthesis, protection from UV radiation, and even attracting pollinators. Pigment molecules are stored inside of **plastids**, a class of cellular organelles that includes **chloroplasts**, the organelles responsible for photosynthesis. Found inside chloroplasts, **chlorophyll** is the most common pigment in a leaf. It comes in two varieties (**chlorophyll *a*** and **chlorophyll *b***). During the spring and summer months, when day length is longest and the sunlight is most direct, plants produce a large amount of chlorophyll, which is used to capture sunlight energy that drives photosynthesis.

All of the wavelengths of energy emitted by the sun are collectively called the **electromagnetic spectrum**. Waves at the low end of the spectrum (those with the longest wavelengths), like radio waves and microwaves, emit less energy, while waves at the high end (those with the shortest wavelengths), like x-rays and gamma rays, emit higher energy. The very small section of the electromagnetic spectrum that humans can see is called the **visible light spectrum**. Plants can use these same wavelengths of light, from red at the low end to violet at the high end of the visible light spectrum, to power photosynthesis (**Fig. 13.1.1**).

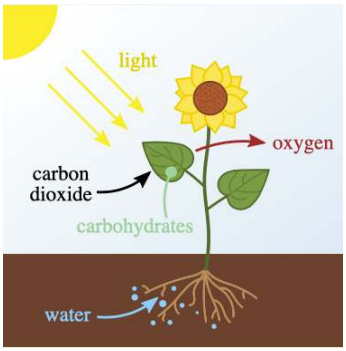


Figure 13.1.1: Photosynthesis Credit: [CC BY-SA 3.0 (https://creativecommons.org/licenses/by-sa/3.0)

Photosynthesis is the process by which plants use energy from the sun and water from the soil to convert atmospheric carbon dioxide into sugar (glucose) that can be used for energy production (**Fig. 13.1.2**).

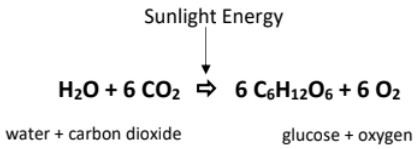


Figure 13.1.2: Chemical Equation for Photosynthesis.

Different pigments absorb and reflect different wavelengths of light and therefore appear different colors to us. During the spring and summer months, when plants are most productive, they produce a huge amount of chlorophyll molecules. Chlorophyll *a* and chlorophyll *b* absorb mostly at the red-to-orange and blue-to-violet ends of the visible light spectrum; they reflect the rest of the wavelengths. The reflected wavelengths, mostly in the yellow-to-green range, are the wavelengths that are detected by the human eye. This is why plants appear green to us (**Fig. 13.1.3**).

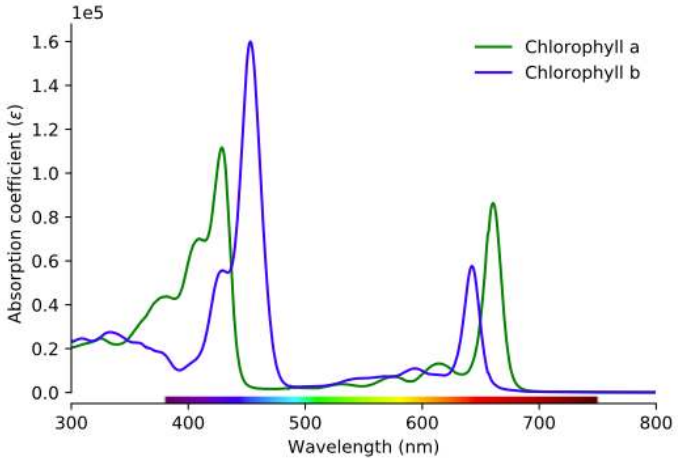


Figure 13.1.3: Absorption spectrum of chlorophyll *a* and chlorophyll *b* Credit: "File:Chlorophyll Absorption Spectrum.svg" by Serge Helfrich is licensed under CC BY-SA 4.0

Another class of pigments found in plants is the **carotenoids.** Carotenoids include **carotenes**, which appear yellow-orange, and **xanthophylls**, which appear mostly yellow. These pigments are found in the plant all year round but are largely masked by the abundant chlorophyll molecules that are present during the spring and summer. Carotenoids play a minor role in photosynthesis, but they play a larger role in protecting the plant tissues from damage caused by UV radiation from the sun.

As summer gives way to fall, day length gets shorter, temperatures decrease, and water may become less available. Plants stop producing new chlorophyll molecules, and the remaining chlorophyll molecules are broken down and reabsorbed to be used again in the following spring. Carotenoids break down more slowly, which is why we can see their yellow and orange colors showing through as chlorophyll is lost from the leaves.

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13.2: Exercise

Exercise 1: Separation of Plant Pigments Using Chromatography

In this lab, you will first extract pigments from spinach leaves and then separate pigments from one another using a technique called **chromatography.** Chromatography is used to separate chemicals based on their varying solubilities in selected solvents. Recall from our discussions of the Chemistry of Life (textbook chapter), molecules and compounds are classified as **polar** or **nonpolar**, and that "like dissolves like." In other words, polar compounds dissolve in polar solvents, but not in non-polar solvents, and vice versa. An example of this concept is seen when oil, a nonpolar substance, comes in contact with water, a polar substance. The two substances do not mix; they are repelled by one another. Chromatography works by separating chemicals according to their varying degrees of polarity. In chromatography experiments, there are two "phases," the stationary phase, which does not move, and the mobile phase, which travels across the stationary phase.

In this experiment, you will use **paper chromatography**. Paper, which is made of cellulose, is very polar and acts as the stationary phase. The solvent you will use is a mixture of petroleum ether and absolute acetone, both of which are nonpolar; the solvent acts as the mobile phase. Extracted plant pigment is applied to the paper, and the paper is placed in the solvent. As the solvent moves up the paper through capillary action, it carries the pigments along with it. Pigments that are more polar are attracted to the polar cellulose molecules in the stationary phase (the paper), so they move more slowly as the solvent travels up the paper. Nonpolar pigments are more strongly attracted to the nonpolar solvent and tend to stay in solution longer, thus moving farther up the paper. The pigments are carried at different rates because they are not equally soluble. Since different pigment molecules have different molecular structures and varying degrees of polarity, this technique works well to separate pigments from one another, giving us a clear look at each pigment individually.

The distance that each pigment travels will be unique to that pigment based on the solvent selected. These migration distances can be used to calculate the (retention factor(opens in new window)) value, which is simply calculated by dividing the distance traveled by the pigment over the distance traveled by the solvent.

Materials

- Spinach leaves
- Electronic balance / weigh boats
- A small amount of clean quartz sand
- Mortar and pestle
- Acetone (keep this in the hood at all times)
- Test tube
- Chromatography paper (11 cm square)
- Metric ruler
- Chromatography jars - glass quart jars (which will hold 11 cm square paper) with lids or can cover with aluminum foil
- Pencil
- Glass capillary tubes
- Small metric rulers
- Chromatography solvent (Use 9 parts petroleum ether and 1 part acetone)
- Waste container for discarded acetone/chlorophyll extract
- Handheld UV light (Turn lights off in lab and shine handheld UV light on students' chlorophyll extracts to cause them to fluoresce red)

Procedures

Pigment Extraction

1. Obtain spinach leaves. Tear leaves into small pieces, discarding the large midvein. Weigh out approximately 4.0 grams of leaf tissue.

2. Place leaf tissue plus a pinch of clean quartz sand into a clean mortar and pestle; grind to a fine pulp.
3. Add 6mL of acetone to the pulp and continue to grind.
4. When leaf tissue is thoroughly ground into a paste-like consistency, allow the solution to rest for 1 minute. Carefully pour off the liquid portion of the mixture into a clean test tube, leaving the pulpy remains in the mortar. *Note: Dispose of acetone/chlorophyll waste in the waste container in the fume hood. Wash the mortar and pestle well with warm soapy water, rinse, and set aside to dry.
5. You may wish to examine your acetone/chlorophyll extract under UV light. Turn off the lights in the lab and shine the fluorescent black light onto the extract. Do not look into the light directly. The chlorophyll will fluoresce red.
6. Can you explain why the extract containing the chlorophyll pigment turns from a green color to a fluorescent red color when exposed to UV light?

Pigment Separation

NOTE: The organic solvents used in this step are extremely volatile and flammable. The chromatography jars must be kept in the fume hood at all times.

Paper chromatography requires that the atmosphere within the chromatography jar be completely saturated with solvent. Be sure that the lid or aluminum foil covering the jar stays in place before and during chromatogram development.

1. **Prepare chromatography sheet:** Use the pencil to draw a line 1.5 - 2 cm from the bottom of the paper. This line will serve as a guide when applying the spinach extract. It will help ensure that the extract is applied evenly, in a straight line, and at a level **above the solvent** in the chromatography jar.
2. **Apply pigment:** Using a glass capillary tube, apply the pigment extract to the paper in a linear series of small dots (follow the line you created as a guide for application). **NOTE: Leave a 1 cm margin at each edge that is free of pigment extract. DO NOT TOUCH THE PAPER; oils from your skin can interfere with the process.**
3. After you have completed the pigment line across the chromatography paper, allow the extract to dry for about 5 min. Go back and re-apply more pigment over the first line, allowing each application to dry before reapplying more extract. Repeat the procedure until ***all*** the pigment extract is used, or until you have applied enough extract (consult with your instructor). Allow the sample to dry.
4. Once your sample has dried, take your chromatography paper to the fume hood. Add approximately 20 mL of chromatography solution to the jar (this may have already been done for you). This should result in the solvent being about 1 cm deep from the bottom of the jar. Roll the paper into a cylinder so that the line faces outward and is toward the bottom of the jar. You can secure this cylinder shape by using a small stapler to staple the sides together at the top and the bottom of the cylinder. Place the rolled paper into the jar so that it is in the chromatography solution. **The chromatography solution should not be higher than the line on your chromatography paper.**
5. Cover the jar with the lid or aluminum foil and observe as the chromatography solvent (the mobile phase) travels up the paper (the stationary phase).
6. When all of the pigments are clearly separated and **before** the solvent front has reached the top edge, remove the chromatogram and allow it to dry in the hood. Make sure to use a pencil to draw a line to indicate where the solvent front ended.
7. You should be able to see at least four distinct bands. (There may be as many as six bands.)
8. Make a sketch of your chromatogram using the template that follows. Using colored pencils, note the color of the bands. Use Table 1 to help you identify each pigment. On your sketch, label each band with the name of the pigment. Mark the distance (in cm) from the initial pigment band to each colored band as well as the total distance from the initial pigment band to the solvent front.

Sketch of Resulting Chromatogram

Table 1: Different classes of pigments found in leaves and their respective colors.

Pigment	Band Color
Beta-carotene	**Orange**
Xanthophylls	**Yellow**
Chlorophyll *a*	**Blue-green**
Chlorophyll *b*	**Yellow-green**

9. Use the data from your sketch to complete the data table below.
10. Calculate the value and include that in your data table.

Data Table for Chromatography Results

A	B	C	D	E
Name of Pigment	Description of color	Distance solvent front traveled from initial pigment band	Distance pigment traveled from initial pigment band	Rf value of pigment (column D / column C)

Exercise 2: Analysis of the Absorption Spectrum of Leaves

While we know most leaves are green, we have now seen that other pigments are also found in the leaves. The green color that we observe is a consequence of reflected light in the wavelengths of about 550-500nm in size (**Fig. 3**). In the autumn months of the year, many plants stop producing chlorophyll, revealing the colors of the other pigments beneath. Every chemical has a specific set of wavelengths it can absorb and thus be identified. By analyzing the absorption spectrum of leaf pigments, we can infer what wavelengths of light will be useful in photosynthesis.

Materials

- PASCO Wireless Spectrometer
- iPad or laptop to connect the spectrometers to
- 4 cuvettes
- Leaf samples
- 3 test tubes
- 100 mL 95% ethanol
- 20 mL graduated cylinder
- Scissors
- Mortar and pestle set up from the previous section

Procedure

1. Connect the spectrometer to a Laptop via a USB cable or wirelessly to an iPad.
2. Open the PASCO spectrometer application.
3. Select the ANALYZE Solution setting from the menu at the top.
4. Choose CALIBRATE DARK from the menu at the bottom. Cover the sample well with your finger to block light until a check mark appears indicating that calibration is complete.

5. Fill a cuvette ¾ full with ethanol. Only handle the cuvettes from the ribbed sides since fingerprint smudges on the clear sides will affect light passing through. Place the cuvette in the well so that the clear side is facing the light source.
6. Choose CALIBRATE REFERENCE from the menu until the checkmark appears. You do not need to cover the cuvette with your finger for this step.

SAMPLE PREPARATION:

1. Obtain 3 different samples of plant leaves.
2. Label 3 test tubes: Sample 1, Sample 2, Sample 3
3. Tear the first sample of plant leaves into small pieces, discarding the large midvein. Weigh out approximately 4.0 grams of leaf tissue.
4. Place the leaf tissue plus a pinch of clean quartz sand into a clean mortar and pestle; grind to a fine pulp.
5. Add 6mL ethanol to the pulp and continue to grind. Make sure to use ethanol and **NOT** acetone. The cuvettes are plastic and acetone will cloud the plastic and not allow light to pass through freely.
6. When the leaf tissue is thoroughly ground into a paste-like consistency, allow the solution to rest for 1 minute. Carefully pour off the liquid portion of the mixture into a clean test tube, labeled sample 1, leaving the pulpy remains in the mortar.
7. Repeat the extraction for the remaining two leaf samples. Be sure to add the appropriate leaf extraction mixture to the correctly labeled test tubes.

DATA COLLECTION

1. Place the first leaf sample extraction mixture into a cuvette in the well and press START RECORDING. The graph that appears is the absorbance spectrum of the extract. If any of the peaks are flattened, add more ethanol to the cuvette to further dilute the sample.
2. When the peaks have stabilized, select STOP RECORDING and name your run for the sample analyzed.
3. Use the ADD COORDINATE tool to find the peak of each sample and SNAPSHOT each of the sample runs. Record the values in a data table.
4. Table 2 shows the absorption spectrum of some common plant pigments. Use this information to predict what pigments were in your samples.

Table 2: Absorption of Plant Pigments

Pigment	Peak Absorption Wavelengths (nm)	Sample 1	Sample 2	Sample 3
Chlorophyll A	430 and 662			
Chlorophyll B	453-642			
Carotenoids	460-550			
Anthocyanins	520*			
Xanthophyll	494			
Betalains	535 or 480*			

*peaks may vary and are pH-dependent.

Exercise 3: Measuring the Rate of Photosynthesis

(remixed from https://batch.libretexts.org/print/Letter/url=https://bio.libretexts.org/Bookshelves/Ancillary_Materials/Laboratory_Experiments/General_Biology_Labs/BCCC_MASTER_Biology_PLC/Biology_102/02%3A_Photosynthesis/2.1%3A_Photosynthesis_Protocol.pdf (opens in new window) CCBY 2.1.1)

Materials:

- Five spinach leaves (Dark green, fresh spinach leaves produce better, faster results.)
- Two cups (Short clear plastic cups which hold ~300 mL work best.)
- Index cards
- Forceps
- Plastic petri dish
- 500 mL of water with 0.2% sodium bicarbonate (NaHCO3) (or baking soda; you may prefer to use 0.3%-0.5% sodium bicarbonate which will result in a faster rate of photosynthesis and more rapid floating for the leaf disks)
- 500 mL of water without sodium bicarbonate (NaHCO3)
- Two drops of dilute detergent (To prepare the dilute detergent, add approximately 5 mL of dishwashing liquid soap to 250 mL of water. Aliquot into microfuge tubes and label with a "DD")
- Hole punch (or a straw)
- Two 10 ml syringes without needles (syringes can be rinsed, dried and reused)
- Lamp with 23 W spiral compact fluorescent bulb (If you have a clamp lamp, obviously this could be attached to a ring stand or other vertical pole; if you don't have enough vertical poles, you could rig up lamp support using a cardboard box.)
- Stopwatch

Employing Steps in the Scientific Method:

1. Record the **Question** that is being investigated in this experiment.

   _____

2. Record a **Hypothesis** for the question stated above.

   _____

3. Predict the results of the experiment based on your hypothesis (if/then).

   _____

4. Perform the experiment below and collect your data.

Procedure:

1. Label one cup **'sodium bicarbonate'** and fill it about one-quarter full (~75 mL) with the sodium bicarbonate solution. Label the second cup **'water'** and fill it about one-quarter full (~75mL) with water. Add one drop of dilute detergent (microfuge tube labeled "DD") to each cup.
2. Next, you will prepare your leaf disks, taking care not to damage them. First, prepare two index cards by folding each of them in half and then unfolding them and laying them flat. Use the hole punch to prepare the leaf disks. Punch out a piece of leaf tissue, avoiding large veins. (If you are using a hole punch, keep it clamped shut as you tap the punch on the table to release the leaf disk onto the piece of paper.) Repeat until you have 10 leaf disks on each index card.
3. Remove the plunger from a syringe, and use one of the folded papers to pour 10 leaf disks into the syringe. Tap them down to the tip of the syringe.
4. Replace the plunger, and push it down to about the 1 mL mark, being careful not to squash the leaf pieces.
5. Suck up ~5 mL of the sodium bicarbonate/detergent solution into the syringe. Hold the syringe upright and push out as much of the air as possible.
6. Put your thumb over the tip of the syringe, and pull back slowly on the plunger to about the 10 mL mark. Additionally, while pulling back on the plunger and keeping your thumb over the tip of the syringe, shake the solution in the syringe. This creates a vacuum and pulls air out of the leaf discs. Tilt and swirl the syringe to make sure all disks are submerged in the solution, then hold it for 10 seconds, and then gently let go of the plunger without removing your thumb from the tip of the syringe. The plunger will "snap" back into position and the solution will enter the leaf disks. If the leaf disks drop to the bottom of the solution in the syringe, you are done. If not, do this again.
7. Remove the plunger and empty the disks into the cup containing sodium bicarbonate solution. Then swirl the cup to dislodge any disks that are stuck to the side of the cup. Make sure that all of the disks are settled on the bottom of the cup.
8. Repeat steps 3 - 7 but use the water/detergent solution instead of sodium bicarbonate.

9. Place both cups under a bright light. Place half a petri dish full of water on top of each cup to act as a temperature buffer.

Safety Precaution

Be careful to keep all liquids away from the light source and electrical cord.

10. At the end of each minute, record the number of floating disks (any disk that is no longer touching the bottom) in the following table.

Table 2: Number of Floating Leaf Disks

Minute	In Water/Detergent	In Bicarbonate/Detergent
1		
2		
3		
4		
5		
6		
7		
8		
9		
10		
11		
12		
13		
14		
15		
16		

Extension Activity: (Optional)

The results of this experiment can be presented graphically. The presentation of your data in a graph will assist you in interpreting your results. Based on your results, you can complete the final step of scientific investigation, in which you must be able to propose a logical argument that either allows you to support or reject your initial hypothesis.

1. Graph your results using the data from Table 2, using different symbols for the leaf disks in water vs. the leaf disks in bicarbonate solution.
2. What is the dependent variable? Which axis is used to graph this data?

   _____
3. What is your independent variable? Which axis is used to graph this data?

   _____

Exercise 4: Observing and Quantifying Stomata (Optional)

Leaves are the primary food factories in a typical plant. Powered by the sun's energy, leaves take in water and carbon dioxide and produce oxygen and glucose through **photosynthesis**. Leaves have specialized cells on their surfaces called **guard cells** which surround openings called **stomata** (singular = stoma). Stomata are tiny openings in the epidermis of terrestrial (land) plants. Gases,

primarily O2 and CO2, can pass through these openings to allow for photosynthesis to occur. Likewise, H2O will evaporate out of the stomata, leading to potential dehydration.

Stomata typically remain open during the day (since sunlight stimulates them to open) and close at night, when photosynthesis doesn't occur, allowing for water conservation. Stomata can also close during especially hot days, during droughts, or as a response to plant growth regulators. Desert plants have a special form of photosynthesis which allows them to open their stomata at night, to perform gas exchange, and close them during the day to conserve water.

Materials:

- Potted plant with leaves
- Bottles of clear nail polish
- Clear packing tape
- Microscopes
- Microscope slides
- **Forceps**
- Scissors
- Calculator

Employing Steps in the Scientific Method:

1. Record the **Question** that is being investigated in this experiment.

   _____

2. Develop a **hypothesis** about the number of open stomata found on the upper side of a leaf as compared to the lower side of the leaf. _____
3. Develop a **hypothesis** about the number of open stomata found on the upper side of a leaf as compared to the lower side of the leaf. Write your hypothesis in the space below. _____
4. Predict the results of the experiment based on your hypothesis (if/then).

   _____

5. Perform the experiment below and collect your data.

Procedure:

1. Obtain 2 cut sections of leaves from the plant provided in the lab.
2. Using a small amount of clear nail polish, coat the underside (lower surface) of one of the leaves with a THIN layer. Set the leaf on a paper towel (painted surface up) and allow it to dry completely (~ 30 min.)
3. Coat the upper surface of the remaining leaf in the same way.
4. As the nail polish dries, it will conform to the surface of the leaf.
5. Place a piece of the clear cellophane tape over the entire surface of the leaf covered with the nail polish. Use your fingertip to gently adhere the tape to the entire surface of the leaf, rubbing back and forth until you feel you have pressed the tape into the surface of the leaf.
6. Use a corner of the tape to gently peel back the tape. While peeling back the tape you will start to separate the dried nail polish from the surface of the leaf. This is the leaf impression you will examine under the microscope.
7. Tape each of your peeled impressions to a clean microscope slide. Use scissors to trim away any excess tape.
8. Examine the leaf cell imprints under a total magnification of 40x. Scan the slide until you find a good area where you can see plenty of the stomata. *Note: You may need to adjust the iris diaphragm to reduce the amount of light and add contrast to the slide to more clearly see the stomata.
9. Increase the total magnification to 100x for counting.
10. Each stoma is surrounded by two sausage-shaped cells that are smaller than the surrounding epidermal cells. These cells are called guard cells and, unlike other cells in the epidermis, contain chloroplasts.
11. Identify the epidermal guard cells and the stomata. Fix the field of your microscope in place, and count the number of visible stomata. Stomata on the edge of the field of view are to be counted! Record this number in Table 3 under "**View 1**".
12. Move the field of view to a new position on the leaf and repeat the procedure. Record this number in Table 3 under "**View 2**".

13. Do a third count in a new position and record this number in Table 3 under "**View 3**". (Do three different counts for the upper surface leaf imprint and the lower surface leaf imprint.)

Table 3: Number of Stomata Observed by Surface

Surface of Leaf	# of Stomata: View 1	# of Stomata: View 2	# of Stomata: View 3	Average # of Stomata
Upper				
Lower				

Extension Activity: (Optional)

The results of this experiment can be presented graphically. The presentation of your data in a graph will assist you in interpreting your results. Based on your results, you can complete the final step of scientific investigation, in which you must be able to propose a logical argument that either allows you to support or reject your initial hypothesis.

1. Graph your results using the data from Table 3.
2. What is the dependent variable? Which axis is used to graph this data?

   _____

3. What is your independent variable? Which axis is used to graph this data?

   _____

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13.3: Lab Report

Exercise 1 Data:

Make a sketch of your chromatogram using the template that follows. Using colored pencils, note the color of the bands. Use Table 1 to help you identify each pigment. On your sketch, label each band with the name of the pigment. Mark the distance (in cm) from the initial pigment band to each colored band as well as the total distance from the initial pigment band to the solvent front.

Sketch of Resulting Chromatogram

- Use the data from your sketch to complete the data table below.
- Calculate the value and include that in your data table.

Data Table for Chromatography Results

A	B	C	D	E
Name of Pigment	Description of color	Distance solvent front traveled from initial pigment band	Distance pigment traveled from initial pigment band	Rf value of pigment (column D / column C)

Exercise 1 Review Questions:

1. Which plant pigments are most polar?

2. Which are least polar?

3. How do these differences in polarity affect the movement of the pigments up the chromatography paper? Explain why this is observed.

4. What does the abbreviation *Rf* stand for? How is it calculated? What does it tell us?

5. If Solution A moves 4 cm and Solution B moves 4.5 cm on a piece of chromatography paper, when the solvent moves 10 cm, which is the most polar solution? Explain your answer.

Exercise 2 Data and Review Questions:

Table 2: Absorption of Plant Pigments

Pigment	Peak Absorption Wavelengths (nm)	Sample 1	Sample 2	Sample 3
Chlorophyll A	430 and 662			
Chlorophyll B	453-642			
Carotenoids	460-550			
Anthocyanins	520*			
Xanthophyll	494			
Betalains	535 or 480*			

*peaks may vary and are pH-dependent.

1. How do you think the knowledge obtained from your chromatogram and the spectrograms relate to our understanding of plant pigments?

2. Why do leaves change color in the fall?

Exercise 3 Employing Steps in the Scientific Method:

1. Record the **Question** that is being investigated in this experiment.

   _____

2. Record a **Hypothesis** for the question stated above.

   _____

3. Predict the results of the experiment based on your hypothesis (if/then).

   _____

Table 2: Number of Floating Leaf Disks

Minute	In Water/Detergent	In Bicarbonate/Detergent
1		
2		
3		
4		
5		
6		
7		
8		
9		
10		
11		
12		
13		
14		
15		
16		

Exercise 3 Review Questions:

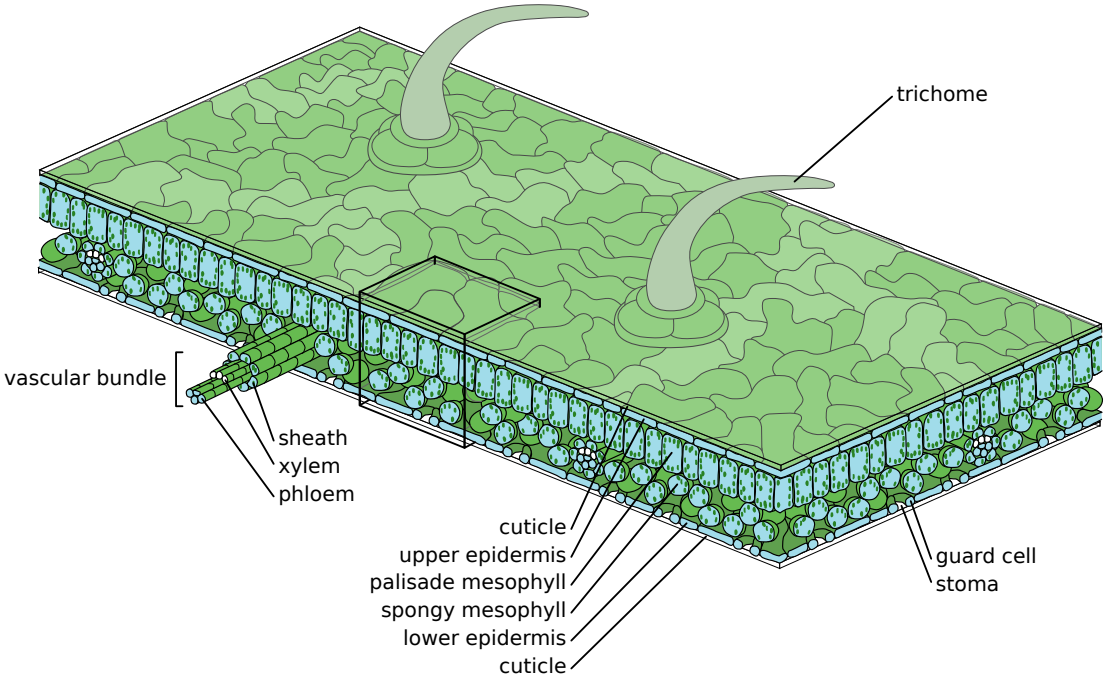
1. Complete the following sentence.

Photosynthesis is a set of _____ in which _____energy is converted to _____energy.

2. Why do some trees appear green in the summer but change colors in the fall?

3. What **optimal wavelengths** (or peaks) did you observe for both chlorophyll *a* and *b*?

4. Do your leaf disks float? Use the information in this diagram of a cross-section of a leaf to explain why a leaf disk would float.



Figure

Figure 13.3.1: Labeled leaf cross section. (CC BY-SA 3.0 Unported; Zephyris(opens in new window) via Wikipedia(opens in new window))

5. Where does photosynthesis occur in a leaf? State which organelles carry out photosynthesis and which type or types of leaf cells have this organelle.

6. Explain why it is useful to the plant to have air spaces around the spongy mesophyll cells in the leaves. (Hint: Recall the chemical equation for photosynthesis.)

7. What was the purpose of the sodium bicarbonate in this experiment?

8. Leaf disks normally float. What caused the leaf disks to sink?

9. To measure the rate of photosynthesis, you replaced the air in the spongy mesophyll in your leaf disks with a liquid. This caused the leaf disks to sink. Then you put these leaf disks in water with dissolved CO_2 and measured the amount of time it took for the leaf disks to float. Which product of photosynthesis accumulated in the spongy mesophyll and caused the leaf disks to float?

10. Suppose that a leaf disk that has had the air sucked out is placed in a bicarbonate solution under a dim light that results in a low rate of photosynthesis that just equals the rate of cellular respiration. Would you expect this leaf disk to float? Explain why or why not.

Exercise 4: Observing and Quantifying Stomata (Optional)

1. Record the **Question** that is being investigated in this experiment.

   _____

2. Develop a **hypothesis** about the number of open stomata found on the upper side of a leaf as compared to the lower side of the leaf. _____
3. Develop a **hypothesis** about the number of open stomata found on the upper side of a leaf as compared to the lower side of the leaf. Write your hypothesis in the space below. _____
4. Predict the results of the experiment based on your hypothesis (if/then).

   _____

Table 3: Number of Stomata Observed by Surface

Surface of Leaf	# of Stomata: View 1	# of Stomata: View 2	# of Stomata: View 3	Average # of Stomata
Upper				
Lower				

Extension Activity: (Optional)

The results of this experiment can be presented graphically. The presentation of your data in a graph will assist you in interpreting your results. Based on your results, you can complete the final step of scientific investigation, in which you must be able to propose a logical argument that either allows you to support or reject your initial hypothesis.

1. Graph your results using the data from Table 3.
2. What is the dependent variable? Which axis is used to graph this data?

   _____

3. What is your independent variable? Which axis is used to graph this data?

   _____

Exercise 4 Review Questions:

1. What are stomata?

2. What is the importance of stomata in photosynthesis?

3. What is the function of the guard cells?

4. During the lab activity, were more **stomata** observed on the upper surface of the leaf or the lower surface of the leaf? Explain why you think this distribution exists.

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CHAPTER OVERVIEW

14: Mitosis

Learning Objectives

Content Objectives

- Examine the processes of the cell cycle and asexual cellular replication.
- Compare and contrast animal and plant mitosis.

Skill Objectives

- Draw and label the stages of Mitosis and identify the important events of each.
- Model the stages of Mitosis using pop-beads to simulate chromosomes.
- Identify the time different cell types spend in the various stages of the cell cycle.

14.1: Introduction
14.2: Exercise
14.3: Lab Report

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14.1: Introduction

Introduction to Mitosis

In eukaryotic cells, the time and phases from the beginning of one cell division until the beginning of the next cell division is called the **cell cycle.** The first phase of the cell cycle is **interphase.** Interphase is the time during which the cell performs its normal functions and prepares for cell division. Cells spend most of their time in this phase. During interphase, **chromosomes** are not visible because they are **decondensed** (present only as a tangled mass of thin threads of DNA with associated proteins, called chromatin). The nuclear membrane is present, and visible, as is the **nucleolus**.

Interphase includes two gap phases, G1 and G2, where the cell increases in size and synthesizes new organelles, enzymes, and other proteins that are needed for cell division. In between the two gap phases, the DNA replicates in preparation for cell division. This stage is called the **S phase**. At the beginning of S phase, chromosomes are single and unreplicated. By the end of S phase, each chromosome has made an exact copy of itself and consists of two **sister chromatids**. At this point in the cell cycle the sister chromatids are held together tightly at the **centromere**. While the two sister chromatids are physically joined together they are still considered one replicated chromosome. When the sister chromatids physically separate, later during the cell cycle, they are then considered to be individual chromosomes.

In animal cells, interphase is also when the **centrosome** (consisting of **two centrioles**) is replicated. Spindle fibers form from and radiate outward from the centrosomes to attach to and move chromosomes during cell division.

Interphase is followed by **mitosis** (in the somatic cells) **or meiosis** (in reproductive cells), which is when replicated chromosomes and cytoplasm separate, during the process of karyokinesis and cytokinesis respectively.

Chromosomes that are the same length, have the same centromere location and the same gene sequences and positions are called homologous chromosomes. Human somatic cells contain pairs of homologous chromosomes. Each homologous pair consists of one maternal chromosome and one paternal chromosome. Cells that contain two copies of each chromosome are called **diploid** (2n, where n is the number of different chromosomes in a single set). Human sex cells (eggs and sperm) contain only one copy of each chromosome. Cells with only one copy of each chromosome are **haploid** (n). When the haploid sperm (n) and egg (n) combine during fertilization this forms a diploid zygote (2n).

MITOSIS

Mitosis is nuclear division that results in two cells containing the same number of chromosomes as the parent cell. Most human cells (skin, muscle, bone, etc.) divide by mitosis. This process is necessary for the normal growth and development of a multicellular eukaryotic organism from a zygote (fertilized egg), as well as growth and the repair and replacement of cells and tissues. At the end of mitosis, two daughter cells are formed that are identical to the original (parent) cell. Mitosis is also a form of asexual reproduction in unicellular eukaryotes.

Mitosis is a complex and highly regulated process. It occurs in the following 4 separate phases: prophase, metaphase, anaphase, and telophase. Telophase is quickly followed by cytokinesis.

Prophase: Cells prepare for division by coiling and condensing their chromatin into chromosomes. By late prophase, individual chromosomes can be seen, each consisting of two sister chromatids joined at a centromere. Spindle fibers begin to form from the centrosomes, which have begun to migrate to opposite “poles” of the cell. The nucleoli and the nuclear membrane degrade.

Metaphase: Spindle fibers (called kinetochore microtubules or kinetochore spindle fibers) that emanate from the centromeres attach to the kinetochore (a proteinaceous area) on the sister chromatids. The fibers pull and otherwise manipulate the chromosomes to align them on the plane that passes through the center of the cell (metaphase plate). This “plate” is not an actual structure; it merely signifies the location of replicated chromosomes prior to their impending separation.

Other non-kinetochore spindle fibers or tubules (aka polar microtubules), emanating from the two centrosomes, elongate and eventually overlap with each other near the metaphase plate.

Anaphase: The centromeres divide, with the help of separase enzymes, and separate the sister chromatids. This happens simultaneously in all the chromosomes. The kinetochore spindles shorten and pull each chromatid to which they are attached

toward the pole (and centrosome) from which they originate. This equally distributes exactly half the chromosomal material to each side of the cell. In late anaphase, the non-kinetochore spindles begin to elongate, lengthening the cell.

Telophase: The non-kinetochore microtubules continue to elongate, further elongating the cell in preparation for cytokinesis (splitting of the cytoplasm). The chromosomes reach their respective poles. The kinetochores disappear. The two nuclear membranes (one in each half of the cell) begin to form around the chromosomes. Nucleoli reappear and the chromosomes in each soon-to-be new cell begin to decondense back into chromatin. Mitosis is complete at the end of this stage.

Cytokinesis (splitting of the cytoplasm):

In animal cells and all other eukaryotes without a cell wall, cytokinesis is achieved by means of a constricting "belt" of protein fibers that slide past each other near the equator of the cell. As this occurs, the diameter of the belt decreases, pinching the cell to form a **cleavage furrow** around the cell's circumference. As constriction proceeds, the furrow deepens until it eventually slices its way into the center of the cell. At this point, the cell is divided into two.

Plant cell walls are far too rigid to be split apart by contracting proteins. Instead, these cells assemble membrane proteins (in vesicles that bud off the Golgi apparatus) in their interior at right angles to the spindle apparatus. This expanding membrane partition, called a cell plate, continues to grow outward until it reaches the interior surface of the plasma membrane and fuses with it. This divides the cell in two.

References

Belwood, Jacqueline; Rogers, Brandy; and Christian, Jason, Foundations of Biology Lab Manual (Georgia Highlands College). "Lab 10: Mitosis & Meiosis," (2019). *Biological Sciences Open Textbooks*. 18. CC-BY

https://oer.galileo.usg.edu/biology-textbooks/18(opens in new window)

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14.2: Exercise

The Phases of Mitosis

Exercise 1: Modeling the Phases of Mitosis with Pop Beads

Materials:

- Chalk (can be used directly on lab bench to draw cellular structures and then washed off)
- Chromosome modeling kits
  - Commercially available pop bead kits (e.g Carolina Biological Supply Company, Item #171100)
- 40 pop beads of one color (red)
- 40 pop beads of another color (yellow)
- 8 magnetic centromeres
- 4 laminated pictures of centrosomes (each consisting of a pair of centrioles)
- Paper towels / Kimwipes

Procedure:

Using models is a great way to represent natural structures and processes that are too small, or too large, or too complex to observe directly. By building chromosomes from the pop beads and manipulating them to model cell division (mitosis and meiosis) you will enhance your understanding of the nature of chromosomes and the cellular structures needed to perform cell division. In this simulation, your cell will be a **diploid (2n)** cell with 4 chromosomes. It will contain 2 homologous pairs of chromosomes. In order to differentiate between the pairs of homologs, one pair of homologous chromosomes will be longer than the other. One chromosome of each pair is red and represents maternal DNA (genetic material contributed by a female's egg). The other chromosome of each pair is yellow and represents paternal DNA (genetic material contributed by a father's sperm). Thus, for each pair of homologous chromosomes, one should be red and one should be yellow. The strands of pop beads represent the DNA in the form of chromatin during the G1, S, and G2 phases. They then represent chromosomes as they enter the phases of mitosis.

This diploid cell with 2 homologous pairs of chromosomes will be modeled as it moves through the following phases of mitosis:

- Interphase (uncondensed DNA) **Before Synthesis** of DNA (G1)
- Interphase (uncondensed DNA) **After Synthesis** of DNA (G2)
- Prophase
- Metaphase
- Anaphase
- Telophase
- Cytokinesis

1. You will build 2 pairs of homologous chromosomes (2n = 4). The first homologous pair of single chromosomes should be constructed using 12 red beads for one member of the long pair and 12 yellow beads for the other member of the pair. You may place the magnetic centromere at any position along the chromosome, but it must be at the same position on both members of the homologous pair.
2. The second homologous pair of single chromosomes should be constructed using 6 red beads for one member of the short pair and 6 yellow beads for the other member of the pair. You may place the magnetic centromere at any position along the chromosome, but it must be at the same position on both members of the homologous pair.
3. Use the pop beads to model the **G1 phase of interphase** (before synthesis of the DNA). On your lab bench, use the chalk to draw the cell membrane, nucleus, and nucleolus. Place a laminated centrosome in the cytoplasm of your cell. Place the 4 assembled chromosomes in the nucleus of your cell drawing. Remember that these represent a mass of chromatin.
4. DNA replication takes place during the S phase of interphase. Model the **S phase of interphase** by assembling a second strand that is identical to each of the 4 single chromosomes. The identical strands are considered sister chromatids, which are held together by their magnetic centromeres. (Note: In a living cell, the centromere is a single unit until it separates in anaphase. Therefore, consider the pair of magnets to be a single centromere.)

5. Model the **G2 phase of interphase** by letting your 4 assembled replicated chromosomes rest in the nucleus of your cell. Duplicate the centrosomes by placing another laminated centrosome in the cytoplasm of your cell drawing. Begin to move them towards opposite poles of the cell drawing.
6. To model the **prophase** stage of mitosis, leave the chromosomes where they are in your cell drawing. In this stage, the chromatin coils and condenses into chromosomes. Using the paper towel (or Kimwipe) start erasing some of the nuclear membrane on your cell drawing. This will simulate the breakdown of the nuclear membrane. Also erase the nucleolus, as the nucleolus disappears in this stage. Continue to move your centrosomes to opposite sides of the cell. Use the chalk to draw spindle fibers beginning to form and radiating outward toward the chromosomes.
7. At **prometaphase** the centrosomes are at opposite poles of the cell. During this phase the chromosomes continue to condense, the nuclear membrane completely breaks down, and the spindle fibers start to attach to the **kinetochores** of the centromeres. These are called **kinetochore microtubules**. Other spindle fibers radiate outward from the centrosomes, but these do not attach to the kinetochores. These spindle fibers are called nonkinetochore microtubules.
8. To model **metaphase**, move the centromeres of your chromosomes to lie on an imaginary plane midway between the centrosomes that are now positioned at opposite poles of the cell. Line up the individual chromosomes in the middle (equator) of the cell. Sister chromatids remain attached at the centromere during metaphase.
9. To model **anaphase**, pull the magnetic centromeres apart and slide them towards opposite poles of the cell. Keep them attached to your drawn kinetochore microtubules, but use the paper towel to erase them, making them shorter. With the chalk, lengthen the nonkinetochore microtubules and have them overlap in the middle of your cell. After separation at the centromere, each sister chromatid is now referred to as an individual chromosome. Anaphase ends when the chromosomes reach the opposite ends of the cell.
10. Model **telophase** by piling up your chromosomes at each pole. Erase your spindle fibers, as they disappear during this stage. Even though we can't simulate it, the chromosomes will start to decondense and uncoil back into chromatin. Redraw a nuclear envelope around each pile of chromosomes. Also add nucleoli to your drawing. The formation of separate nuclear envelopes divide the nuclei and marks the end of telophase.
11. The division of the cytoplasm, or cytokinesis, results in the formation of two separate cells. To model **cytokinesis** in animal cells, fungi, and slime molds, leave the piles of chromosomes at their separate poles. Draw indentations of the cell membrane inwards, towards the cytoplasm, on the sides of the cell where there are no centrosomes. This represents a **cleavage furrow**,which eventually "pinches" the cell's cytoplasm into two separate cells. The proteins actin and myosin contribute to the formation of the cleavage furrow. In plant cells, membrane-bound vesicles migrate to the center of the cell (the equatorial plane) and fuse together to form a **cell plate**. The cell plate eventually divides the cytoplasm into two separate cells. Materials needed to build the cell wall are released from the vesicles at the cell plate, forming a new cell wall.

- How do the daughter cells you formed compare to the original parent cell?

_____

Observe the phases of Mitosis in Plant Cells

Exercise 2: Observing the Phases of Mitosis in the Onion Root Tip

Materials:

- Prepared slide of the onion root tip
- Compound light microscope

Procedure:

1. Examine a slide of a longitudinal section of an onion root tip. Adjust the slide to view the region just above the root cap, where there are likely to be dividing cells.
2. Focus on the dividing cells using the 4x scanning objective lens, then switch to the 10x objective and then the 40x objective.
3. Survey the slide to find a cell in each phase of mitosis. Draw a cell for each phase in the boxes provided.

Interphase

DNA is uncondensed and in the form of chromatin. Individual chromosomes are not visible. The nuclear membrane is intact. The nucleolus is visible.

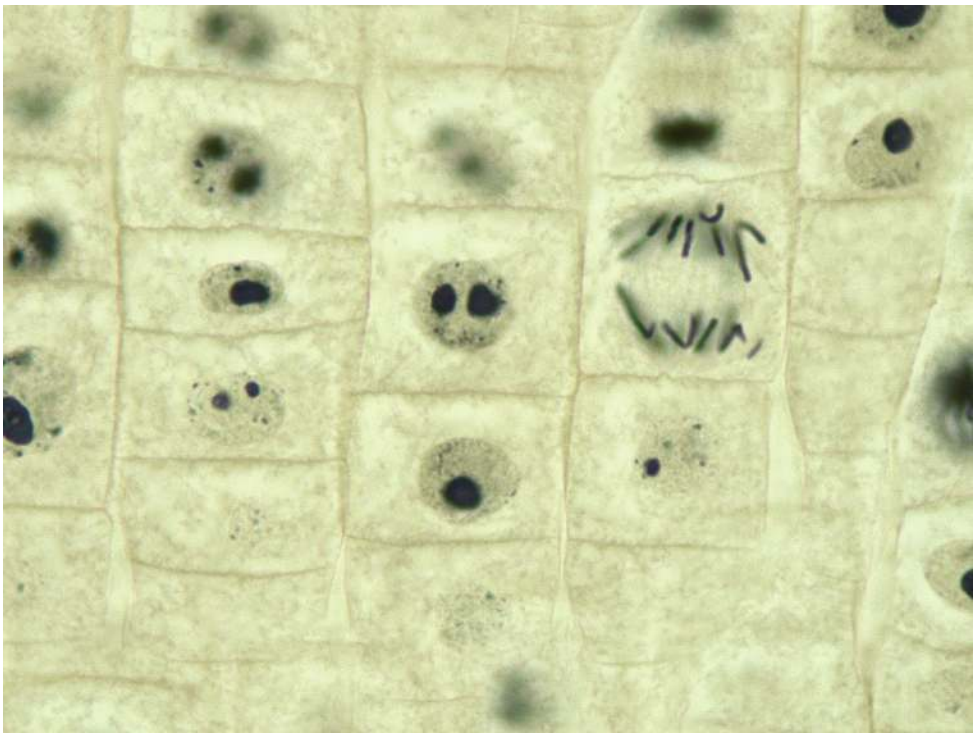


Figure 14.2.1: Interphase in plant cells.

Prophase

Chromatin begins to condense into distinguishable chromosomes. These “puffy” structures are seen throughout the nucleus. Nucleoli begin to disappear. In late prophase (often called prometaphase) the nuclear membrane is no longer visible.

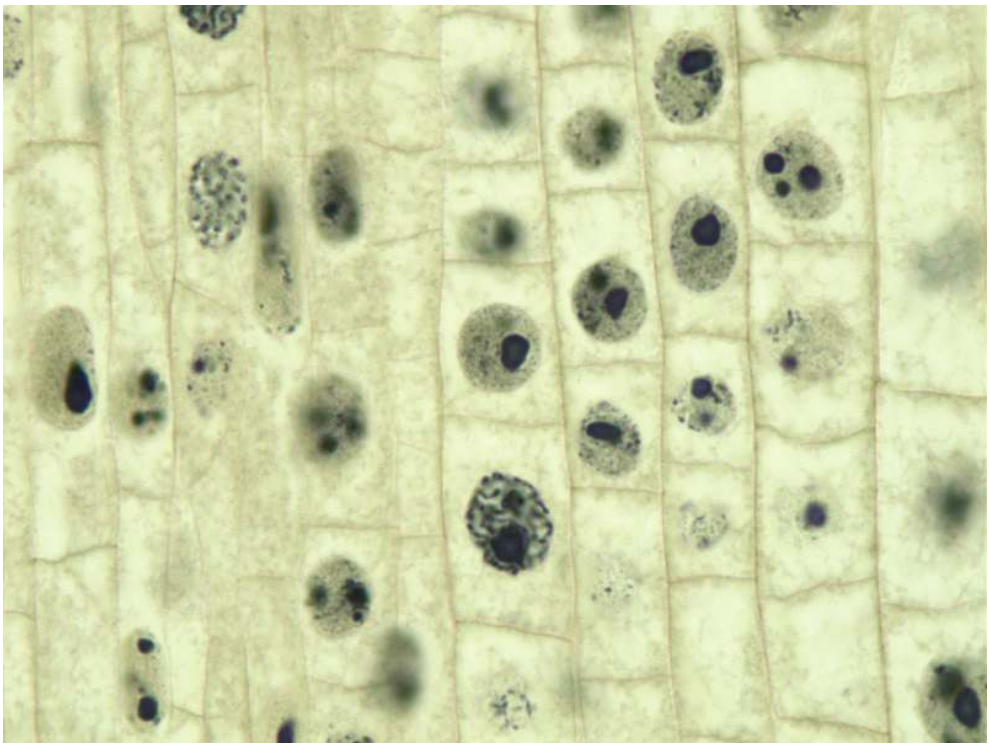


Figure 14.2.2: Prophase in plant cells.

Metaphase

The chromosomes line up in the middle of the cell. Spindle fibers attach to kinetochores at the centromere and extend to the poles of the cell.



Figure 14.2.3: Metaphase in plant cells.

Anaphase

Centromeres split, separating each former chromatid into two individual chromosomes. The chromosomes move toward opposite poles.

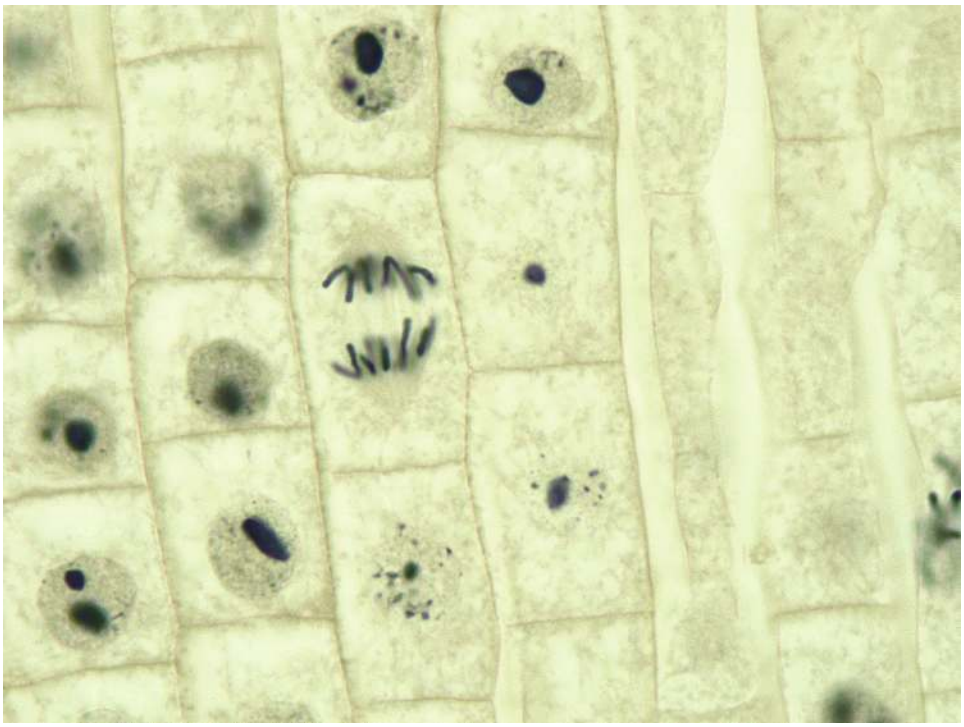


Figure 14.2.4: Anaphase in plant cells.

Telophase and Cytokinesis

Chromosomes reach the poles. The nuclear envelopes begin to reform. The formation of a cell plate forms between the two cells to carry out cytokinesis.

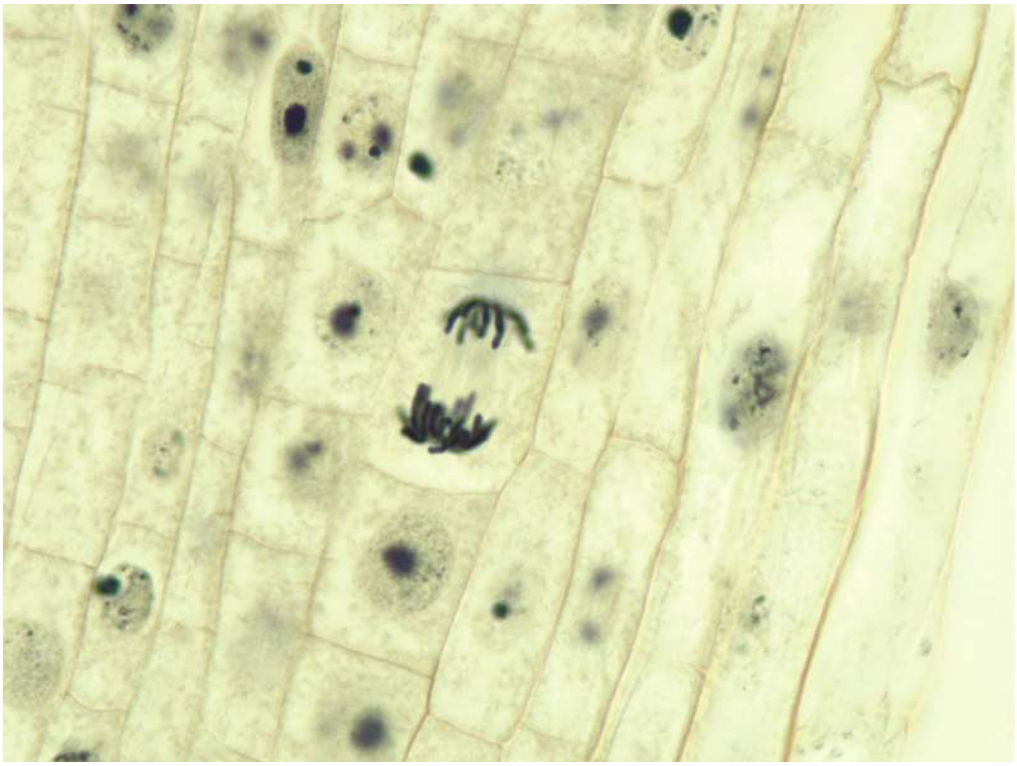


Figure 14.2.5: Telophase in plant cells.

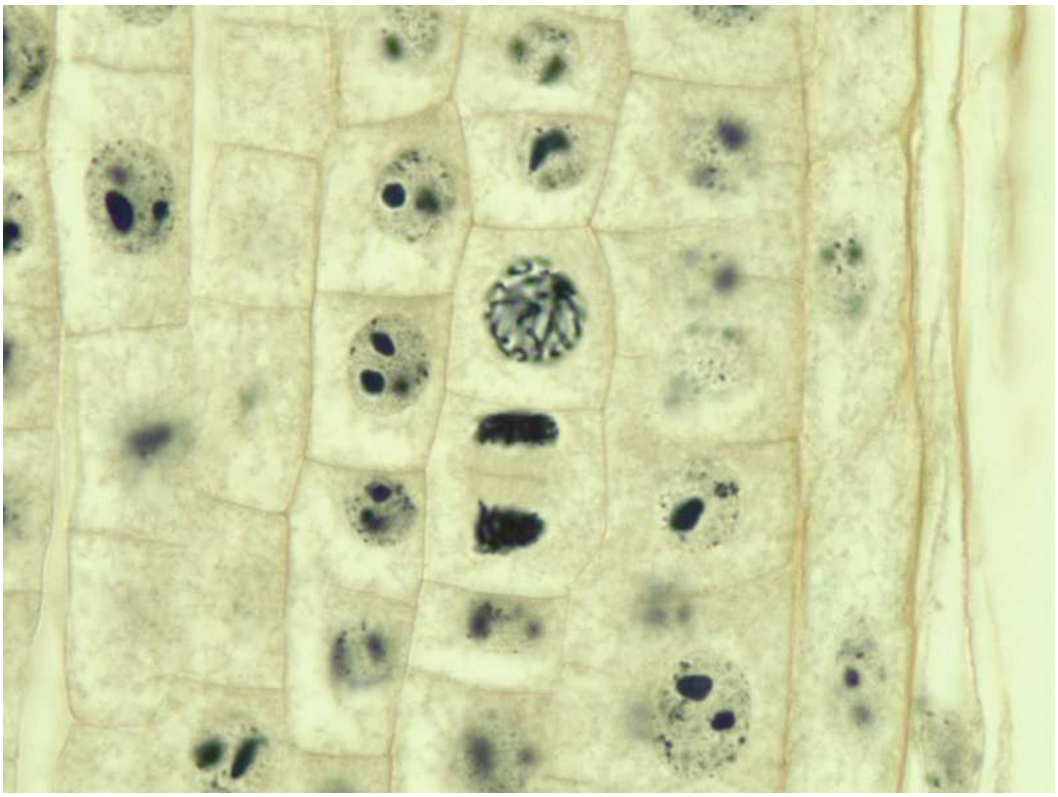


Figure 14.2.6: Cytokinesis in plant cells.

Observe the phases of Mitosis in Animal Cells

Exercise 3: Observing the Phases of Mitosis in the Whitefish Blastula

Materials:

- Prepared slide of whitefish blastula
- Compound light microscope

Procedure:

The blastula is an early embryonic stage where many of the cells are dividing at any one time.

1. Focus on the dividing cells using the 4x scanning objective lens, then switch to the 10x objective and then the 40x objective.
2. Survey the slide to find a cell in each phase of mitosis. Draw a cell for each phase in the boxes provided.

Interphase

The DNA is uncondensed and in the form of chromatin. Individual chromosomes are not visible. The nuclear membrane is intact. The nucleolus is visible.

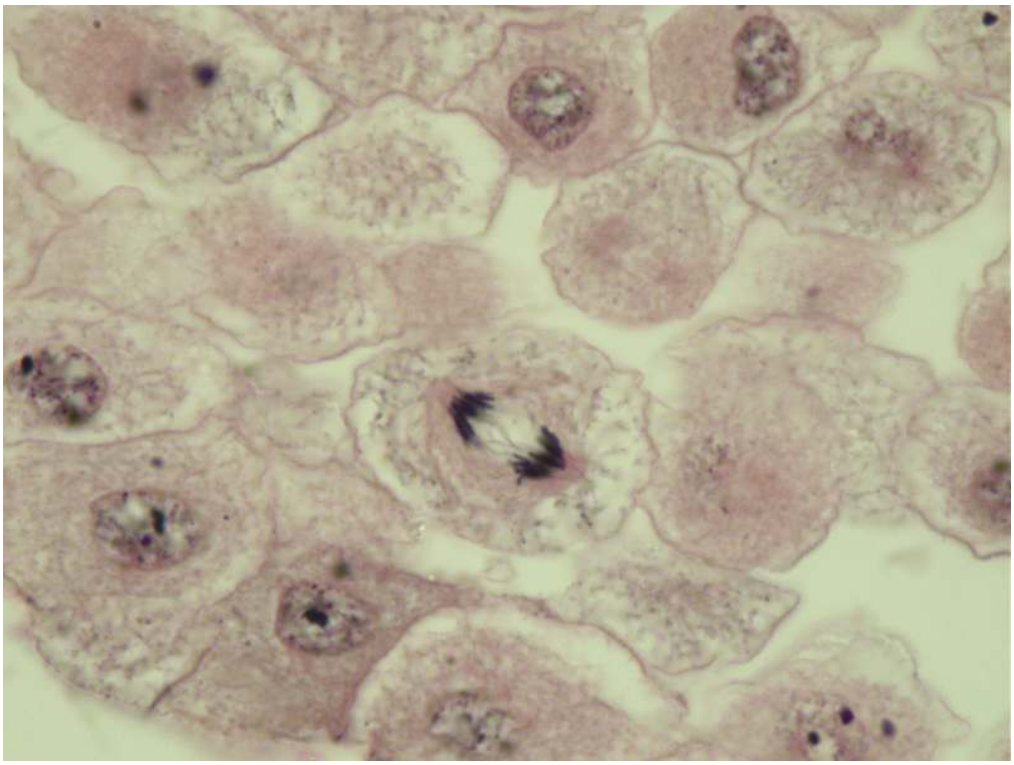


Figure 14.2.7: Interphase in animal cells.

Prophase

Chromatin begins to condense and chromosomes are distinguishable. These “puffy” structures are seen throughout the nucleus. The nucleoli begin to disappear. In late prophase (often called prometaphase) the nuclear membrane is no longer visible.

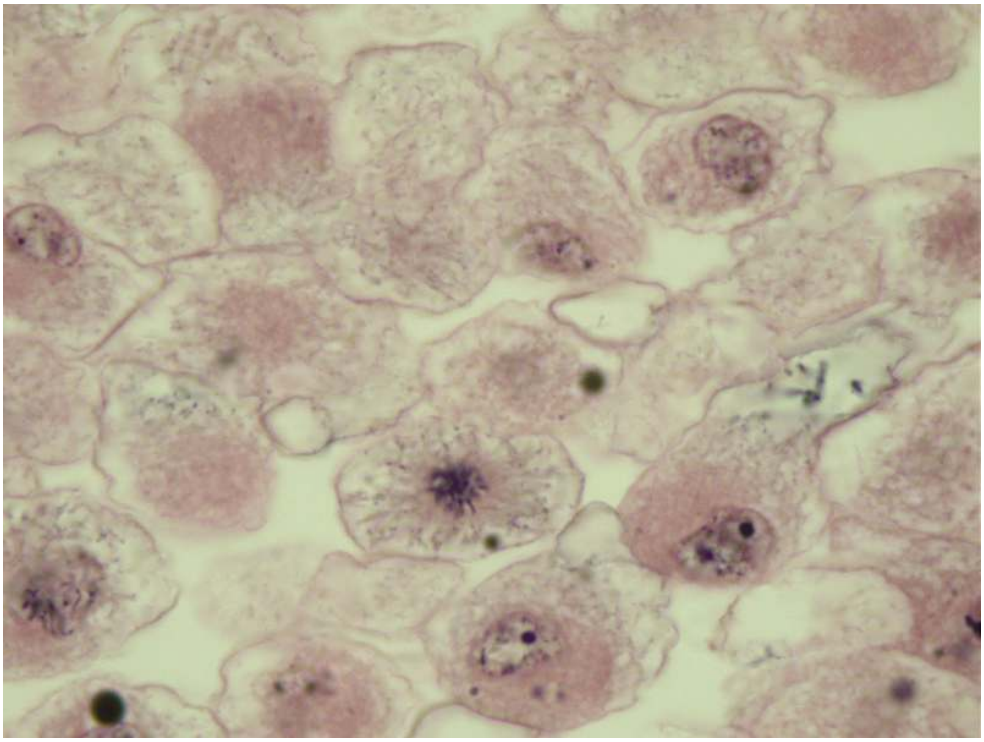


Figure 14.2.8: Prophase in animal cells.

Metaphase

The chromosomes line up in the middle of the cell. Spindle fibers attach to kinetochores at the centromere and extend to the poles of the cell.

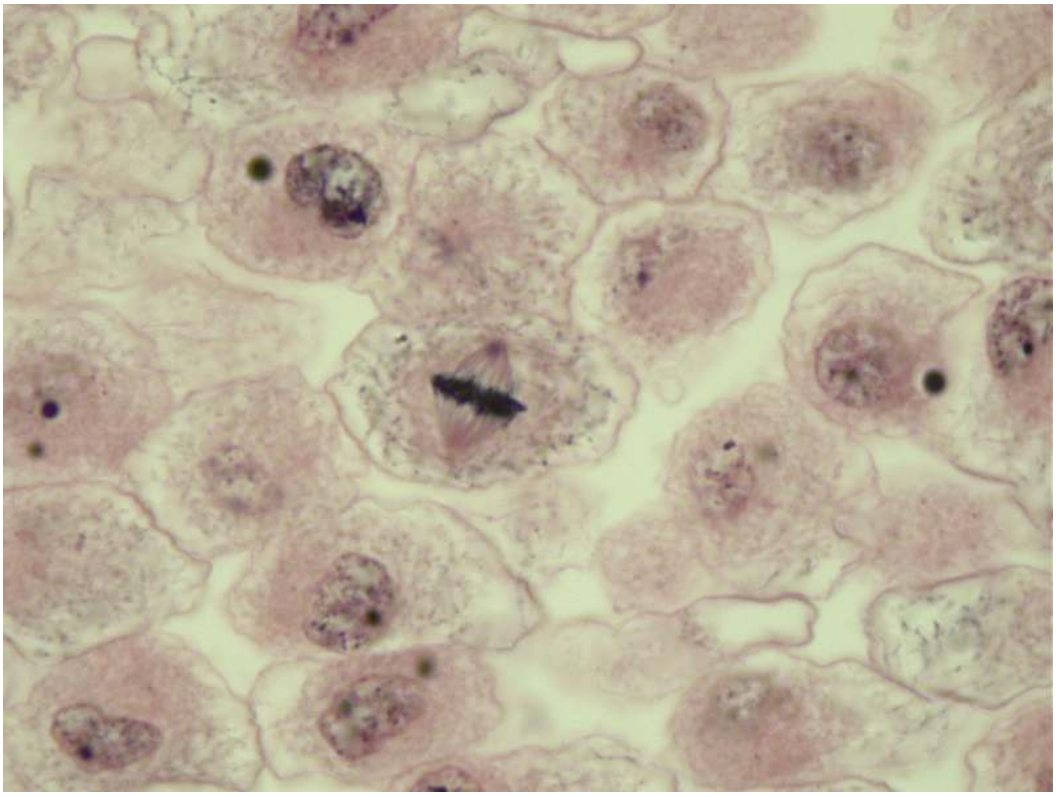


Figure 14.2.9: Metaphase in animal cells.

Anaphase

Centromeres split, separating each former chromatid into two individual chromosomes. The chromosomes move toward opposite poles.

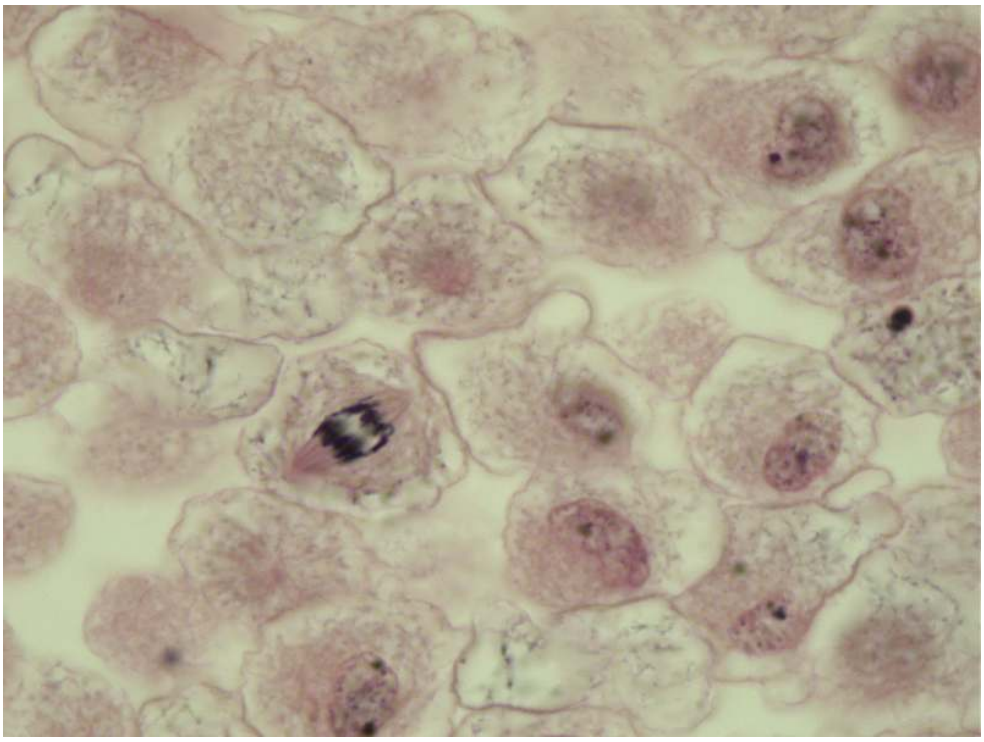


Figure 14.2.10: Anaphase in animal cells.

Telophase and Cytokinesis

Chromosomes reach the poles. The nuclear envelopes begin to reform. A **cleavage furrow** forms between the two cells to carry out cytokinesis.

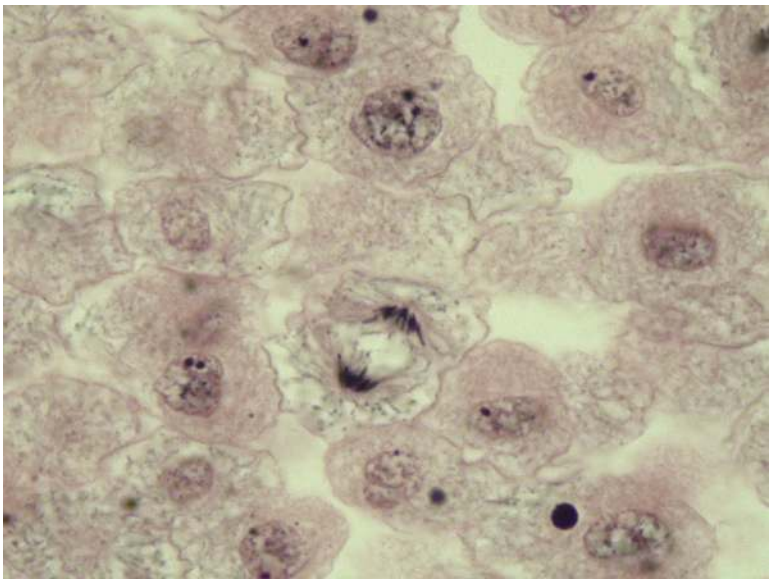


Figure 14.2.11: Telophase in animal cells.

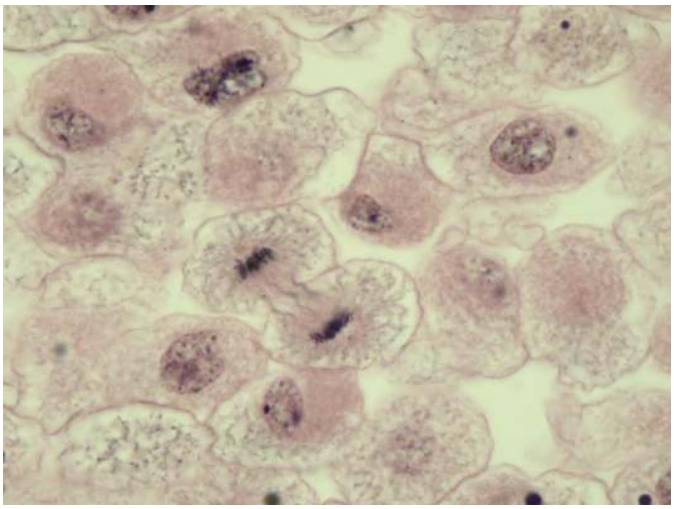


Figure 14.2.12: Cytokinesis in animal cells.

- Why would the method of cytokinesis in animal cells not work in plant cells? Explain.

_____

How Long Does a Cell Spend in Each Phase of the Cell Cycle?

Exercise 4: Determining Time Spent in Different Phases of the Cell Cycle (Optional)

Materials:

- Laptop
- Calculator

Procedure:

1. Obtain a laptop.
2. Open a web browser and go to the following site: **http://www.biology.arizona.edu(opens in new window)**
3. This site will provide an interactive test of your ability to identify the stages of mitosis. It will also allow you to calculate the duration of the stages identified in the laboratory exercise you just completed, but the website will give standard results for the entire class.
4. Go to the **Cell Biology** section, and find the activity "**Online Onion Root Tips**".
5. Keep clicking on "Next" at the bottom of the page until you get to the screen: **Determining Time Spent in Different Phases of the Cell Cycle**
6. Click on "Next" at the bottom of the page.
7. Identify each stage shown to you by the program. When a picture of a cell pops up in a stage of mitosis, simply click on the phase in which the cell belongs. If you make a mistake, read the explanation for why you were mistaken before making a new selection.
8. When you are finished, use the formula given below and record your results in the table.
9. The duration of each stage of mitosis can be determined by using the following formula. Compute the length of time for each stage and place your calculations in the table below.

(Number of cells in a stage ÷ Total number of cells) x 1440 (min in a day) = minutes a cell spends

in each stage in one day

Data Table for Mitosis Stages

Number of cells in each stage	Interphase _____	Prophase _____	Metaphase _____	Anaphase _____	Telophase _____	Total 36
Percent of cells (# of cells / Total)						100%
Time (in minutes) spent in Stage – use calculation above						

References

Belwood, Jacqueline; Rogers, Brandy; and Christian, Jason, Foundations of Biology Lab Manual (Georgia Highlands College). "Lab 10: Mitosis & Meiosis," (2019). *Biological Sciences Open Textbooks*. 18. CC-BY

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14.3: Lab Report

Practical Challenge Questions: Mitosis

1. In the circle below, sketch a 2n=6 diploid cell in metaphase of mitosis. Be sure to label the centromere, centrioles, and spindle fibers.

Questions for Review

1. What is the meaning of diploid? What abbreviation do we use to represent diploid? Name 2 diploid cells in humans.
2. What is the meaning of haploid? What abbreviation do we use to represent haploid? Name 2 haploid cells in humans.
3. In what stage of the cell cycle does S phase occur? Explain why the DNA must be duplicated during the S phase of the cell cycle, prior to mitosis taking place.
4. What specific feature of cytokinesis in animal cells can you use to distinguish this process from cytokinesis in plant cells?

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CHAPTER OVERVIEW

15: Meiosis

Learning Objectives

Content Objectives

- Describe the stages of Meiosis.
- Compare and contrast Meiosis and Mitosis.

Skill Objectives

- Draw and label the stages of Meiosis and identify the important events of each.
- Model the stages of Meiosis using pop-beads to simulate chromosomes.

15.1: Introduction
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15.1: Introduction

Meiosis

Introduction to Meiosis (aka “Reduction Division”)

Meiosis is a special type of cell division in which the daughter cells produced have half the number of chromosomes (n) as their parent cell. In animals, this division occurs in the reproductive organs (gonads -- testes of males or ovaries of females) of species that reproduce sexually, and results in the formation of gametes (eggs or sperm) that contain half the number of chromosomes as the original cell. Sexual reproduction involves the joining of gametes (fertilization) to form a zygote, which then has two copies of each chromosome (2n). Meiosis is a critical process, as it increases genetic diversity within a species.

Cells that divide by meiosis prepare for cellular division (during interphase) much like every other cell. Meiosis progresses through the same phases as mitosis (prophase, anaphase, metaphase, telophase, and cytokinesis). However, unlike mitosis, meiosis involves two rounds of cellular division (meiosis I and meiosis II). Meiosis involves only one round of DNA replication where each chromosome replicates to form sister chromatids. Therefore, when meiosis is completed, each daughter cell contains only half the number (n) of chromosomes as the original cell.

The stages of meiosis:

Meiosis I

- Prophase l
- Metaphase l
- Anaphase l
- Telophase l
- Cytokinesis l

Meiosis II

- Prophase ll
- Metaphase ll
- Anaphase ll
- Telophase ll
- Cytokinesis ll

Prophase I: During prophase of meiosis I, the chromosomes join in homologous pairs. Homologous chromosomes (aka homologs) are the same length, have their centromere in the same position, and carry genetic information (genes) for the same traits, but not necessarily the same versions (alleles) of the gene.

For example, human chromosome #19 contains a gene for eye color. In one person, one allele might code for blue eyes and the other allele codes for green eyes. Since every human inherits two copies of chromosome 19 (one from the mother’s egg and one from the father’s sperm) a person could have 2 blue alleles, 2 green alleles, or one of each.

Paired homologous chromosomes are called tetrads and are said to be in synapsis. During synapsis, equivalent pieces of homologous chromatids are exchanged between the chromosomes. This is called **crossing-over** and can occur several times along the length of the chromosomes. As occurs in the mitotic division, prophase of meiosis I also involves the degradation of the nuclear membrane, disassembly of the nucleolus, and formation of spindle fibers.

Metaphase I: Metaphase of meiosis I occurs when the joined homologous chromosome pairs are moved to the center of the cell by spindle fibers. The fibers arrange the pairs so that homologs are on opposite sides of the metaphase plate (aka equatorial plane).

Anaphase I follows, as homologs are pulled apart, toward opposite poles of the cell. [*Note: this is significantly different from the separation of sister chromatids that occurs during mitosis].

Telophase I marks the end of meiosis I, as new nuclei form and cytokinesis separates the cytoplasm forming two daughter cells.

At the end of meiosis I, the two daughter cells have half the number of chromosomes as did their parent cell. Thus, the cells have been reduced from diploid (2n) to haploid (n). [n refers to the number of chromosomes in a set that are characteristic for a species.

Humans have one set (n) of 23 unique chromosomes (n = 23). A diploid human cell has 2 sets (2n) of 23 unique chromosomes (2n = 46).

*Meiosis II follows meiosis I, which proceeds very much like mitosis.

During **Prophase II**, chromosomes containing two sister chromatids are lined up on the equator of each daughter cell by the spindle fibers. This is completed by the end of **Metaphase II.**

The centromeres separate and sister chromatids are pulled to each pole of the cell during **Anaphase ll.** Cytokinesis II occurs after Telophase II to complete cell division and ultimately the production of four (4) daughter cells (Figure 11). The cells produced (egg or sperm, in humans) are haploid (n rather than 2n) and will either unite (via fertilization) or die. They do not divide further on their own as meiosis is not a cycle.

References

Belwood, Jacqueline; Rogers, Brandy; and Christian, Jason, Foundations of Biology Lab Manual (Georgia Highlands College). "Lab 10: Mitosis & Meiosis," (2019). *Biological Sciences Open Textbooks*. 18. CC-BY

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15.2: Exercise

The Phases of Meiosis

Exercise 1: Modeling the Phases of Meiosis

Materials:

- Chalk (can be used directly on lab bench to draw cellular structures and then washed off)
- Chromosome modeling kits
  - Commercially available pop bead kits (e.g Carolina Biological Supply Company, Item #171100)
- 40 pop beads of one color (red)
- 40 pop beads of another color (yellow)
- 8 magnetic centromeres
- 8 laminated pictures of centrosomes (each consisting of a pair of centrioles)
- Paper towels / Kimwipes
- Fine point sharpie
- Small round stickers

Procedure:

A diploid cell with 2 homologous pairs of chromosomes (as in the previous modeling exercise) will be modeled as it moves through the meiosis. First, you will model meiosis l. Then, you will model meiosis ll as described below.

Model Meiosis l (1 diploid cell → 2 haploid cells)

- tetrads form, crossing over occurs, homologues separate
  - Interphase Before Synthesis of DNA (G1)
  - Interphase After Synthesis of DNA (G2)
  - Prophase l
  - Metaphase l
  - Anaphase l
  - Telophase l
  - Cytokinesis l

Model Meiosis ll (2 haploid cells → 4 haploid cells)

- sister chromatids separate
  - Prophase ll
  - Metaphase ll
  - Anaphase ll
  - Telophase ll
  - Cytokinesis ll

Modeling Meiosis l

1. You will build 2 pairs of homologous chromosomes (2n = 4). The first homologous pair of single chromosomes should be constructed using 12 red beads for one member of the long pair and 12 yellow beads for the other member of the long pair. You may place the magnetic centromere at any position along the chromosome, but it must be at the same position on both members of the homologous pair.
2. The second pair of single chromosomes will represent the X and Y sex chromosomes in a male. The X chromosome should be constructed using 8 red beads. To make manipulation of the chromosomes easier, place the magnetic centromere in the middle of the X chromosome so that you have 4 red beads on one side of the centromere and 4 red beads on the other side. The Y chromosome should be constructed using 2 yellow beads with a magnetic centromere between them.

3. Alternative forms of genes are called alleles. We will use the letter “B” or “b” to represent the gene for eye color. Labeled stickers will be used to represent alleles. With the Sharpie, label one small sticker with a “B” and another small sticker with a “b”. “B” will represent the dominant allele for brown eyes and “b” will represent the recessive allele for blue eyes. You may place the “B” sticker on any of the red beads of the long red chromosome. The “b” sticker must be placed at the same bead position of the “B” sticker, but on the long yellow chromosome.
4. To model the **G1 phase of interphase** (before synthesis of the DNA). On your lab bench, use the chalk to draw the cell membrane, nucleus, and nucleolus. Place a laminated centrosome in the cytoplasm of your cell. Place the 4 assembled chromosomes in the nucleus of your cell drawing. Remember that these represent a mass of chromatin.
5. DNA replication takes place during the S phase of interphase. Model the **S phase of interphase** by assembling a second strand that is identical to each of the 4 single chromosomes. The identical strands are considered sister chromatids, which are held together by their magnetic centromeres. (Note: In a living cell, the centromere is a single unit until it separates in anaphase. Therefore, consider the pair of magnets to be a single centromere.)
6. Model the **G2 phase of interphase** by letting your 4 assembled replicated chromosomes rest in the nucleus of your cell. Duplicate the centrosomes by placing another laminated centrosome in the cytoplasm of your cell drawing. Begin to move them towards opposite poles of the cell drawing.
7. To model the **prophase I** stage of meiosis, leave the chromosomes where they are in your cell drawing. In this stage, the chromatin coils and condenses into chromosomes. Using the paper towel (or Kimwipe) start erasing some of the nuclear membrane on your cell drawing. This will simulate the breakdown of the nuclear membrane. Also erase the nucleolus, as the nucleolus disappears in this stage. Continue to move your centrosomes to opposite sides of the cell. Use the chalk to draw spindle fibers beginning to form and radiating outward toward the chromosomes.
8. **Prophase l** of meiosis has some notable differences compared to prophase of mitosis. To further model **prophase l** of meiosis, arrange the condensed chromosomes so that homologous chromosomes are paired. Put homologous chromosomes next to each other in your cell drawing to simulate this process of **synapsis** (homologs pairing). Homologous pairs of chromosomes paired in this way are called **tetrads**. When tetrads form, the inner non-sister chromatids of the tetrad pair can exchange DNA by a process known as **crossing over**. Pieces of equivalent segments of non-sister chromatids can be exchanged from one non-sister chromatid to the other. Crossing over can occur several times along the length of the chromosomes.

- How many tetrad complexes do you have in your cell, which is 2n = 4?

_____

9. Exchange segments of the inner non-sister chromatids of the red and yellow beads that contain the two different alleles, “B” and “b”. Remember that the red beads represent DNA from the maternal chromatid and the yellow beads represent DNA from the paternal chromatid. You now have one crossover event for this tetrad.
10. **Metaphase l** of meiosis is also notably different than metaphase of mitosis. To model **metaphase l** of meiosis, move your tetrads to the equator, midway between the two centrosomes, which should now be positioned at the two opposite poles of the cell. Draw spindle fibers radiating out from the centrosomes to the homologous pairs (tetrads) which are lined up along the equatorial plane of the cell. Homologs are paired together but the maternal and paternal chromosomes are on opposite sides of the equatorial plate along the middle of the cell. Every pair of chromosomes is arranged independent of another. The side of the equatorial plate where each chromosome is arranged is completely random and independent of the side of the equatorial plate on which other chromosomes are located. Therefore, there are several different arrangements that can occur.
11. In **anaphase l** of meiosis, homologous chromosomes separate toward opposite poles of the cell. **Centromeres do not split as they do in mitosis.** For each homologous pair, move the duplicated maternal chromosome and duplicated paternal chromosome to opposite poles of the cell. Remember that in our model, the two magnets (one of each of the two sister chromatids) represent one centromere. Erase the spindle fibers and redraw them shorter and shorter as each homologous pair moves away from one another towards opposite poles of the cell.

- How does the structure of chromosomes in anaphase I of meiosis differ from that in anaphase of mitosis?

_____

12. Anaphase ends and **telophase l** begins when chromosomes reach opposite poles of the cell. Arrange the chromosomes in groups at opposite ends of the cell. You should have one long chromosome of 12 beads and either the X chromosome or the Y chromosome at each pole. Nuclear division happens in telophase. The formation of separate nuclear envelopes divide the nuclei and mark the end of telophase.
13. Model **cytokinesis l** by drawing the formation of a cleavage furrow to divide the cytoplasm into two and form two separate cells. Redraw the nuclear membrane around the chromosomes and draw a nucleolus inside of each nucleus. These two cells will now enter meiosis ll. Note that each daughter cell has half the number of chromosomes as the parental cell. Thus, the cells have been reduced from **diploid (2n)** to **haploid (n)**. **[n** refers to the **number of *pairs* of chromosomes that are characteristic for a species**. Humans have a "n" of 23, so a diploid human cell has 2(23), or 46 chromosomes].

Modeling Meiosis ll

1. There is no DNA replication before the second cell division stage of meiosis. The stages of meiosis ll proceed very much like mitosis. The two cells created in meiosis l will enter into **prophase ll**. The chromosomes in each cell contain two sister chromatids, which are condensed and distributed throughout the nucleus. Add another laminated centrosome. There should be two centrosomes in each new cell. In your drawing be sure to note that the nuclear envelope begins to break down, the nucleolus disappears, the centrosomes move towards opposite poles, and spindle fibers begin to form and radiate toward the chromosomes. Spindle fibers attach to **kinetochore** proteins at the centromeres of the chromosomes.
2. To model **metaphase ll**, line up the individual chromosomes on the equator (middle) of each cell. Sister chromatids remain attached at the centromere during metaphase ll.
3. Model **anaphase ll** by pulling the two magnetic centromeres of each duplicated chromosome apart. The sister chromatids should be separated and moved toward opposite poles of each cell. After separation at the centromere, the chromatids are now called chromosomes. In anaphase II single chromosomes move towards opposite poles.
4. Anaphase ll ends and **telophase ll** begins when the chromosomes reach opposite poles of the cells. Nuclear division happens in telophase. The spindle fibers disassemble. Nuclear envelopes and nucleoli reappear. Condensed chromosomes begin to decondense and uncoil. The formation of separate nuclear envelopes divide the nuclei and marks the end of telophase.
5. Model **cytokinesis ll** by drawing the formation of a cleavage furrow to divide the cytoplasm of each cell into two separate cells.

- What is the total number of nuclei and cells now present? _____
- How many cells were present when meiosis began? _____
- How many chromosomes were present in the original parental cell? _____
- How many chromosomes are in each new daughter cell? _____
- Are the chromosomes in the new daughter cells identical to the chromosomes in the original parental cell? Explain your results in terms of independent assortment and crossing over. _____

Questions for Review

1. What is the meaning of diploid? What abbreviation do we use to represent diploid? Name 2 diploid cells in humans.
2. What is the meaning of haploid? What abbreviation do we use to represent haploid? Name 2 haploid cells in humans.
3. In what stage of the cell cycle does S phase occur? Explain why the DNA must be duplicated during the S phase of the cell cycle, prior to mitosis taking place.
4. What specific feature of cytokinesis in animal cells can you use to distinguish this process from cytokinesis in plant cells?

Practical Challenge Questions

1. In the circle below, sketch a 2n=6 haploid cell in metaphase l of meiosis.
2. A monogenic gene gives rise to a trait from a single set of alleles. A polygenic gene gives rise to a trait from several sets of alleles. Give an example of a monogenic and polygenic trait.

References

Belwood, Jacqueline; Rogers, Brandy; and Christian, Jason, Foundations of Biology Lab Manual (Georgia Highlands College). "Lab 10: Mitosis & Meiosis," (2019). *Biological Sciences Open Textbooks*. 18. CC-BY

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15.3: Lab Report

- How many tetrad complexes do you have in your cell, which is 2n = 4?

  _____

- How does the structure of chromosomes in anaphase I of meiosis differ from that in anaphase of mitosis?

  _____

- What is the total number of nuclei and cells now present? _____
- How many cells were present when meiosis began? _____
- How many chromosomes were present in the original parental cell? _____
- How many chromosomes are in each new daughter cell? _____
- Are the chromosomes in the new daughter cells identical to the chromosomes in the original parental cell? Explain your results in terms of independent assortment and crossing over. _____

1. What is the meaning of diploid? What abbreviation do we use to represent diploid? Name 2 diploid cells in humans.
2. What is the meaning of haploid? What abbreviation do we use to represent haploid? Name 2 haploid cells in humans.
3. In what stage of the cell cycle does S phase occur? Explain why the DNA must be duplicated during the S phase of the cell cycle, prior to mitosis taking place.
4. What specific feature of cytokinesis in animal cells can you use to distinguish this process from cytokinesis in plant cells?

1. In the circle below, sketch a 2n=6 haploid cell in metaphase l of meiosis.
2. A monogenic gene gives rise to a trait from a single set of alleles. A polygenic gene gives rise to a trait from several sets of alleles. Give an example of a monogenic and polygenic trait.

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CHAPTER OVERVIEW

16: Genetics

Learning Objectives

Content Objectives

- Identify and distinguish the different genetic terminology of Mendelian inheritance.
- Give examples of **monohybrid** and **dihybrid** crosses.

Skill Objectives

- Practice identifying and combining **dominant** and **recessive** alleles.
- Examine the allele frequency of the class for selected genetic traits.
- Complete Punnett squares for **monohybrid** and **dihybrid** crosses.

16.1: Introduction
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16.1: Introduction

Introduction



Figure 16.1.1: Experimenting with thousands of garden peas, Mendel uncovered the fundamentals of genetics. (credit: modification of work by Jerry Kirkhart)

Genetics is the study of heredity. Johann Gregor Mendel set the framework for genetics long before chromosomes or genes had been identified, at a time when meiosis was not well understood. Mendel selected a simple biological system and conducted methodical, quantitative analyses using large sample sizes. Because of Mendel's work, the fundamental principles of heredity were revealed. We now know that genes, carried on chromosomes, are the basic functional units of heredity with the ability to be replicated, expressed, or mutated. Today, the postulates put forth by Mendel form the basis of classical, or Mendelian, genetics. Not all genes are transmitted from parents to offspring according to Mendelian genetics, but Mendel's experiments serve as an excellent starting point for thinking about inheritance.

Mendel's Experiments



Figure 16.1.2: Johann Gregor Mendel set the framework for the study of genetics.

Johann Gregor Mendel (1822–1884) (**Figure** 16.1.2) was a lifelong learner, teacher, scientist, and man of faith. As a young adult, he joined the Augustinian Abbey of St. Thomas in Brno in what is now the Czech Republic. Supported by the monastery, he taught physics, botany, and natural science courses at the secondary and university levels. In 1856, he began a decade-long research pursuit involving inheritance patterns in honeybees and plants, ultimately settling on pea plants as his primary **model system** (a system with convenient characteristics that is used to study a specific biological phenomenon to gain understanding to be applied to other systems). In 1865, Mendel presented the results of his experiments with nearly 30,000 pea plants to the local natural history society. He demonstrated that traits are transmitted faithfully from parents to offspring in specific patterns. In 1866, he published his work, *Experiments in Plant Hybridization*, in the proceedings of the Natural History Society of Brünn.

Mendel's work went virtually unnoticed by the scientific community, which incorrectly believed that the process of inheritance involved a blending of parental traits that produced an intermediate physical appearance in offspring. This hypothetical process appeared to be correct because of what we know now as continuous variation. **Continuous variation** is the range of small differences we see among individuals in a characteristic like human height. It does appear that offspring are a "blend" of their parents' traits when we look at characteristics that exhibit continuous variation. Mendel worked instead with traits that show **discontinuous variation**. Discontinuous variation is the variation seen among individuals when each individual shows one of two —or a very few—easily distinguishable traits, such as violet or white flowers. Mendel's choice of these kinds of traits allowed him

to see experimentally that the traits were not blended in the offspring as would have been expected at the time, but that they were inherited as distinct traits. In 1868, Mendel became abbot of the monastery and exchanged his scientific pursuits for his pastoral duties. He was not recognized for his extraordinary scientific contributions during his lifetime; in fact, it was not until 1900 that his work was rediscovered, reproduced, and revitalized by scientists on the brink of discovering the chromosomal basis of heredity.

Mendel's Crosses

Mendel's seminal work was accomplished using the garden pea, *Pisum sativum*, to study inheritance.

This species naturally self-fertilizes, meaning that pollen encounters ova within the same flower. The flower petals remain sealed tightly until pollination is completed to prevent the pollination of other plants. The result is highly inbred, or "true-breeding," pea plants. These are plants that always produce offspring that look like the parent. By experimenting with true-breeding pea plants, Mendel avoided the appearance of unexpected traits in offspring that might occur if the plants were not true breeding. The garden pea also grows to maturity within one season, meaning that several generations could be evaluated over a relatively short time. Finally, large quantities of garden peas could be cultivated simultaneously, allowing Mendel to conclude that his results did not come about simply by chance.

Mendel performed **hybridizations**, which involve mating two true-breeding individuals that have different traits. In the pea, which is naturally self-pollinating, this is done by manually transferring pollen from the anther of a mature pea plant of one variety to the stigma of a separate mature pea plant of the second variety.

Plants used in first-generation crosses were called **P**, or parental generation, plants (**Figure** 16.1.3). Mendel collected the seeds produced by the P plants that resulted from each cross and grew them the following season. These offspring were called the **F1**, or the first filial (filial = daughter or son), generation. Once Mendel examined the characteristics in the F1 generation of plants, he allowed them to self-fertilize naturally. He then collected and grew the seeds from the F1 plants to produce the **F2**, or second filial, generation. Mendel's experiments extended beyond the F2 generation to the F3 generation, F4 generation, and so on, but it was the ratio of characteristics in the P, F1, and F2 generations that were the most intriguing and became the basis of Mendel's postulates.

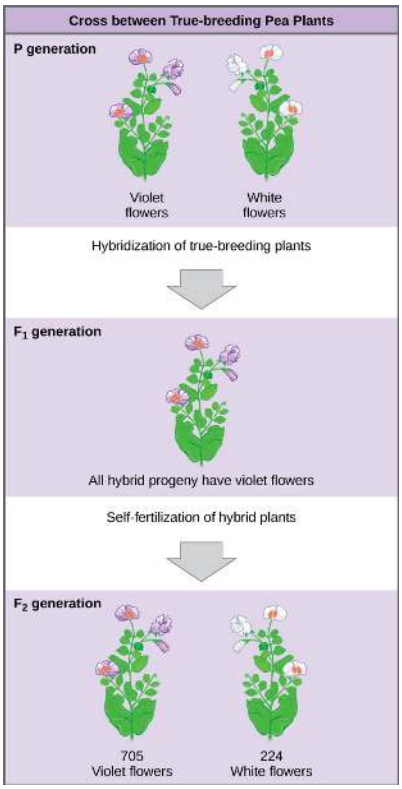


Figure 16.1.3: Mendel's process for performing crosses included examining flower color.

References

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OpenStax, Biology. OpenStax CNX. May 27, 2016 http://cnx.org/contents/GFy_h8cu@10....nts-and-the-La

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16.2: Exercise

Introduction to Mendelian Genetics - Part 1

Genes occur in pairs due to the diploid nature of human somatic cells, with one gene on each homologous chromosome. The DNA sequence of each gene in this pair may be identical or different in sequence. These possible forms are called **alleles**. When a person has two identical alleles, they are **homozygous** (i.e., AA or aa). When a person has two different alleles, they are **heterozygous** (i.e., Aa). Our genetic makeup, or **genotype**, is what determines our physical characteristics, the **phenotype**.

In some cases, one allele masks the expression of the other allele. This allele is called the **dominant** allele, and is expressed an observed in the phenotype. The allele that is masked and not observed is known as the **recessive** allele. Dominant traits are represented by capital letters, such as 'A', whereas recessive traits are represented by lowercase letters, such as 'a'.

1. Use the letter 'A' to represent the following:
   - Homozygous dominant organism: _____ Color: _____
   - Homozygous recessive organism: _____ Color: _____
   - Heterozygous organism: _____ Color: _____

2. If the letter 'A' represents flower color, where 'A' represents red flowers and 'a' represents white flowers, what color would each flower be? Fill-in the blanks above.

In somatic cells, both alleles are present in each diploid cell. However, during the formation of haploid gametes, the alleles for each trait separate so that each gamete only contains one allele for each particular trait. This is known as Mendel's **law of segregation**, which contributes to the haploid nature of our gametes, the sperm and egg. Additionally, Mendel's law of **independent assortment** tells us that each trait is inherited independently of every other trait.

If we know the genotypes of both parents, we can calculate the expected genotypes and phenotypes of their offspring. **Punnett squares** allow us to calculate expected results.

3. Use Punnett Squares to calculate the expected genotypes and phenotypes of the offspring from the following crosses:

AA x aa AA x Aa

Aa x Aa (monohybrid cross) **Aa x aa**

Traits are not always dominant or recessive, however. Some traits exhibit **incomplete dominance**, where the heterozygous genotype has an intermediate phenotype. For example, in certain flowers like the snapdragon flower, a heterozygous condition results in pink flowers.

4. Use Punnett Squares to calculate the expected genotypes and phenotypes of the offspring from the following crosses, if trait 'A' exhibits incomplete dominance:

AA x aa AA x Aa

Aa x Aa Aa x aa

A condition known as codominance also occurs, where both alleles in a heterozygous individual are expressed equally. The most common example of codominance are the ABO blood groups, where a person with type AB blood expresses both A and B alleles equally, and O is recessive to both the A and B alleles. (Note: The alleles are simplified here. We will see them written as I^A, I^B, and i in lecture.)

5. Use Punnett Squares to calculate the expected genotypes and phenotypes of the offspring from the following monohybrid crosses, if traits 'A' and 'B' exhibit codominance:

AO x BO AO x OO

AA x BO BB x OO

Working with your lab partners, determine your phenotypes for the traits discussed in the next section. These traits are based upon two alleles and follow the normal dominance rules of Mendelian inheritance. Record your phenotype and possible genotype(s) in

the table below. Once each group has completed their data collection, we will combine our class results and place it on a chart on the board.

Traits:

1. Bent little finger – bent is dominant “C”, straight is recessive “c” (clinodactyly)
2. Tongue rolling – tongue rolling is “R”, non-tongue rolling is “r”
3. Widow’s peak – V-shaped forehead hairline is “W”, straight forehead hairline is “w”
4. Free earlobe – free earlobes are “E”, attached earlobes are “e”
5. Finger hair – middle segment hair is “H”, no hair is “h”
6. Dimpled cheeks – dimples on one or both cheeks is “D”, dimple absence is “d”
7. Eyebrow raising – ability to raise eyebrows is “Y”, inability is “y”
8. Ear wiggling – ability to wiggle ears is “G”, inability is “g”
9. Long toe – if your second toe is longer than your big toe = “L”, a shorter second toe is “l”
10. Curly hair – curly hair is “A”, straight hair is “a”
11. Freckles – presence of freckles is “Z”, no freckles is “z”
12. PTC tasting – ability to taste is “P”, inability is “p” (phenylthiocarbamide)

Data Table for Student Genotypes and Phenotypes

Trait	Your Phenotype	Your Genotype(s)	Possible	# Students Dominant Phenotype	# Students Recessive Phenotype	Class % Dominant Phenotype	Class % Recessive Phenotype
Bent little finger							
Tongue rolling							
Widow’s peak							
Free earlobe							
Finger hair							
Dimpled cheeks							
Eyebrow raising							
Ear wiggling							
Long toe							
Curly hair							
Freckles							
PTC tasting							

Number of students in our class: _____

Introduction to Mendelian Genetics Part 2 – Dihybrid Cross

Dihybrid crosses involve analyzing possible combinations of two traits, ‘A’ and ‘B’.

In the problem below, the letter ‘A’ represents flower color, where ‘A’ represents red flowers and ‘a’ represents white flowers. The letter ‘B’ represents stem height, where ‘B’ represents long stems and ‘b’ represents short stems.

1. What are the possible combinations of alleles that may be found in the gametes of an organism that is heterozygous for both traits, ‘AaBb’? Remember that the gametes must have one allele for each trait:

   _____

2. Use a Punnett square to calculate the expected genotypes and phenotypes of the offspring from the following dihybrid cross: AaBb x AaBb

Data Table for Dihybrid Cross

Gametes:				

Expected genotypes and ratio: _____

Expected phenotype and ratio: _____

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16.3: Lab Report

Introduction to Mendelian Genetics - Part 1

1. Use the letter 'A' to represent the following:
   - Homozygous dominant organism: _____ Color: _____
   - Homozygous recessive organism: _____ Color: _____
   - Heterozygous organism: _____ Color: _____

2. If the letter 'A' represents flower color, where 'A' represents red flowers and 'a' represents white flowers, what color would each flower be? Fill-in the blanks above.

3. Use Punnett Squares to calculate the expected genotypes and phenotypes of the offspring from the following crosses:

AA x aa AA x Aa

Aa x Aa (monohybrid cross) **Aa x aa**

4. Use Punnett Squares to calculate the expected genotypes and phenotypes of the offspring from the following crosses, if trait 'A' exhibits incomplete dominance:

AA x aa AA x Aa

Aa x Aa Aa x aa

5. Use Punnett Squares to calculate the expected genotypes and phenotypes of the offspring from the following monohybrid crosses, if traits 'A' and 'B' exhibit codominance:

AO x BO AO x OO

AA x BO BB x OO

Traits:

1. Bent little finger – bent is dominant "C", straight is recessive "c" (clinodactyly)
2. Tongue rolling – tongue rolling is "R", non-tongue rolling is "r"
3. Widow's peak – V-shaped forehead hairline is "W", straight forehead hairline is "w"
4. Free earlobe – free earlobes are "E", attached earlobes are "e"
5. Finger hair – middle segment hair is "H", no hair is "h"
6. Dimpled cheeks – dimples on one or both cheeks is "D", dimple absence is "d"
7. Eyebrow raising – ability to raise eyebrows is "Y", inability is "y"
8. Ear wiggling – ability to wiggle ears is "G", inability is "g"
9. Long toe – if your second toe is longer than your big toe = "L", a shorter second toe is "l"
10. Curly hair – curly hair is "A", straight hair is "a"
11. Freckles – presence of freckles is "Z", no freckles is "z"
12. PTC tasting – ability to taste is "P", inability is "p" (phenylthiocarbamide)

Data Table for Student Genotypes and Phenotypes

Trait	Your Phenotype	Your Genotype(s)	Possible	# Students Dominant Phenotype	# Students Recessive Phenotype	Class % Dominant Phenotype	Class % Recessive Phenotype
Bent little finger							
Tongue rolling							
Widow's peak							
Free earlobe							
Finger hair							
Dimpled cheeks							
Eyebrow raising							
Ear wiggling							
Long toe							
Curly hair							
Freckles							
PTC tasting							

Number of students in our class: _____

Introduction to Mendelian Genetics Part 2 – Dihybrid Cross

1. What are the possible combinations of alleles that may be found in the gametes of an organism that is heterozygous for both traits, 'AaBb'? Remember that the gametes must have one allele for each trait:
   _____
2. Use a Punnett square to calculate the expected genotypes and phenotypes of the offspring from the following dihybrid cross: AaBb x AaBb

Data Table for Dihybrid Cross

Gametes:				

Expected genotypes and ratio: _____

Expected phenotype and ratio: _____

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CHAPTER OVERVIEW

17: DNA and Chromosomes

Learning Objectives

Content Objectives

- Compare and contrast DNA, mRNA, and protein.
- List and describe the steps of transcription and translation.

Skill Objectives

- Transcribe DNA to mRNA and use the genetic code to identify the corresponding tRNA anticodons and amino acids.
- Use a sequence of amino acids to derive potential mRNA and DNA sequences corresponding to the polypeptide.

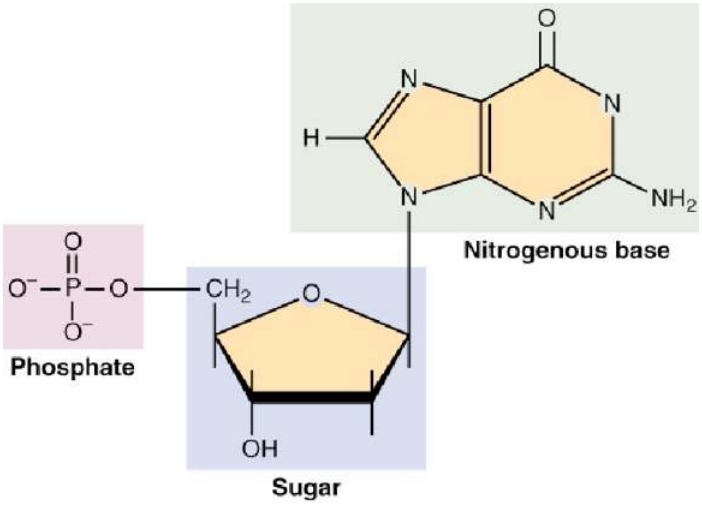
17.1: Introduction
17.2: Exercise
17.3: Lab Report

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17.1: Introduction

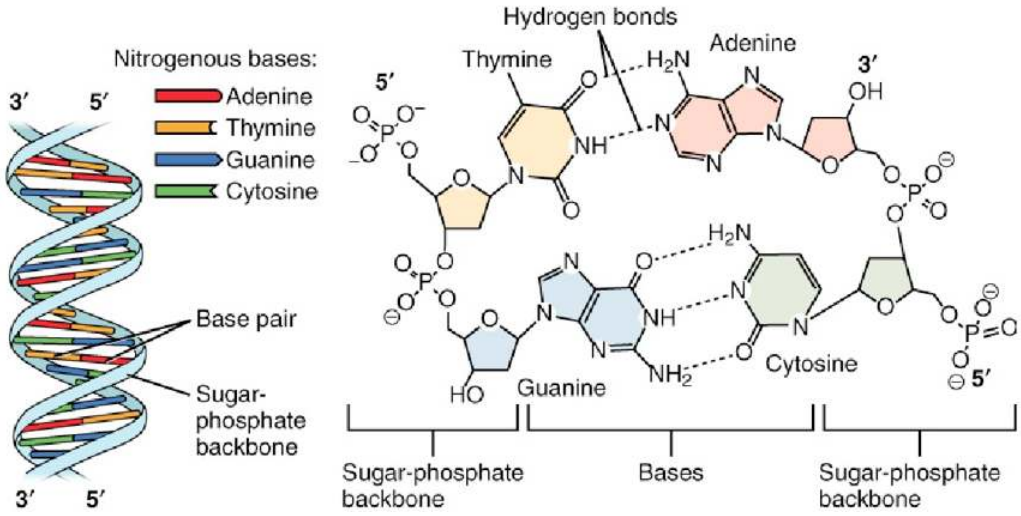
Introduction:

Our genetic information is coded within the macromolecule known as deoxyribonucleic acid (DNA). DNA belongs to a class of organic molecules called **nucleic acids**. The building block, or monomer, of all nucleic acids is a structure called a nucleotide. A nucleotide has three parts: **phosphate**, **deoxyribose sugar**, and a **nitrogen base**.



There are four different nucleotides that make up a DNA molecule, each differing only in the type of nitrogenous base. These include **adenine** (A), **thymine** (T), **cytosine** (C), and **guanine** (G), often indicated by their first letters only.

James Watson and Francis Crick discovered the three-dimensional shape of DNA in the early 1950s. The shape, which they described as a double helix, has the shape of a twisted ladder.



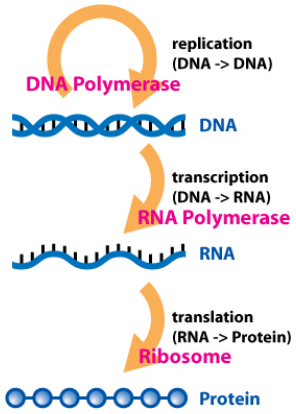
The Genetic Code:

Think of the four nucleotides that make up DNA as the letters of an alphabet. To spell out a word (in this case an amino acid) three "letters" from our alphabet are required. Since only about 20 amino acids make up all the proteins, having a four-letter alphabet is more than sufficient to spell out the 20 "words" (see the calculations that follow). The genetic code is universal (almost) for all living things. What this means is that the triplet code spells the same amino acid in different organisms, from dolphins to plants to bacteria!

Sequence of Nucleotides	# Amino Acids Coded
one	4^1 = 4 (not enough)
two	4^2 =16 (not enough)
three	**4^3 =64 (more than enough)**

The Gene Concept:

Think of a gene as a segment of DNA on a chromosome that codes for a series of amino acids that when linked together makes up what is known as a **polypeptide**. Polypeptides are then folded into complex three-dimensional shapes that become functional proteins.



The Central Dogma:

All organisms use the same fundamental mechanism for gene expression.

DNA → RNA → Polypeptide → Protein

Protein Synthesis:

Protein synthesis is a two-step process.

DNA —(transcription)→ RNA —(translation)→ Polypeptide

Transcription happens when the information from the DNA template is transcribed onto another form of nucleic acid known as ribonucleic acid or RNA (actually messenger RNA).

Translation happens when the information from the language of nucleic acid is translated into the language of proteins.

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17.2: Exercise

Part 1: DNA to Protein Exercise

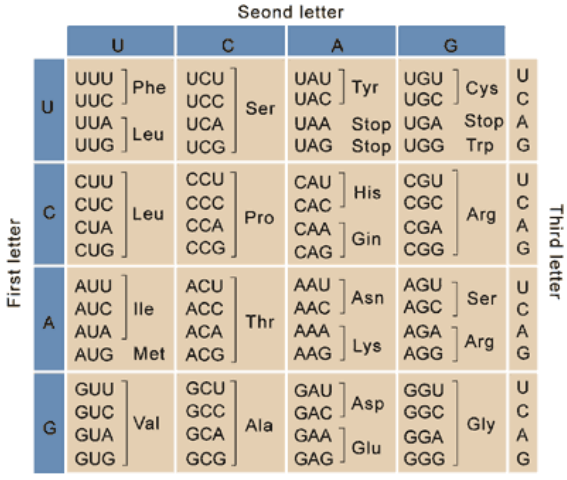
The following DNA sequence is part of the gene that controls dimples. Decode the DNA message into mRNA, tRNA and finally amino acids. Use the genetic code chart to fill in the table below.

> **Note**
> The genetic code is based on mRNA (not DNA or tRNA). When you have finished this, you will be able to determine the phenotype of the person the DNA came from. (If arginine is the 3rd amino acid, the person will have dimples.)

Data Table for Codons, Anticodons, and Amino Acids

DNA	mRNA Codon	tRNA Anticodon	Amino Acid
C G A			
G T C			
G C A			
T A A			

1. Does the person with the sequence above have dimples?
2. What two great tasks are carried out by our genetic machinery?
3. What name do we call a three-nucleotide sequences of mRNA?
4. How many DNA bases does it take to code for an RNA codon?
5. How many amino acids does an RNA codon code for?
6. What brings amino acids to the ribosome?
7. What is the difference between transcription and translation?
8. True or false: Most of the DNA in the human genome codes for proteins.



Part 2: Protein Synthesis Exercise

DNA: 3′ A G C C G T A G A A T T 5′

1. Using this strand of DNA as a template, **draw a picture** of the complete DNA molecule. Include **all** parts of the DNA molecule. You do not need to draw your molecule with atomic accuracy.
2. Now draw a complete picture of the mRNA strand that will be made from this DNA. *Label* the 5′ and 3′ ends of your mRNA strand. (Use the given DNA strand at the top of this page as your template . . .)
3. Carefully indicate the codons present in the mRNA strand from question 2.

4. Draw a **complete** picture of all the tRNA molecules that will match up with the codons from the previous question. Include **all appropriate** amino acids in your picture, and do not mix up their order!

5. Draw a picture of the completed protein coded for by this strand of DNA (abbreviations are fine). Show the amino acids in the same order they would be observed in the finished protein.

Part 3: Protein Synthesis Bingo

Fill in the boxes with 16 of the 20 amino acids. Every bingo square will be unique. Then listen as random nucleotide sequences are pulled from the hat. Listen carefully to what kind of sequence is called! Use the mRNA codon chart on the previous page to determine the amino acid associated with each sequence. (**Printable version here.**)

Table of Amino Acids

alanine—ala—A	cysteine—cys—C	histidine—his—H	methionine—met—M	threonine—thr—T
arginine—arg—R	glutamine—gln—Q	isoleucine—ile—I	phenylalanine—phe—F	tryptophan—trp—W
asparagine—asn—N	glutamic acid—glu—E	leucine—leu—L	proline—pro—P	tyrosine—tyr—Y
aspartic acid—asp—D	glycine—gly—G	lysine—lys—K	serine—ser—S	valine—val—V

Data Table for Codons and AAs

	Sequence Called	DNA? mRNA? tRNA?	Codon	AA
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				
11				
12				
13				
14				
15				
16				
17				
18				

Part 4: Wheat Germ Extraction

Wheat germ is the sprouting embryo contained within a wheat kernel (the wheat seed). The remainder of the wheat kernel is called the endosperm, and is the food storage site for the developing embryo. Our task today is to break down the cells within the wheat germ and remove the DNA.

Materials:

- Raw, Untoasted Wheat Germ (2 g.)
- Adolph's Natural Meat Tenderizer (unseasoned)
- Liquid Dishwashing Detergent (Palmolive) (3 mL.)
- 1M Sodium Bicarbonate—$NaHCO_3$ (5 mL.)
- Ice Cold 95% Ethanol (20 mL.)
- Tap Water
- Water Bath (55° C)
- 250 mL. Beaker
- Thermometer
- Graduated Cylinder (10 mL.)
- Serological Pipette, 10 mL.
- Glass Stirring Rod
- Glass DNA Hook
- Ice Bath

Procedure:

1. Measure 45 mL. of tap water into your beaker using the graduated cylinder, and place it in the warm water bath (55°C). Allow it a few minutes to warm up. Do **not** allow the temperature of the bath to exceed 60° C!
2. Sprinkle in 2 g of wheat germ into the beaker and gently stir in 3 mL. of detergent. Incubate this mixture in the warm water bath for 5 minutes.
    1. Detergents dissolve lipids and proteins that form the cell membranes found in the wheat germ by disrupting the chemical bonds that hold the membrane together. This releases the cell's contents, including the DNA held within the nucleus, into the solution.
    2. The warm water bath denatures enzymes that might otherwise damage the DNA, and it also helps the detergent work more effectively. If your water bath is too hot, then your DNA will become damaged.
3. After 5 minutes, gently stir in 2 g of meat tenderizer and 5 mL. of the 1M sodium bicarbonate solution. Incubate this mixture at 55° C for an additional 15–20 minutes.
    1. Eventually, even at 55° C, the DNA would be damaged, so this additional incubation period must not exceed 15–20 minutes.
    2. The sodium bicarbonate acts as a buffer that maintains a near-neutral pH in the solution. This ensures the DNA remains stable, and it also enables the enzyme found in the meat tenderizer to be most effective.
    3. The meat tenderizer contains a proteolytic enzyme that degrades the proteins found in the nuclear membrane, ultimately freeing the DNA into solution.
4. Transfer the beaker containing the wheat germ mixture to an ice bath for a few minutes to quickly cool it to room temperature. Gently stir during this time.
    1. The ice bath cools down the mixture so that the DNA is not damaged by the heat!
5. Using the serological pipet, carefully layer 10 mL of ice-cold alcohol over the wheat germ solution in the beaker. Allow the alcohol to flow from the pipet with the pipet tip held against the inside surface of the beaker, just above the liquid level. If the DNA does not appear, repeat this step.
    1. When the dissolved DNA makes contact with the very cold alcohol, the alcohol effectively dehydrates the DNA and it precipitates from the solution. This is because DNA is insoluble in the alcohol (and this is especially true of ice COLD alcohol).
    2. If carried out accurately, long strands of DNA will form at the interface between the alcohol and the original solution. These can be physically spooled using the glass DNA hook.
6. Using the DNA hook, attempt to spool the DNA using slow, twirling motion.

Show your DNA to your instructor for credit!

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17.3: Lab Report

Part 1: DNA to Protein Exercise

The following DNA sequence is part of the gene that controls dimples. Decode the DNA message into mRNA, tRNA and finally amino acids. Use the genetic code chart to fill in the table below.

> **Note**
> The genetic code is based on mRNA (not DNA or tRNA). When you have finished this, you will be able to determine the phenotype of the person the DNA came from. (If arginine is the 3rd amino acid, the person will have dimples.)

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DNA	mRNA Codon	tRNA Anticodon	Amino Acid
C G A			
G T C			
G C A			
T A A			

1. Does the person with the sequence above have dimples?

2. What two great tasks (the central dogma of molecular biology) are carried out by our genetic machinery?

3. What name do we call a three-nucleotide sequences of mRNA?

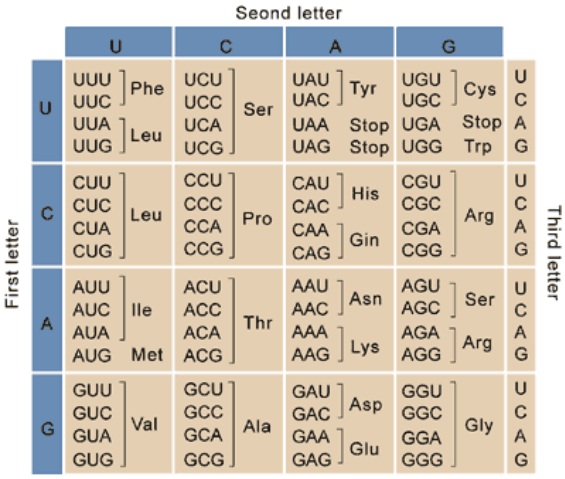
4. How many DNA bases does it take to code for an RNA codon?

5. How many amino acids does an RNA codon code for?

6. What brings amino acids to the ribosome?

7. What is the difference between transcription and translation?

8. True or false (circle one): Most of the DNA in the human genome codes for proteins.



Part 2: Protein Synthesis Exercise

DNA: 3′ A G C C G T A G A A T T 5′

1. Using this strand of DNA as a template, **draw a picture** of the complete DNA molecule. Include **all** parts of the DNA molecule. You do not need to draw your molecule with atomic accuracy.

2. Now draw a complete picture of the mRNA strand that will be made from this DNA. *Label* the 5′ and 3′ ends of your mRNA strand. (Use the given DNA strand at the top of this page as your template . . .)

3. Using the space below, carefully indicate the codons present in the mRNA strand from question 2.

4. Using the space below question 3 (above), draw a **complete** picture of all the tRNA molecules that will match up with the codons from the previous question. Include **all appropriate** amino acids in your picture, and do not mix up their order!

5. Using the space below question 3 (but below the tRNA-amino acid complexes), draw a picture of the completed protein coded for by this strand of DNA (three letter abbreviations are fine). Show the amino acids in the same order they would be observed in the finished protein.

Part 3: Protein Synthesis Bingo

Fill in the boxes with 16 of the 20 amino acids. Every bingo square will be unique. Then listen as random nucleotide sequences are pulled from the hat. Listen carefully to what kind of sequence is called! Use the mRNA codon chart on the previous page to determine the amino acid associated with each sequence. (**Printable version here.**)

Table of Amino Acids

alanine—ala—A	cysteine—cys—C	histidine—his—H	methionine—met—M	threonine—thr—T
arginine—arg—R	glutamine—gln—Q	isoleucine—ile—I	phenylalanine—phe—F	tryptophan—trp—W
asparagine—asn—N	glutamic acid—glu—E	leucine—leu—L	proline—pro—P	tyrosine—tyr—Y
aspartic acid—asp—D	glycine—gly—G	lysine—lys—K	serine—ser—S	valine—val—V

Data Table for Codons and AAs

	Sequence Called	DNA? mRNA? tRNA?	Codon	AA
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				
11				
12				
13				
14				
15				
16				
17				
18				

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CHAPTER OVERVIEW

18: Recombinant DNA and Bacterial Transformation

Learning Objectives

Content Objectives

- Analyze the process of bacterial transformation.
- Describe the formation and significance of recombinant DNA.

Skill Objectives

- Perform bacterial transformation.
- Use restriction enzymes to create recombinant DNA.

18.1: Introduction
18.2: Exercise
18.3: Lab Report

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18.1: Introduction

18.1.1: Recombinant DNA Technology & Bacterial Transformation

In addition to the normal circular chromosome found in bacterial cells, some bacteria have a small extrachromosomal DNA molecule known as a **plasmid**. Plasmids replicate independently of the main chromosome and are not required for survival, growth, or reproduction. However, plasmids can confer survival advantages. Certain plasmids, for example, called **R-plasmids**, carry genes for resistance against antibiotics such as penicillin, tetracycline, or ampicillin. These genes encode enzymes that inactivate the antibiotic, allowing the bacterial cells to survive and grow.

In lecture, we introduced Frederick Griffith's experiment on transformation. Bacterial transformation involves the transfer of foreign genetic information into bacterial cells. Transformed cells acquire new traits when they incorporate and express the genes found on the newly acquired DNA. In the laboratory, the type of DNA used most frequently for transformation experiments is bacterial plasmid DNA. The presence of antibiotic-resistance genes on plasmids makes it possible for scientists to select for bacteria containing the plasmid of interest. Bacteria that contain the plasmid will be **resistant** and able to grow in the presence of the antibiotic, whereas bacteria lacking the plasmid will be **sensitive** to the antibiotic and die.

Some bacteria, known as **competent bacteria**, are naturally receptive to foreign DNA. For other cells, competency can be generated artificially by changing the temperature and/or salt concentration of the medium. In today's exercise, we will induce competent ampicillin-sensitive *Escherichia coli* cells to take up the plasmid pAMP, which contains a gene for ampicillin resistance. We will be able to **select for** transformed cells because only cells that have successfully taken up the plasmid will be able to grow on agar plates containing ampicillin. Any *E. coli* that haven't been transformed will be killed by the ampicillin.

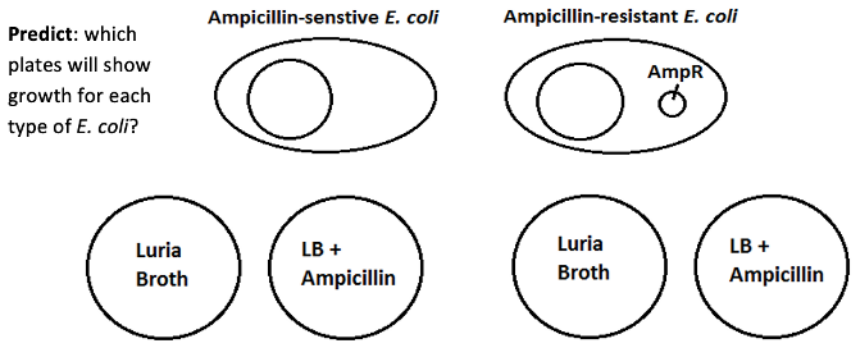


Figure 18.1.1: Predict the activity of competent, ampicillin-sensitive *Escherichia coli* cells when induced to take up the plasmid pAMP. Original image created by M. Chen.

18.1.2: Create a hypothesis and prediction for this experiment:

Hypothesis:

Prediction:

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18.2: Exercise

18.2.1: Exercise A

18.2.1.1: Procedure for bacterial transformation:

1. Using a micropipette and sterile tip, add 250 µL cold 0.05 M $CaCl_2$ into two microcentrifuge tubes (microtubes). Label both microtubes with your group name.
2. Obtain a plate of *E. coli*. Sterilize an inoculation loop by flaming it from the base to the tip slowly and then cooling it by placing the loop gently onto the agar plate where no bacteria are growing. Transfer a large colony of *E. coli* to one of the microtubes. Be careful not to pick up any agar.
3. Place your loop into the $CaCl_2$ solution and mix well to dislodge the cells into the solution.
4. Immediately suspend the cells by pipetting up and down 10 times using a 100 µL micropipette with sterile tip. Mark this first tube "+" and return it to the ice.
5. Repeat steps 2 to 5 for the second tube. Mark this tube "-". Sterilize your loop again once you are finished. Make sure your loop has cooled before the next step.
6. Using a micropipette and sterile tip, transfer 10 µL of pAMP plasmid directly into the cell suspension in tube "+" only. Be sure the volume on your micropipette is set correctly. Then, immerse the tip into the "+" solution and pipette up and down several times. Make sure your micropipette tip is placed directly into the solution and does **not** touch the wall of the microtube. Mix well. **Return the tube to ice for 15 minutes**.
7. While the tubes are incubating on ice, obtain two Luria Broth (LB) agar and two LB/Amp agar (Luria Broth containing ampicillin) plates. Label one LB agar plate "LB+" and the other "LB-". Label one LB/Amp plate "LB/Amp+" and the other "LB/Amp-". Label all plates with your group name, *E.coli*, the date, and 37°C. All labels should be written on the base of the plates, not the lids.
8. Heat-shock the cells in both the "+" and "-" microtubes by transferring the tubes **rapidly** from ice into the 42°C water bath for 90 seconds. This transfer must be rapid for a sharp and distinct shock, which optimizes transformation of the pAMP plasmid into *E. coli*. Immediately return the cells to ice for 2 minutes.
9. Use a micropipette and sterile tip to add 250 µL cold Luria broth to each tube. Mix well and leave the tubes at room temperature for recovery for **10 minutes**. During this time, the Amp^R gene that has been newly introduced into *E. coli* is expressed. As a result, β-lactamase, an enzyme that cleaves the functional group used by some antibiotics to kill bacteria, is produced. The transformed cells are now resistant to ampicillin since they contain the gene whose product renders the antibiotic ineffective.
10. Place 100 µL of the "-" cells onto the "LB-" plate and 100 µL of the "-" cells onto the "LB/Amp-" plate. Place 100 µL of "+" cells onto the "LB+" plate and 100 µL of the "+" cells onto the "LB/Amp+" plate.
11. Immediately spread the cells using a sterile spreading rod. Immerse and remove the spreading rod from ethanol and briefly pass it through a flame. (Do NOT flame sterilize if you are using plastic single use spreaders.) Cool the rod by gently touching it to the agar on a region of the plate away from the bacteria. Spread the cells throughout the plate. **Sterilize the rod again** before you spread the "+" cells and once more when you are finished spreading the cells. Allow the plates to set for 5 minutes right-side up (lid up). Then, tape your plates together and incubate upside-down (lid down) at 37°C for 24-48 hours.

18.2.2: **Exercise B**

18.2.2.1: **Procedure for making a recombinant plasmid model via restriction enzyme digest**

Your instructor will provide a paper replica of the *E. coli* plasmid. The unshaded piece of DNA represents a circular *E. coli* plasmid. The shaded pieces of DNA represent a segment of DNA taken from a human chromosome. **Restriction enzyme** *Hin*dIII is isolated from *Haemophilus influenza*e and recognizes the following restriction site and produces fragments with "sticky ends":

5' A A G C T T 3'

3' T T C G A A 5'

Cut and tape the unshaded strand together to form a circular plasmid of bacterial DNA.

Tape the shaded (human DNA) pieces together to form a long, linear, strand.

Locate the cut site(s) on the plasmid DNA and on the linear strand of DNA. Draw it in pencil as they would be cut by *Hin*dIII and then show them to your instructor for approval.

Once the instructor confirms that you have the correct cut sites, use a pair of scissors to cut the DNA of both DNA molecules. Note, your scissors represent the restriction enzyme *Hin*dIII.

Lastly, insert the isolated (target) human DNA into the plasmid and tape the fragments together. You now have a recombinant DNA plasmid.

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18.3: Lab Report

18.3.1: Exercise A

18.3.1.1: Transformation Day 2: Results, Analysis, and Critical Thinking

After the plates have been incubated for 24-48 hours, observe for growth. Which plates did *E. coli* grow on? Indicate growth by circling the plates below:

LB- LB+ LB/Amp- LB/Amp+

A1. What was the purpose of the "LB-" plate?

A2. What was the purpose of the LB/Amp- plate?

A3. Why was there no growth observed on the LB/Amp- plate?

A4. Did your results support your hypothesis? Yes / No (circle one)

A5. Based on your results, would it be possible for ampicillin resistance to be transferred (horizontally) from one bacterium to another?

Explain how this might occur:

Transformation efficiency is expressed as the number of antibiotic-resistant colonies per microgram of pAMP. On which plate will **only** the antibiotic-resistant colonies grow? _____ Use this plate to determine transformation efficiency below:

A6. Determine the total amount of pAMP you added to the "+" microtube: _____µg

(Note: one loopful = 10 µL, and the concentration of the pAMP stock solution was 0.2 µg / µL)

A7. Determine the total concentration of pAMP (in µg / µL) in the total suspension of cells plus Luria broth used for recovery (250 µL $CaCl_2$ + 10 µL pAMP + 250 µL Luria broth): _____ µg / µL

A8. Total amount of pAMP in the 100 µL spread on the "+" plates: _____ µg pAMP/ 100 µL

A9. Count the number of colonies on the plate: _____ colonies. (If there are too many colonies to count, divide the plate into quarters or eighths, count one section, and multiply by the number of sections.)

A10. Divide the number of colonies by the amount of pAMP in the 100 µL of cell suspension spread on the plate (step 8) to find the colonies/µg pAMP (scientific notation): _____ colonies / µg pAMP. This is the transformation efficiency for your group.

A11. Record the transformation efficiencies for the other groups in our lab:

A12. Find the average transformation efficiency for our class: _____ colonies / µg pAMP

18.3.2: Exercise B

B1. What is the name of the enzyme that connects, or seals, the DNA fragments together?

B2. What do you notice about the 3' and 5' ends of the target gene and the plasmid cut site?

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CHAPTER OVERVIEW

19: Molecular Biology

Learning Objectives

Content Objectives

- Describe the purpose and functions of restriction enzymes.
- Describe the purpose and process of DNA electrophoresis.

Skill Objectives

- Learn how to cut DNA into fragments with enzymes.
- Load and separate DNA fragments by electrophoresis.
- Analyze electrophoresis gels.

19.1: Introduction
19.2: Exercise
19.3: Lab Report

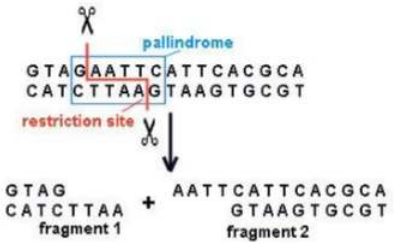
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19.1: Introduction

Introduction

DNA RESTRICTION ANALYSIS

In this experiment, DNA from the bacteriophage Lambda (48,502 base pairs in length) is cut with a variety of restriction enzymes and the resulting fragments are separated using gel electrophoresis. Three samples of Lambda (phage) DNA are incubated at 37º C, each with one of the 3 restriction endonuclease enzymes: Pst1, EcoRI, and HindIII. A fourth sample will be the negative control in that is will be incubated without any endonuclease. Each of the 3 enzymes recognizes a different sequence of bases on DNA called a **palindrome** , and cuts within it at a specific site called a "restriction site."



The DNA samples are then loaded into wells of an agarose gel and electrophoresed, along with **loading dyes** (see procedure below). An electrical field applied across the gel causes the DNA fragments in the samples to move from their origin (a sample well) through the gel matrix toward the positive electrode. Small DNA fragments migrate faster than larger ones, so restriction fragments of differing sizes separate into distinct bands during electrophoresis. The loading dyes are of 2 different sizes, corresponding to very small DNA fragments and very large DNA fragments. They can be seen as the electrophoresis progresses, and they form a 'bracket' in between which the DNA fragments are moving. Otherwise, one cannot tell how far the DNA fragments have moved through the agar. The characteristic number and pattern of bands produced by each restriction enzyme are made visible by staining with a compound that binds to the DNA molecule---**methylene blue.**

Contributors and Attributions

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19.2: Exercise

MATERIALS NEEDED

- restriction buffer
- loading dye
- ice
- 37º C water bath
- Lambda phage DNA
- TBE buffer mix
- restriction enzymes: Pst1, EcoR1, Hind111
- agarose to be poured (in 60º C water bath)
- reaction tubes
- 4 microtubes: clear, violet, green, orange
- staining tray

PROCEDURES

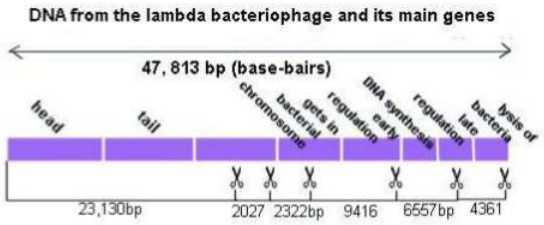
Use of the micropipetter

1. Be sure that the amount set on the pipetter is correct.
2. Place new tip firmly on micropipetter.
3. Depress plunger to **first stop**, and hold this position. This step eliminates any air in tip.
4. Dip tip into solution to be pipetted, and draw fluid into tip by releasing plunger. Always touch pipette tip to side of tube to dislodge any small amount stuck to tip. You now have a sample inside of your pipette tip.
5. To expel sample, touch pipette tip to inside wall of tube into which you want to empty sample. This creates a capillary effect which will help draw fluid out of tip.
6. Slowly depress plunger to first stop and then depress to second stop to blow out last bit of fluid in tip. **DO NOT release plunger before removing tip from fluid in tube. Otherwise, it will suck fluid back into tip.**
7. When taking a sample, **always** check for air at the tip. If it is present, put the sample back and begin again.

Digest DNA with restriction endonucleases (keep all enzymes on ice)

1. Label four 1.5ml tubes, in which you will perform restriction digestion: "P" for **Pst1** enzyme, "E" for **EcoRI** enzyme, "H" for **HindIII** enzyme, and "L" for **Lambda** DNA uncut.
2. Using table below, add reagents to each tube in this order: DNA, restriction buffer, water, and enzymes last (ask for them). Use a fresh pipette tip for each different reagent. The amounts are given in microliter (millionths of a liter).
3. Pool and mix reagents by tapping the tube bottom on counter a couple of times (or use a microcentrifuge).
4. Incubate all reaction tubes for 30 minutes at 37º C.
5. The tubes will go into the freezer until the next period when the electrophoresis will be done.

color	tube code	enzyme	DNA	buffer	Pst1	EcoRI	HindIII
clear	L	uncut DNA	4	6	---	---	---
violet	P	Pst1	4	5	1	---	---
green	E	EcoRI	4	5	---	1	---
orange	H	HindIII	4	5	---	---	1



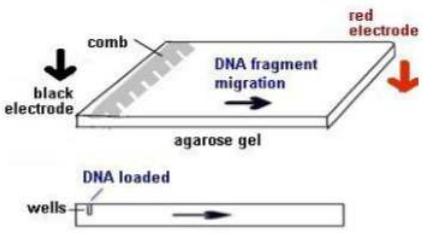
How many DNA fragments are produced when HindIII endonuclease cuts the lambda virus DNA? 6

Casting agarose gel

1. Set gel-casting tray into the tray apparatus, screw tight, and insert well-forming comb into space marked with red line. There is a leveling bubble which can be used to level the tray (by turning knobs at bottom).
2. Place tray FLAT where agarose can be poured and allowed to set UNDISTURBED.
3. Carefully pour 40 ml of agarose solution (liquified in 60º C water bath) into casting tray. Use a toothpick to move any bubbles to edges (this must be done BEFORE gel hardens).
4. Gel will solidify within 20 minute. Do NOT move tray while agarose is solidifying.
5. Gently remove comb, pulling it straight up and taking care not to rip wells.
6. When solidified, remove the gel tray from the gel-casting tray and place on platform of electrophoresis box, so that comb is at negative (BLACK) cathode end. The - charged DNA fragments will migrate towards the + anode end.
7. Fill box with TBE buffer, to level that just covers entire surface of gel by about 2 mm.
8. Make certain that sample wells left by comb are completely submerged by buffer.
9. The gel is now ready to load with DNA.

Loading gel with DNA

1. Remove the 4 tubes from the fridge and pulse spin the tubes in a centrifuge or tap the tubes firmly down on the table top so that all contents go down to the bottom of the tube.
2. Add 2 µl loading dye to each reaction tube and tap contents of tube on table top.
3. Use pipette to load contents of each reaction tube into a separate well in gel (total of 4 wells). **Set your micropipetter on 12 µl** (that should be the total contents in the tube).
4. You will need to remember the order of your tubes since there is NO way that the gel can be marked. Use a clean pipette tip for each different tube.
   - Steady pipette over well using 2 hands.
   - Expel any air first from pipette tip.
   - Dip pipette tip through buffer, positioned over the well, and slowly expel the mixture (do not punch thru bottom of gel).
5. The loading dye contains sucrose which is heavier than the DNA. It weighs the mixture down so it will sink into the bottom of the well.





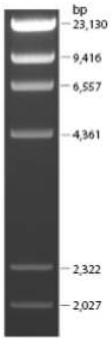
Electrophoresis

1. The electrophoresis chamber top is placed on the chamber, the electrodes connected to power supply--anode to anode (red-red) and cathode to cathode (black-black).

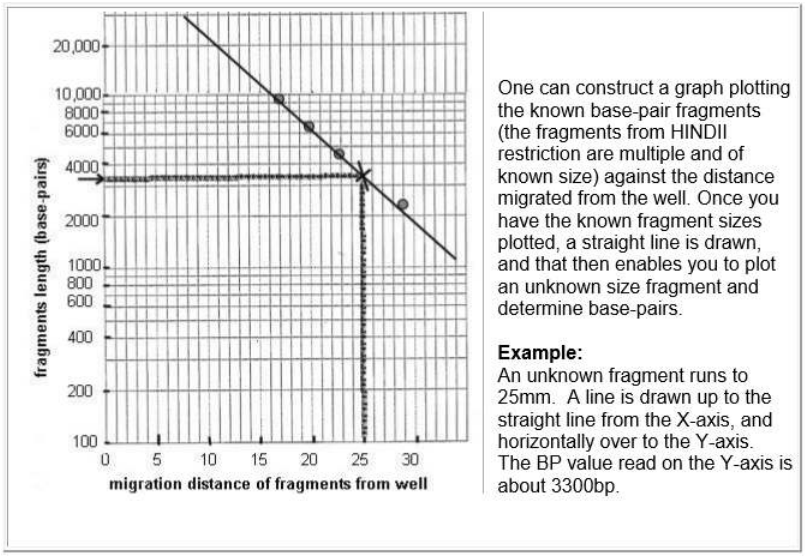
2. Power supply is turned on and voltage set---120 V. The higher the voltage, the faster the electrophoresis time. In a few minutes, you should begin to notice the loading dye moving through the gel toward the + pole (anode). We will let it run for about 1 hour.
3. The **loading dye** will resolve into 2 bands of color. The faster-moving, purplish band is the dye **bromophenol blue,** the slower-moving, aqua band is **xylene cyanol** . Bromophenol blue migrates through the gel at the same rate as a DNA fragment approximately 300 base pairs long. Xylene cyanol migrates at the a rate equivalent to approximately 2,000 base pairs long.
4. After 1 hour, the bromophenol band should be nearing the end of the gel. Turn off the power supply, disconnect the leads, and remove the top of chamber.
5. Carefully remove the casting tray and keep it horizontal until you are ready to put into the plastic container. Slide gel easily into staining tray labeled with your group name.
6. Add enough methylene blue DNA stain to cover the gel and place cover on it. They will sit in the dye overnight.
7. The gels will be washed a couple of times in distilled water (standing for 10-20 min), and will refrigerate until next lab period when we will look at them.
8. The gels can then be placed on gel support film, which binds the gel and dehydrates it, if your instructor so chooses.

INTERPRETATION

Examine your stained gel on a light box, overhead projector, or a UV box. Which restriction enzyme produced the most restriction sites on the lambda DNA?



The HindIII digest of lambda DNA yields at least 6 fragments suitable for use as molecular weight standards for gel electrophoresis. This is called a ladder. After the sample is ran, the unknown fragments can be compared with the ladder fragments to determine the approximate size of the unknown DNA bands by how they match up to the known bands of the ladder.



QUESTIONS

1. What is the source of the DNA being used in this exercise?
2. The speed of the DNA fragment migration correlates directly with the _____ of the fragment.
3. Why use 3 different restriction enzymes to cut DNA?
4. How can you account for differences in band separation and intensity between your gel and the ideal gel?
5. Small restriction fragments of nearly the same length will appear as a single band on this gel, even though it may be run to the very end. Why?

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19.3: Lab Report

DNA RESTRICTION ANALYSIS

QUESTIONS

1. What is the source of the DNA being used in this exercise?

2. The speed of the DNA fragment migration correlates directly with the _____ of the fragment.

3. Why use 3 different restriction enzymes to cut DNA?

4. How can you account for differences in band separation and intensity between your gel and the ideal gel?

5. Small restriction fragments of nearly the same length will appear as a single band on this gel, even though it may be run to the very end. Why?

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CHAPTER OVERVIEW

20: Scientific Method

Learning Objectives

Content Objectives

- Gain proficiency in the use of the scientific method to answer questions and design experiments.
- Learn how to use a dichotomous key to identify the taxonomy of species.

Skill Objectives

- Practice the steps of the scientific method by completing various case studies.
- Use the scientific method to design an experiment to study termite behavior.
- Practice using dichotomous keys to identify organisms.
- Design your own dichotomous key to identify emojis.

20.1: Introduction
20.2: Exercise
20.3: Lab Report

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20.1: Introduction

Scientific Method

Adapted from BiologyCorner [www.biologycorner.com]

The scientific method is central to the study of biology: it is a process of acquiring and verifying information through experimentation. The general steps of the scientific method are depicted in the figure below. The **hypothesis**, or suggested explanation for the observation, is the basis for setting up experiments. Based on your hypothesis you can write a specific **prediction**: an outcome of your experiment that would support your hypothesis. A good experimental design is essential to the scientific method. A few keys to good experimental design include effective use of controls, reproducibility, a large sample size, and multiple trials.

In an experiment, in order to determine that any changes that occur are due to investigator manipulation only, there must be some basis for comparison. A **control group** is necessary to establish this basis of comparison. In the control group, everything is kept the same as the experimental group except for the **independent variable**.

The experimental group is actually being experimented upon. For example, in a drug trial, there will be a group that receives the drug (the experimental group) and a group that receives a placebo (the control group). The drug itself is considered the independent variable and any change(s) that occur because of the drug are considered the **dependent variable**.

In order to ensure that it is only the drug causing changes, all other variables must be tightly controlled (such as diet, exercise, smoking, etc.). These are referred to as **controlled variables**.

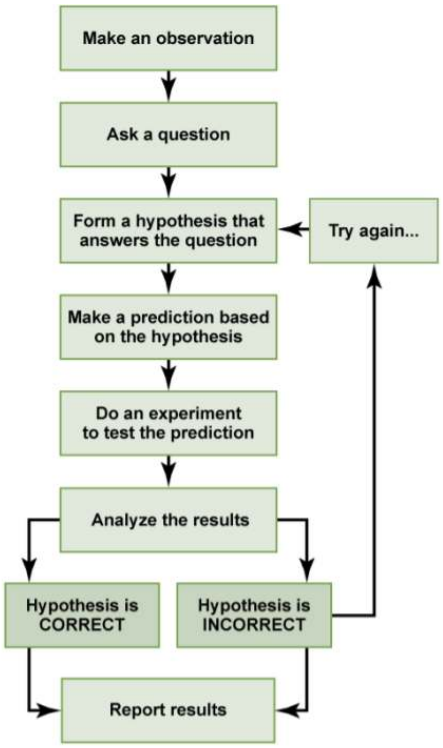


Figure 20.1.1: The Scientific Method

Using the Scientific Method to Investigate Termite Behavior

Termites are insects closely related to ants and, like ants, follow trails that other termites produce. Termites produce trails by laying down chemicals called **pheromones** on substrate.

Taxonomy

Because the diversity of life on Earth is so vast, biologists use a general system of classification and naming organisms (taxonomy) to track and organize species based on evolutionary relatedness. The broadest taxon is the domain; organisms belong to one of the three domains (Bacteria, Archaea, and Eukarya). Within the domains are increasingly specific taxa, usually following the order in the table below.

The scientific name of an organism is given using binomial nomenclature; the genus and species of an organism give its specific scientific name. These names are usually derived from Greek or Latin, and therefore must be italicized when written. The genus is to be capitalized and the species is lower case. For example, the scientific name of a common wombat (top) is *Vombatus ursinus*.

Let us compare the wombat to a similar species, a quokka (bottom).

Note that both animals differ only when we reach the family level. If you knew that a kangaroo was in the same family as a quokka, would you assume the quokka was more closely related to a kangaroo or a wombat?

Scientific names might seem confusing, but are useful for several reasons. Common names tend to vary according to region (crawfish, crayfish, mudbug, crawdad), but the scientific name is always the same.

Comparison of wombat and quokka taxonomy

	Wombat	Quokka
Domain	Eukarya	Eukarya
Kingdom	Animalia	Animalia
Phylum	Chordata	Chordata
Class	Mammalia (Marsupialia)	Mammalia (Marsupialia)
Order	Diprotodontia	Diprotodontia
Family	*Vombatidae*	*Macropodidae*
Genus	*Vombatus*	*Setonix*
Species	*ursinas*	*brachyurus*

Questions:

1. Llamas, alpacas, and camels are all in the same family: Camelidae. Therefore, it is reasonable to assume that these animals will also be in the same...

2. The scientific name of the brown-throated three-toed sloth is named Bradypus variegatus. What is the genus of the organism? The species?

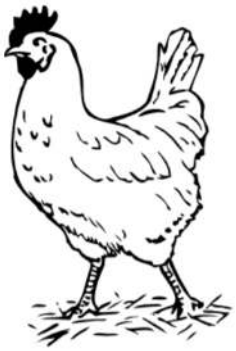
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- **1.2: Taxonomy** by Susan Burran and David DesRochers is licensed CC BY-SA 4.0. Original source: https://libguides.daltonstate.edu/PrinciplesofBiology/labmanual.

20.2: Exercise

Scientific Method

Part 1: The Strange Case of BeriBeri:



In 1887 a strange nerve disease attacked the people in the Dutch East Indies. The disease was beriberi. Symptoms of the disease included weakness and loss of appetite, victims often died of heart failure. Scientists thought the disease might be caused by bacteria. They injected chickens with bacteria from the blood of patients with beriberi. The injected chickens became sick. However, so did a group of chickens that were not injected with bacteria. One of the scientists, Dr. Eijkman, noticed something. Before the experiment, all the chickens had eaten whole-grain rice, but during the experiment, the chickens were fed polished rice. Dr. Eijkman researched this interesting case and found that polished rice lacked thiamine, a vitamin necessary for good health.

1. State the problem.

2. What was the hypothesis?

3. How was the hypothesis tested?

4. Do the results indicate that the hypothesis should be rejected?

5. What should be the new hypothesis and how would you test it?

Part 2: How Penicillin Was Discovered:

In 1928, Sir Alexander Fleming was studying Staphylococcus bacteria growing in culture dishes. He noticed that a mold called Penicillium was also growing in some of the dishes. A clear area existed around the mold because all the bacteria that had grown in this area had died. In the culture dishes without the mold, no clear areas were present. Fleming hypothesized that the mold must be producing a chemical that killed the bacteria. He decided to isolate this substance and test it to see if it would kill bacteria. Fleming transferred the mold to a nutrient broth solution. This solution contained all the materials the mold needed to grow. After the mold grew, he removed it from the nutrient broth. Fleming then added the nutrient broth in which the mold had grown to a culture of bacteria. He observed that the bacteria died which was later used to develop antibiotics used to treat a variety of diseases.

1. Identify the problem.

2. What was Fleming's hypothesis?

3. How was the hypothesis tested?

4. Do the results indicate that the hypothesis should be rejected?

5. This experiment led to the development of what major medical advancement…?

Part 3: Identify the Controls and Variables



Smithers thinks that a special juice will increase the productivity of workers. He creates two groups of 50 workers each and assigns each group the same task (in this case, they're supposed to staple a set of papers). Group A is given the special juice to drink while they work. Group B is not given the special juice. After an hour, Smithers counts how many stacks of papers each group has made. Group A made 1,587 stacks; Group B made 2,113 stacks.

Identify the:

- Control Group:
- Independent Variable:
- Dependent Variable:

What should Smithers' conclusion be?

How could this experiment be improved?



Homer notices that his shower is covered in a strange green slime. His friend Barney tells him that coconut juice will get rid of the green slime. Homer decides to check this out by spraying half of the shower with coconut juice. He sprays the other half of the shower with water. After 3 days of "treatment", there is no change in the appearance of the green slime on either side of the shower.

What was the initial observation?

Identify the:

- Control Group:
- Independent Variable:
- Dependent Variable:

What should Homer's conclusion be?



Bart believes that mice exposed to radio waves will become extra strong (maybe he's been reading too much Radioactive Man). He decides to perform this experiment by placing 10 mice near a radio for 5 hours. He compared these 10 mice to another 10 mice that had not been exposed. His test consisted of a heavy block of wood that blocked the mouse food. He found that 8 out of 10 of the exposed mice were able to push the block away, while 7 out of 10 of the other mice were able to do the same.

Identify the:

- Control Group:
- Independent Variable:
- Dependent Variable:

What should Bart's conclusion be?

How could Bart's experiment be improved?



Krusty was told that a certain itching powder was the newest best thing on the market: it even claims to cause 50% longer lasting itches. Interested in this product, he buys the itching powder and compares it to his usual product. One test subject (A) is sprinkled with the original itching powder, and another test subject (B) was sprinkled with the Experimental itching powder. Subject A reported having itches for 30 minutes. Subject B reported having itches for 45 minutes

Identify the:

- Control Group:
- Independent Variable:
- Dependent Variable:

Explain whether the data supports the advertisement's claims about its product.

How could this experiment be improved?



Lisa is working on a science project. Her task is to answer the question: "Does Rogooti (which is a commercial hair product) affect the speed of hair growth". Her family is willing to volunteer for the experiment.

Design Lisa's experiment.

Using the Scientific Method to Investigate Termite Behavior

Procedure:

1. Obtain a termite from the class supply. You will use a paintbrush to gently put your termite in a cup and bring it to your lab bench.
2. Obtain a piece of clean white paper and place your termite on the paper. Observe it for a bit, noting any interesting behaviors.
3. You will do two experiments with your termite, one introductory experiment and one of your own design.

Experiment 1:

1. Obtain a clean piece of white paper, a pencil, a ballpoint pen, and a sharpie.
2. Draw three circles on your piece of paper, one circle with each type of writing implement.
3. Write a hypothesis and prediction for this experiment: How do you think the termite will react to each circle? Why?
4. Place your termite into each circle and record the results. What happens when the termite encounters each circle? Does it follow each of the trails?

Experiment 2:

1. You have many options of writing implements: pens, pencils, markers, etc. with which to make trails.
2. Write a hypothesis and a prediction. Questions to consider as you write your hypothesis:
    - What types of trails do you think your termite will follow or avoid, and why?
    - Does shape matter?
    - Can you trick your termite?
3. Design your experiment: what is your independent variable? What is your dependent variable? What are your control and experimental groups?
4. Check in with your instructor before you start your experiment.
5. Conduct your experiment.
6. Write a brief conclusion. What did you find? Did your results support your hypothesis?

Taxonomy

Part 1: A Simple Dichotomous Key

A dichotomous key is a tool used to determine the identity of species that have been previously described. You can think of it as a series of questions in which each question only has two possible answers.

In the table below, you have been given a list of creatures and their descriptions. The different characteristics, behaviors, and habitats of the creatures can be used in the dichotomous key to differentiate among them.

Mythical creatures with descriptions

Creature	Description
Jackelope	Mean-spirited horned jack rabbit
Chupacabra	Reptilian creature covered in scales with spines along the dorsal ridge; likes to eat goats
Altamaha-ha	Water monster with an alligator-like head and long neck; lives in the marshes of Coastal Georgia
Sasquatch	Stinky giant humanoid covered in brown fur; found in the forests of North America
Yeti	Giant mountain humanoid covered in white fur; prefers the snow
Kraken	Giant octopus-like creature; takes down ships in the open ocean
Nessie	Water monster with a snake-like head and long neck; lives in Loch Ness, in the Scottish highlands

Below, you will find the dichotomous key used to identify a folkloric creature you may come across. On the left is the list of questions and on the right, the same list is represented as a flowchart. Both are useful representations of the same dichotomous key.

1. Does the creature live on land?

- a. Yes: go to question 2
- b. No: go to question 5

2. Does the creature resemble a human?

- a. Yes: go to question 3
- b. No: go to question 4

3. Does the creature have brown fur?

- a. Yes: Sasquatch!
- b. No: Yeti!

4. Does the creature have scales?

- a. Yes: Chupacabra!
- b. No: Jackelope!

5. Does the creature have tentacles?

- a. Yes: Kraken!
- b. No: go to question 6

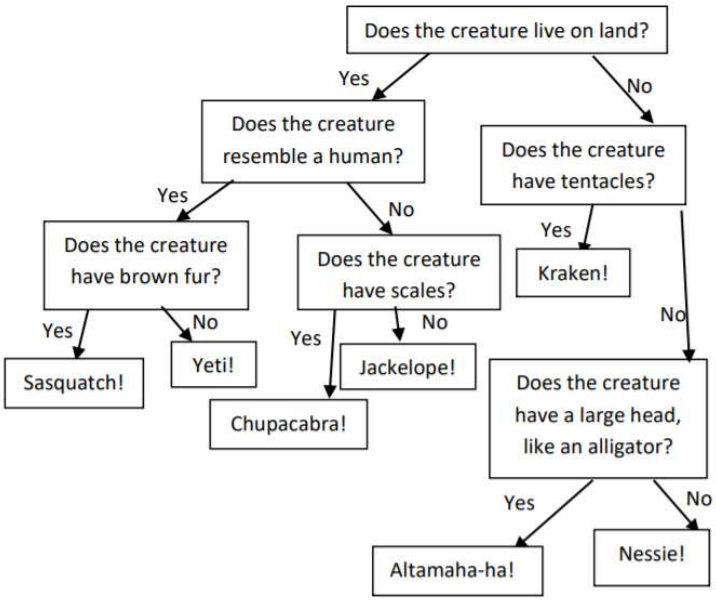
6. Does the creature have a large head, resembling an alligator?

- a. Yes: Altamaha-ha!
- b. No: Nessie!



"Patterson–Gimlin film frame 352" by Patterson-Gimlin film. Via Wikipedia

Using the dichotomous key, identify the creature on the right.



Part 2: Building a Dichotomous Key

In the table below, there are several different emojis. Your job is to build a dichotomous key that would help distinguish among them. There is space in the table to write out a description of each emoji, if necessary, as well as a name for each. Record your question series in the space below.

Emoji	Description	Name
😂		
😏		
😘		
😋		
😤		

Emoji	Description	Name
		

Dichotomous Key Questions:

1.
a.
b.
2.
a.
b.
3.
a.
b.
4.
a.
b.
5.
a.
b.

Part 3: Using a Dichotomous Key to Identify Trees

In this section, a dichotomous key will be used to identify tree species based on samples provided by your instructor. Refer to the guide provided should any terms about the characteristics used to distinguish among trees be unfamiliar.

Plant Number	Scientific/Common Name
Plant 1	
Plant 2	
Plant 3	
Plant 4	
Plant 5	
Plant 6	
Plant 7	

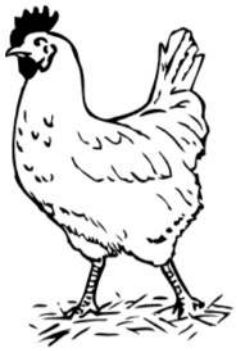
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20.3: Lab Report

Scientific Method

Part 1: The Strange Case of BeriBeri:



1. State the problem.

2. What was the hypothesis?

3. How was the hypothesis tested?

4. Do the results indicate that the hypothesis should be rejected?

5. What should be the new hypothesis and how would you test it?

Part 2: How Penicillin Was Discovered:

1. Identify the problem.

2. What was Fleming's hypothesis?

3. How was the hypothesis tested?

4. Do the results indicate that the hypothesis should be rejected?

5. This experiment led to the development of what major medical advancement…?

Part 3: Identify the Controls and Variables



1. Identify the:
    - Control Group:
    - Independent Variable:
    - Dependent Variable:
2. What should Smithers' conclusion be?

3. How could this experiment be improved?



4. What was the initial observation?

5. Identify the:
    - Control Group:
    - Independent Variable:
    - Dependent Variable:
6. What should Homer's conclusion be?



7. Identify the:

- Control Group:
- Independent Variable:
- Dependent Variable:

8. What should Bart's conclusion be?

9. How could Bart's experiment be improved?



10. Identify the:
    - Control Group:
    - Independent Variable:
    - Dependent Variable:
11. Explain whether the data supports the advertisement's claims about its product.

12. How could this experiment be improved?

_____

_____



13. Design Lisa's experiment.

Using the Scientific Method to Investigate Termite Behavior

Experiment 1:

1. Write a hypothesis and prediction for this experiment: How do you think the termite will react to each circle? Why?

2. Place your termite into each circle and record the results. What happens when the termite encounters each circle? Does it follow each of the trails?

Experiment 2:

1. Write a hypothesis and a prediction.

2. Design your experiment: what is your independent variable? What is your dependent variable? What are your control and experimental groups?

3. Write a brief conclusion. What did you find? Did your results support your hypothesis?

Part 1: A Simple Dichotomous Key

Mythical creatures and their descriptions

Creature	Description
Jackelope	Mean-spirited horned jack rabbit
Chupacabra	Reptilian creature covered in scales with spines along the dorsal ridge; likes to eat goats
Altamaha-ha	Water monster with an alligator-like head and long neck; lives in the marshes of Coastal Georgia
Sasquatch	Stinky giant humanoid covered in brown fur; found in the forests of North America
Yeti	Giant mountain humanoid covered in white fur; prefers the snow
Kraken	Giant octopus-like creature; takes down ships in the open ocean
Nessie	Water monster with a snake-like head and long neck; lives in Loch Ness, in the Scottish highlands

Below, you will find the dichotomous key used to identify a folkloric creature you may come across. On the left is the list of questions and on the right, the same list is represented as a flowchart. Both are useful representations of the same dichotomous key.

1. Does the creature live on land?

- a. Yes: go to question 2
- b. No: go to question 5

2. Does the creature resemble a human?

- a. Yes: go to question 3
- b. No: go to question 4

3. Does the creature have brown fur?

- a. Yes: Sasquatch!
- b. No: Yeti!

4. Does the creature have scales?

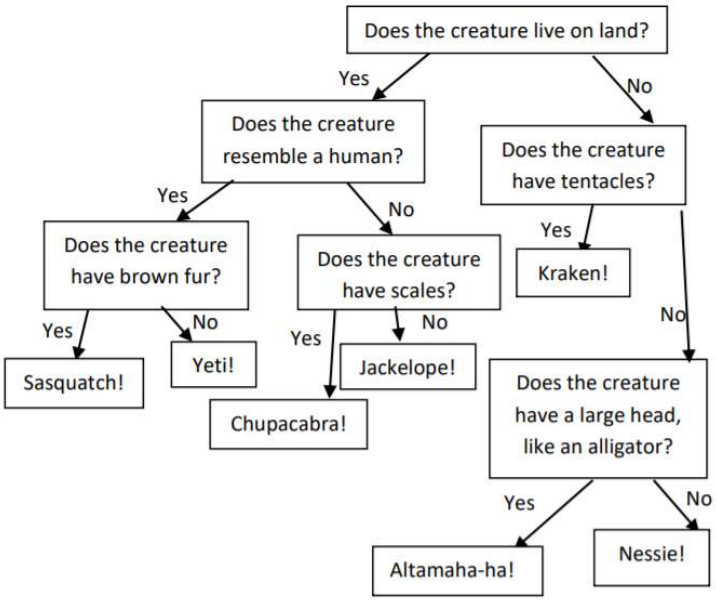
- a. Yes: Chupacabra!
- b. No: Jackelope!

5. Does the creature have tentacles?

- a. Yes: Kraken!
- b. No: go to question 6

6. Does the creature have a large head, resembling an alligator?

- a. Yes: Altamaha-ha!
- b. No: Nessie!



Using the dichotomous key, identify the creature on the right.

Part 2: Building a Dichotomous Key



"Patterson–Gimlin film frame 352" by Patterson-Gimlin film. Via Wikipedia

Table for building an emoji dichotomous key

Emoji	Description	Name
😂		
😏		
😘		
😋		
😤		
😭		

Dichotomous Key Questions:

1.
a.
b.

2.
a.
b.

3.
a.
b.

4.
a.
b.

5.
a.
b.

Part 3: Using a Dichotomous Key to Identify Trees

In this section, a dichotomous key will be used to identify tree species based on samples provided by your instructor. Refer to the guide provided should any terms about the characteristics used to distinguish among trees be unfamiliar.

Table for dichotomous key to identify trees

Plant Number	Scientific/Common Name
Plant 1	
Plant 2	
Plant 3	
Plant 4	
Plant 5	
Plant 6	
Plant 7	

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CHAPTER OVERVIEW

21: Evolution and Natural Selection

Learning Objectives

Content Objectives

- Identify the three requirements for Natural Selection.
- Examine the various processes that can cause changes in allele frequency.

Skill Objectives

- Model different ways that Natural Selection affects populations.
- Use cladograms and phylogenetic trees to determine evolutionary relationships among organisms.

21.1: Introduction
21.2: Exercise
21.3: Lab Report

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21.1: Introduction

Natural Selection

In Bio 4A you studied genetics and the biological explanations for variation of traits in living things. Recall that Mendel described "particles" that were passed from parent to offspring. We now know those particles were **genes**, and that variation in the traits he studied was the result of inheriting different variants, **alleles** of each gene. It is important to have a good foundation in genetics to understand evolution.

Evolution is formally defined as a **change in allele frequency over time**. In other words, if you analyze a population of organisms today, and then sometime in the future, you may note a shift in the percentages of specific alleles. Since evolution is merely a change in allele frequency over time, then all life is constantly evolving. What fuels these changes?

The principal process involved in evolutionary change is **natural selection**, which is defined as "differential survival and/or reproduction."

There are three requirements that must be met for natural selection to actually take place:

1. There must be variation among the members in the population for the trait in question
2. The variants of the trait must result in differences in survival and/or reproduction.
3. The trait in question must be controlled to some degree by genes (e.g., when you dye your hair, this new variant is not controlled by genes).

There is one well-known classic case of natural selection: melanism mutation in peppered moths. Prior to the Industrial Revolution in England, nearly every peppered moth had light-colored speckled wings, only some months had the mutated black peppered phenotype. This coloration enabled the moths to blend in on the lichen-covered tree trunks. The much rarer black peppered moths were so obvious that birds (the primary killer [i.e., the important "selector"] of peppered moths) easily caught and killed them. The result is that these forms differed in survival probability and thus the light-colored moths survived and reproduced. Black moths were only maintained because of the rare mutation.

The Industrial Revolution was responsible for the production of huge amounts of soot (from factory smokestacks). The soot was produced so rapidly that it covered the once-light-colored tree trunks. The now-dark tree trunks exposed the light-colored moths and the dark moths now were blending in. The birds now caught mostly light-colored moths, thus dark moths had higher survival and reproduction. Because they were producing more offspring (which had better survival probability), the dark moths now became abundant. The population now contained a larger fraction of dark moths, and this follows the definition of evolution (i.e., change in allele frequencies).

Note that if no variation exists in a population, then natural selection cannot work. Other modes of evolution exist as well (mutation, genetic drift, migration). These can work alone or in conjunction with one another:

Mutations

in a broad sense, is the only source of evolution novelties. However, most of mutations are results of somehow broken DNA and therefore probability of useful mutation is similar to probability to upgrade your smartphone with a hammer.

Migration

brings new genotypes to the population and therefore able to change the local course of evolution.

Genetic drift

is usually a result of catastrophic factors applied to small populations. The smaller is a population, the bigger is probability that it will simply die out. Another name of this thing is an evolution *bottleneck*: few will survive, and these few will be chosen not because they are better but simply by random.

Phylogenetics and Cladistics

In scientific terms, **phylogeny** is the evolutionary history and relationship of an organism or group of organisms. A phylogeny describes the organism's relationships, such as from which organisms it may have evolved, or to which species it is most closely

related. Phylogenetic relationships provide information on shared ancestry but not necessarily on how organisms are similar or different.

Phylogenetic Trees

Scientists use a tool called a phylogenetic tree to show the evolutionary pathways and connections among organisms. A **phylogenetic tree** is a diagram used to reflect evolutionary relationships among organisms or groups of organisms. Scientists consider phylogenetic trees to be a hypothesis of the evolutionary past since one cannot go back to confirm the proposed relationships. In other words, we can construct a “tree of life” to illustrate when different organisms evolved and to show the relationships among different organisms (Figure 20.2).

Unlike a taxonomic classification diagram, we can read a phylogenetic tree like a map of evolutionary history. Many phylogenetic trees have a single lineage at the base representing a common ancestor. Scientists call such trees **rooted**, which means there is a single ancestral lineage (typically drawn from the bottom or left) to which all organisms represented in the diagram relate. Notice in the rooted phylogenetic tree that the three domains— Bacteria, Archaea, and Eukarya—diverge from a single point and branch off. The small branch that plants and animals (including humans) occupy in this diagram shows how recent and miniscule these groups are compared with other organisms. Unrooted trees do not show a common ancestor but do show relationships among species.

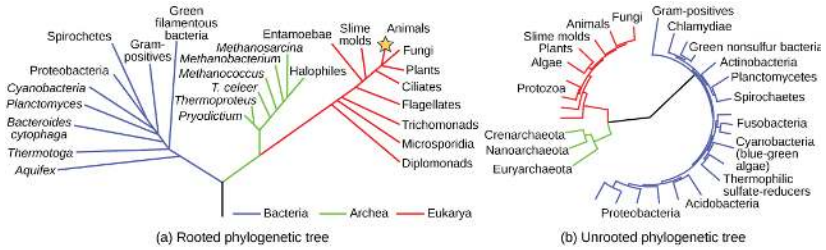


Figure 21.1.1: Both of these phylogenetic trees show the relationship of the three domains of life—Bacteria, Archaea, and Eukarya—but the (a) rooted tree attempts to identify when various species diverged from a common ancestor while the (b) unrooted tree does not. (credit a: modification of work by Eric Gaba)

In a rooted tree, the branching indicates evolutionary relationships (Figure 21.1.2:). The point where a split occurs, a **branch point**, represents where a single lineage evolved into a distinct new one. We call a lineage that evolved early from the root that remains unbranched a **basal taxon**. We call two lineages stemming from the same branch point **sister taxa**. A branch with more than two lineages is a **polytomy** and serves to illustrate where scientists have not definitively determined all of the relationships. Note that although sister taxa and polytomy do share an ancestor, it does not mean that the groups of organisms split or evolved from each other. Organisms in two taxa may have split at a specific branch point, but neither taxon gave rise to the other.

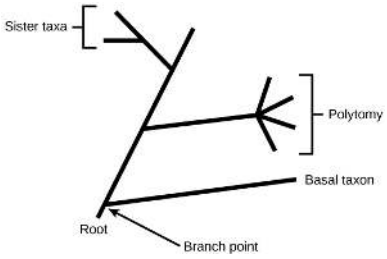


Figure 21.1.2: A phylogenetic tree's root indicates that an ancestral lineage gave rise to all organisms on the tree. A branch point indicates where two lineages diverged. A lineage that evolved early and remains unbranched is a basal taxon. When two lineages stem from the same branch point, they are sister taxa. A branch with more than two lineages is a polytomy.

The diagrams above can serve as a pathway to understanding evolutionary history. We can trace the pathway from the origin of life to any individual species by navigating through the evolutionary branches between the two points. Also, by starting with a single species and tracing back towards the "trunk" of the tree, one can discover species' ancestors, as well as where lineages share a common ancestry. In addition, we can use the tree to study entire groups of organisms.

Another point to mention on phylogenetic tree structure is that rotation at branch points does not change the information. For example, if a branch point rotated and the taxon order changed, this would not alter the information because each taxon's evolution from the branch point was independent of the other.

Many disciplines within the study of biology contribute to understanding how past and present life evolved over time; these disciplines together contribute to building, updating, and maintaining the “tree of life.” **Systematics** is the field that scientists use to organize and classify organisms based on evolutionary relationships. Researchers may use data from fossils, from studying the body part structures, or molecules that an organism uses, and DNA analysis. By combining data from many sources, scientists can construct an organism's phylogeny. Since phylogenetic trees are hypotheses, they will continue to change as researchers discover new types of life and learn new information.

Limitations of Phylogenetic Trees

It may be easy to assume that more closely related organisms look more alike, and while this is often the case, it is not always true. If two closely related lineages evolved under significantly varied surroundings, it is possible for the two groups to appear more different than other groups that are not as closely related. For example, the phylogenetic tree in Figure 21.1.3: shows that lizards and rabbits both have amniotic eggs; whereas, frogs do not. Yet lizards and frogs appear more similar than lizards and rabbits.

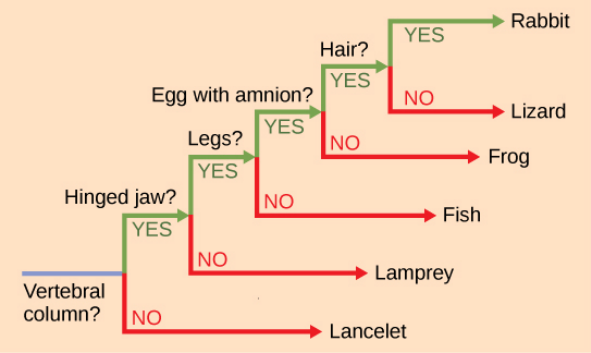


Figure 21.1.3: An organism that lacked a vertebral column roots this ladder-like phylogenetic tree of vertebrates. At each branch point, scientists place organisms with different characters in different groups based on shared characteristics.

Another aspect of phylogenetic trees is that, unless otherwise indicated, the branches do not account for length of time, only the evolutionary order. In other words, a branch's length does not typically mean more time passed, nor does a short branch mean less time passed— unless specified on the diagram. For example, in Figure 20.4, the tree does not indicate how much time passed between the evolution of amniotic eggs and hair. What the tree does show is the order in which things took place. Again using Figure 20.4, the tree shows that the oldest trait is the vertebral column, followed by hinged jaws, and so forth. Remember that any phylogenetic tree is a part of the greater whole, and like a real tree, it does not grow in only one direction after a new branch develops. Thus, for the organisms in Figure 20.4, just because a vertebral column evolved does not mean that invertebrate evolution ceased. It only means that a new branch formed. Also, groups that are not closely related, but evolve under similar conditions, may appear more phenotypically similar to each other than to a close relative.

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21.2: Exercise

Part 1: Natural Selection Exercise—Generation 1

This exercise illustrates the effect of natural selection on populations of predators and prey. Students will represent predators, each with a different adaptation for capturing their prey. The prey will consist of different species represented by different colored beans.

Procedure:

1. Each team of 4 students will count out exactly 100 dried beans of each color.
2. Thoroughly mix the beans and spread them evenly over your "habitat." Your habitat depends on the weather.
    1. If the weather is poor, it is dark outside, or your instructor would rather, your habitat will be a tray of sediment in the classroom.
    2. If the weather is lovely, or your instructor is adventurous, you will do this lab outside. Each team will mark off a 1m × 1m "habitat" in the grass using yarn, a meter stick, and wood stakes.
    3. All "preys" are confined to the habitat, wherever it is!
3. Each student (predator) will have a different feeding apparatus: A fork, spoon, knife or forceps.

Before you begin, hypothesize which predator will be the most successful. Hypothesize which prey species will be the most successful. As with all hypotheses, be sure to include a reason for your thoughts. Write these two hypotheses down in your lab notebook.

1. When everyone is ready, predators will spend 60 seconds capturing prey with their devices and depositing them into a cup while obeying the following rules:
    1. Predators must only use their capture device to capture prey.
    2. Predators may not scoop prey up with their cup.
    3. If predators "eat" too much of the environment, they will become constipated and DIE.
2. Each predator determines the number of prey captured and records results in Data Sheet: Generation 1.
3. Calculate and fill in the remaining statistics on the data sheet (see example below).

Data Sheet: Generation 1

Data Sheet: Generation 1

Prey Type	Black bean	Pinto bean	Red bean	White bean	Total	% Captured
Population Size	100	100	100	100	400	—
Forceps						
Spoon						
Fork						
Knife						

Prey Type	Black bean	Pinto bean	Red bean	White bean	Total	% Captured
Total Kills						
# Survived						
% Survived						
% Total Population						

Example of Data Collection and Analysis for Generation 1

Prey Type	Black bean	Pinto bean	Red bean	White bean	Total	% Captured
Population Size	100	100	100	100	400	—
Forceps	8	15	22	12	57	14%
Spoon	14	29	21	18	82	21%
Fork	10	20	14	19	63	15%
Knife	15	30	20	10	75	19%

Prey Type	Black bean	Pinto bean	Red bean	White bean	Total	% Captured
Total Kills	47[1]	94	77	59	—	—
# of This Bean That Survived	53[2]	6	23	41	123[3]	—
% of This Bean That Survived	53%[4]	6%	23%	41%	—	—
% Total Population	43%[5]	5%	19%	3%	—	—

of This Bean That Survived = population size – total kills

% of This Bean That Survived = (# survived/population size) x 100

% Total Population = (# survived/total survived) x 100

Part 2: Natural Selection Exercise—Generation 2

The predator with the lowest capture percentage will go “extinct” and will not participate in the next exercise. The predator with the highest capture percentage will reproduce itself and the “offspring” will participate in the next exercise. The surviving prey will also survive and reproduce.

Procedure:

1. The person with the lowest capture percentage (as calculated in the previous exercise) will “die” and turn in their feeding device.
2. The person with the highest capture percentage will reproduce by having the “dead” person use their same feeding device in the next round.
    1. If the Fork won the first round and the Spoon lost, then in the second round, there will be **two** Forks and **zero** Spoons. There will also be one Knife and one pair of Forceps.
3. Now the surviving prey will reproduce and double.
    1. If there are 40 surviving black beans, you will add another 40 black beans to the habitat, so there are a total of 80 black beans in the habitat for round 2.
4. Repeat the procedure you carried out in Part 1. Collect data for Generation 2.

Data Sheet: Generation 2

Prey Type	Black bean	Pinto bean	Red bean	White bean	Total	% Captured
Population Size	100	100	100	100	400	—
Forceps						
Spoon						
Fork						
Knife						

Prey Type	Black bean	Pinto bean	Red bean	White bean	Total	% Captured
Total Kills						
# Survived						
% Survived						
% Total Population						

> Note
>
> For population size in generation 2, multiply the number that survived in generation 1 by two.

Part 3: Natural Selection Exercise—Generation 3

The winning predator will reproduce again and the surviving prey will also reproduce (just like they did in the previous exercise). Collect and record new data.

Data Sheet: Generation 3

Prey Type	Black bean	Pinto bean	Red bean	White bean	Total	% Captured
Population Size	100	100	100	100	400	—
Forceps						
Spoon						
Fork						
Knife						

Prey Type	Black bean	Pinto bean	Red bean	White bean	Total	% Captured
Total Kills						
# Survived						
% Survived						
% Total Population						

> Note
>
> For population size in generation 2, multiply the number that survived in generation 1 by two.

Part 4: Pie Chart Analysis of Predator and Prey Populations

Now that you have collected data from three generations of predator and prey populations, you will use the data to create a set of pie charts to help you interpret your results. The first pair of pie charts represent the data from the original predator and prey populations. Use these examples to create your own charts using your group's data.

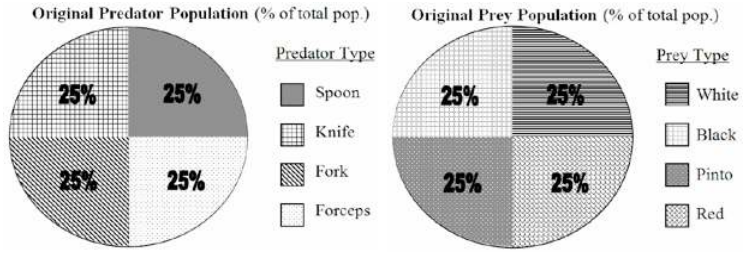
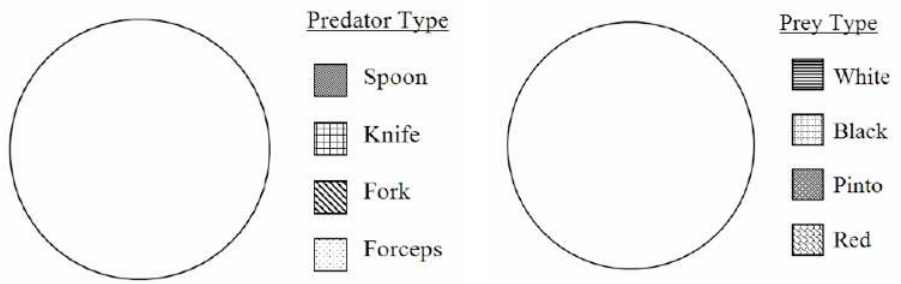
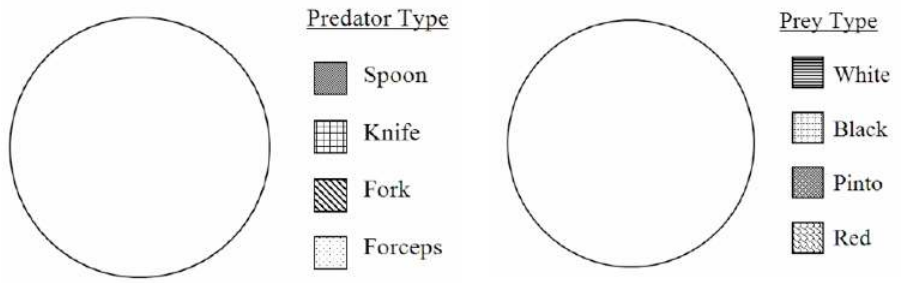


Figure 21.2.1: data from the original predator and prey populations.

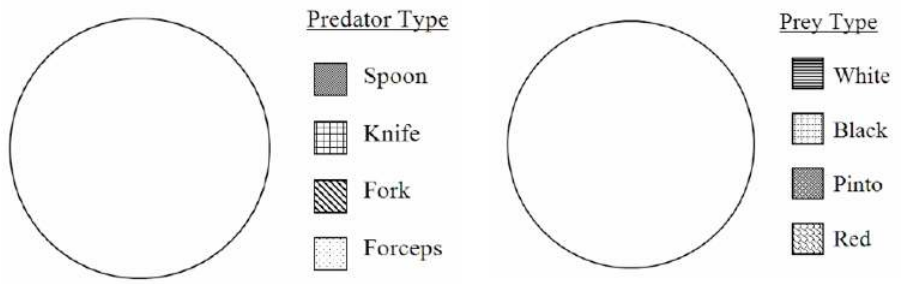
End of First Generation:



End of Second Generation:



End of Third Generation:



Questions:

1. Explain in your own words the process of natural selection.
2. What conclusions can you draw regarding the effect of natural selection on the predator populations in this exercise?
3. What conclusions can you draw regarding the effect of natural selection on the prey populations in this exercise?

4. Why did we start with 4 predators but 200 prey? What happen if numbers of predators and prey are equal? Explain
5. Imagine a scenario where the one of the predator groups has very low genetic variability. A disease comes through and modifies the prey-capturing tool, decreasing the predator's ability to hunt. Predict what would happen to that particular predator group.
6. Relate the concept of **natural selection** to the process of **evolution**.
7. Apart from natural selection, the real evolution process will also be influenced by (a) mutations, (b) migrations from other populations and (c) random processes ("genetic drift"). How you would change the rules of game in order to accommodate one or more of these processes?
    - mutations
    - migration
    - genetic drift

---

1. 8 + 14 + 10 +15 ↵
2. 100 – 47 ↵
3. 53 + 6 + 23 + 41 ↵
4. (53/100) × 100 (6/100) × 100 ↵
5. (53/123) × 100 ↵

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Cladistics and Phylogenetics

A new system of phylogenetic classification, called cladistics, is currently in practice today. A cladogram is a hypothesis about the evolutionary relationships between the organisms depicted on the tree. In this way, a cladogram illustrates the lines of descent for these organisms. A cladogram proposes an answer to the question "Which groups of organisms share a common ancestry?"

Take a look at these two identical, generic cladograms. The capital letters indicate the terminal organisms represented in the tree. The numbers indicate characters present in organisms beyond that point. And the nodes (indicated by the lowercase letters and the dots) represent the common ancestors of the terminal organisms. Even though they look different, if you examine them closely, these two cladograms are depicting the same relationships between critters A, B and C.

In this example (Fig 2) you see that A is more closely related to B than C based on the shared derived characteristic 1. Note that at each branch a derived characteristic is indicated that separates the left branch from the right branch of the evolutionary tree.**(opens in new window)**

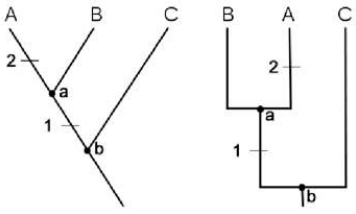


Figure 21.2.2: Two cladograms.

Now examine the cladogram at the bottom of this page illustrating the evolutionary relationships between a hagfish, shark, bony fish, frog, rat, bird, and lizard.

Procedure:

1. Name each organism on the cladogram.
2. Place a dot at every point that represents a common ancestor.
3. Indicate one shared derived characteristic that distinguishes each branch.
4. Who is more closely related: the shark and bony fish, or the bony fish and frog?

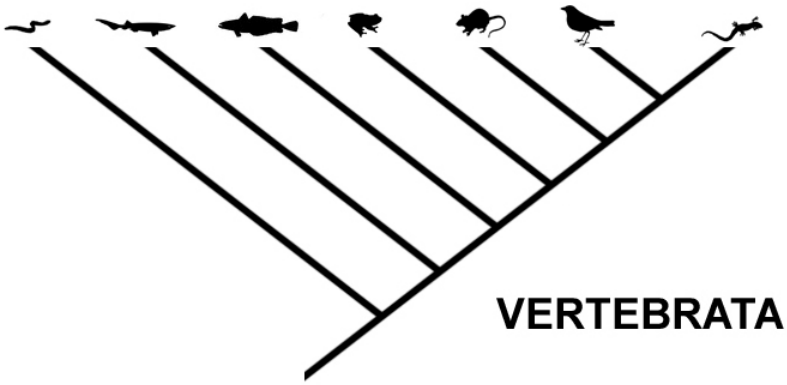


Figure 21.2.3: Cladogram for Subphylum Vertebrata.

Phylogenetics of Metal Objects

Define phylogenetics

You'll be provided with a set of metal objects. With your group, create a tree/cladogram of the proposed evolutionary relationships between your objects.

You may receive some or all of the following objects:

- 75 mm tack [A]
- 20 mm nail [B]
- 20 mm screw [C]
- Hairpin (50 mm) [D]
- Staple (25 mm) [E]
- Safety pin (40 mm) [F]
- Split rivet (20 mm) [G]
- Paperclip (32 mm) [H]
- 25 mm tack [J]
- Upholstery pin (20 mm) [K]
- 13 mm nail [L]
- Mirror screw (20 mm) [M]
- Insulated staple (13 mm) [N]
- Round-headed paper fastener (20 mm) [O]
- Flat-headed paper fastener (20 mm) [P]
- Round-headed screw (25 mm) [Q]
- 50 mm nail [R]
- Drawing pin (6 mm) [S]
- Hook (20 mm) [T]
- Kirby grip [W]
- Bolt (65 mm) [Z]

Questions:

1. What object did you choose for your common ancestor? Why?
2. Record your tree.
3. Choose one path and explain the sequence of events/line that led to the “most recent” object. For example, L → B → R is a line that depicts an increase in size.
4. Does your tree illustrate an example of convergent evolution? Explain.

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21.3: Lab Report

Part 1: Natural Selection Exercise—Generation 1

1. Hypothesize which predator will be the most successful? (As with all hypotheses, be sure to include a reason for your thoughts.)

2. Hypothesize which prey species will be the most successful? (As with all hypotheses, be sure to include a reason for your thoughts.)

Data Sheet: Generation 1

Prey Type	Black bean	Pinto bean	Red bean	White bean	Total	% Captured
Population Size	100	100	100	100	400	—
Forceps						
Spoon						
Fork						
Knife						

Prey Type	Black bean	Pinto bean	Red bean	White bean	Total	% Captured
Total Kills						
# Survived						
% Survived						
% Total Population						

Part 2: Natural Selection Exercise—Generation 2

Data Sheet: Generation 2

Prey Type	Black bean	Pinto bean	Red bean	White bean	Total	% Captured
Population Size	100	100	100	100	400	—
Forceps						
Spoon						
Fork						
Knife						

Prey Type	Black bean	Pinto bean	Red bean	White bean	Total	% Captured
Total Kills						
# Survived						
% Survived						
% Total Population						

Part 3: Natural Selection Exercise—Generation 3

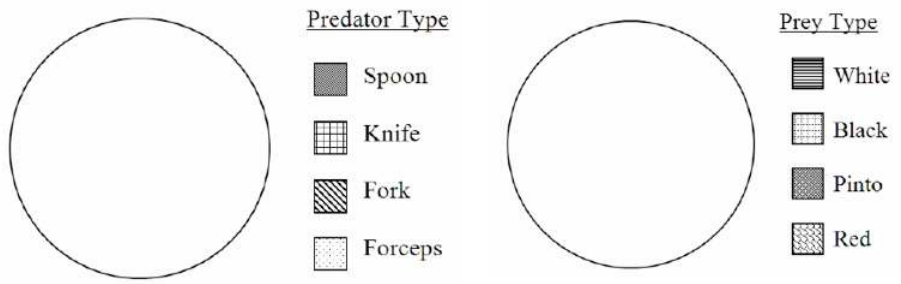
Data Sheet: Generation 3

Prey Type	Black bean	Pinto bean	Red bean	White bean	Total	% Captured
Population Size	100	100	100	100	400	—
Forceps						
Spoon						
Fork						
Knife						

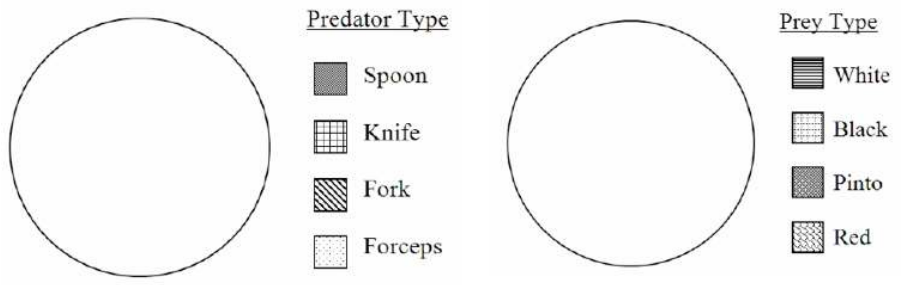
Prey Type	Black bean	Pinto bean	Red bean	White bean	Total	% Captured
Total Kills						
# Survived						
% Survived						
% Total Population						

Part 4: Pie Chart Analysis of Predator and Prey Populations

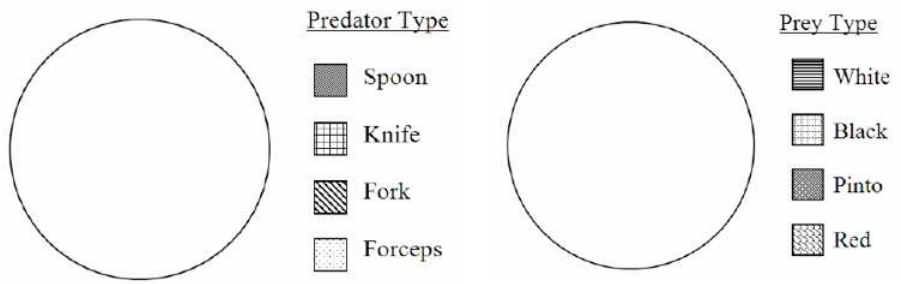
End of First Generation:



End of Second Generation:



End of Third Generation:



Questions:

1. Explain in your own words the process of natural selection.

2. What conclusions can you draw regarding the effect of natural selection on the predator populations in this exercise?

3. What conclusions can you draw regarding the effect of natural selection on the prey populations in this exercise?

4. Why did we start with 4 predators but 200 prey? What happen if numbers of predators and prey are equal? Explain.

5. Imagine a scenario where the one of the predator groups has very low genetic variability. A disease comes through and modifies the prey-capturing tool, decreasing the predator's ability to hunt. Predict what would happen to that particular predator group.

6. Relate the concept of **natural selection** to the process of **evolution**.

7. Apart from natural selection, the real evolution process will also be influenced by (a) mutations, (b) migrations from other populations and (c) random processes ("genetic drift"). How you would change the rules of game in order to accommodate one or more of these processes?
   - mutations
   - migration
   - genetic drift

---

1. 8 + 14 + 10 +15 ↵
2. 100 – 47 ↵
3. 53 + 6 + 23 + 41 ↵
4. (53/100) × 100 (6/100) × 100 ↵
5. (53/123) × 100 ↵

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Cladistics and Phylogenetics

1. Name each organism on the cladogram.
2. Place a dot at every point that represents a common ancestor.
3. Indicate one shared derived characteristic that distinguishes each branch.
4. Who is more closely related: the shark and bony fish, or the bony fish and frog?

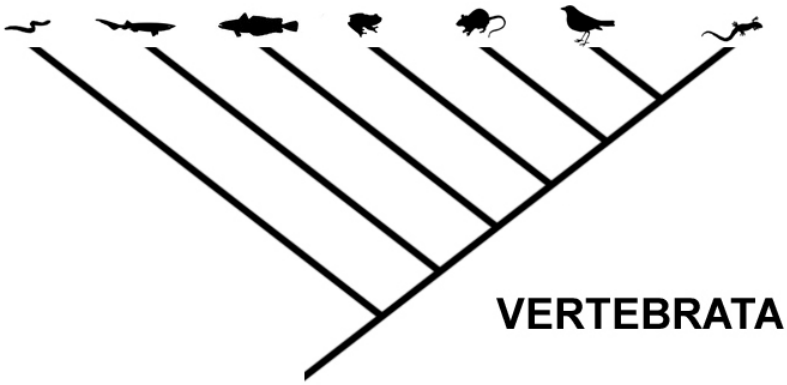


Figure 21.3.1: Cladogram for Subphylum Vertebrata.

Phylogenetics of Metal Objects

1. Define phylogenetics:

2. What object did you choose for your common ancestor? Why?

3. Record your tree.

4. Choose one path and explain the sequence of events/line that led to the “most recent” object. For example, L → B → R is a line that depicts an increase in size.

5. Does your tree illustrate an example of convergent evolution? Explain.

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CHAPTER OVERVIEW

22: Microscopy Part III

Learning Objectives

Content Objectives

- Describe the processes of primary succession and secondary succession
- Explain the stages of succession of milk
- Describe how pH changes as milk goes through successional stages

Skill Objectives

- Draw and describe the shapes of bacteria
- Identify gram negative and positive bacteria

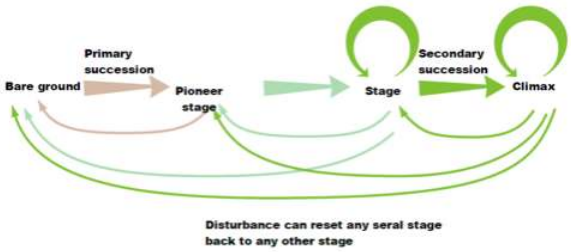
22.1: Introduction
22.2: Exercise
22.3: Lab Report

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22.1: Introduction

Ecological Succession of Bacteria in Milk

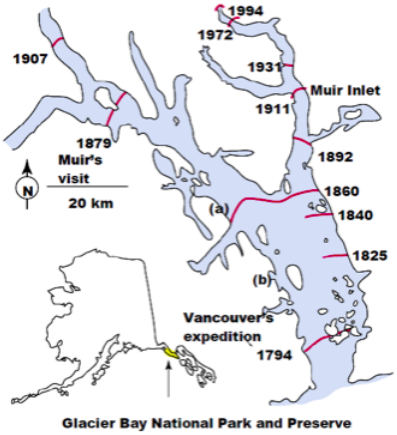
The communities within ecosystems develop over time, from very simple species assemblages, to complex, rich ecosystems. In this process, called succession, each *succeeding* species facilitates changes in environment which allow new species to come into the ecosystem. As the community becomes more and more complex, the biodiversity of the ecosystem also increases. Both biotic and abiotic processes can reset the succession process. That is, events cause by both the community itself, and outside events can return the community to an earlier succession state. The gradual changes in the community are both orderly and predictable in many ecosystems. The peak or most complex, advanced community that can develop in any abiotic environment is called the climax community. The picture below describes the developing communities as a series of steps, each of which can be driven against the succession process by disturbances:



QUESTION

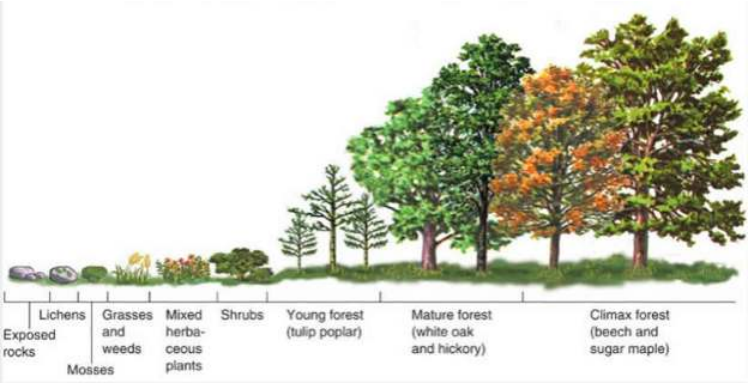
1. What types of events could “reset” a succession process? Name at least one biotic and one abiotic disturbance.

One example of a severe disturbance, reducing the land to bare ground, is the passage of a glacier. Though glaciers have not covered this part of North America for ten thousand years, there are parts of the continent that are even now becoming uncovered by receding glaciers. One area is the pacific northwest. From Juneau to Glacier Bay, many glaciers which have previously fallen directly into the ocean are now leaving bare soil which has not been exposed for more than fifty thousand years. Because the glaciers retreat very slowly, we can watch communities change across time in a single snap shot. Take the glaciers of Glacier Bay National Park, midway between Juneau and Anchorage, Alaska. The glaciers there have been retreating since the explorer Vancouver’s first expedition in 1794. Since then, the retreat has covered over 100 km, including new coastline, meadows and mountains:



QUESTION

1. Explain how this retreat will result in different communities along the glacier’s retreat, though in similar environments. Hint: what is the difference between exposed soil at point A and point B.



As the glacier retreats, it leaves nutrient poor soil which can only support simple plants such as liverworts, lichens, and other primitive plants. As they photosynthesize and die, we see them enter the decomposer pathway and increase the quality of the soil for later plants. However, this slow glacier retreat is a unique situation. To set up an experiment to test our understanding of succession would require hundreds of years, longer than a scientists lifetime. However, some organisms and communities proceed at a much faster rate, within your own refrigerators. The process of milk decomposition from a community of bacteria can test the same processes and theories in a much more reasonable time frame. This substitution of a simpler and faster community for experimental purposes is called a 'model' system.

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22.2: Exercise

Ecological Succession of Bacteria in Milk

Milk is a highly nutritious food containing carbohydrates (lactose, or milk sugar), proteins (casein, or curd), and lipids (butterfat). This high level of nutrition makes milk an excellent medium for the growth of bacteria. Pasteurizing milk does not sterilize it (sterilizing kills *all* bacteria) but merely destroys pathogenic bacteria, leaving many bacteria that can multiply and these bacteria will begin to grow and bring about milk spoilage. Biologists have discovered that as milk ages, changing conditions in the milk bring about a predictable, orderly succession of microorganism communities.

In this laboratory exercise, you will observe successional patterns in several types of milk. You will record changes in the environmental conditions of the types of milk as they age. These changes are a result of changes in the bacterial communities. Here are some of the major bacteria found at various stages.

Stages of Milk Succession

1. Psuedomonas and *Achromobacter* (gram-negative rods) digest butterfat and give milk a putrid smell
2. *Lactobacillus* (gram-positive rod) and *Streptococcus* (gram-positive coccus) ferment lactose to lactic acid and acetic acid.
3. Acidity sours milk and converts casein to curd.
4. Yeast (fungi) thrive in acidic conditions and metabolize the acids into non-acidic compounds.
5. *Bacillus* metabolize proteins into ammonia products and raise the milk's pH. Spoiled milk odor is very noticeable at this stage.

QUESTIONS

1. What are some advantages to using bacteria as opposed to plants in this experiment?
2. What factors might speed or delay a successional process? Apply your example to succession in milk.

Locate the milk samples available in the lab. Take a look at the samples and form two conditions you would like to study. Develop a short design for studying the first condition, state the dependent and independent variable, control and experimental group, the hypothesis and any variables which have been controlled. Then develop a short design for studying the second condition, state the dependent and independent variable, control and experimental group, the hypothesis and any variables which have been controlled.

Just as in any of our experiments, you must use effective scientific method**.** Develop hypotheses (at least 2—one with different milk types and one with either temperature or time as an independent variable) that you can test in the process of the milk community succession.

We will be performing a gram stain on our milk samples. Remember from our microbiology section that bacteria can be either gram negative (pink) or gram positive (purple). We will also look at these bacteria under a microscope to identify their shapes. Recall that bacteria can be cocci, bacilli, or spirillum.

QUESTIONS

1. What hypotheses are you testing? List both here.
2. What information led you to ask these hypotheses?
3. Make predictions about your hypotheses. i.e. How will you know if the data supports or refutes your hypotheses?
4. Be sure to identify the variables you will *test*, and those you will *control* for each experiment.
5. Prepare a table for data collection. You will be recording the pH, smell, consistency, and bacteria shapes and colors present in your milk samples.

On each lab bench are several small beakers. You will obtain a sample of the milk samples you need for your experimental design and test the pH, color, consistency, smell and other characteristics of each sample. For each milk sample:

1. Using the Vernier, take the pH of each flask. Record your results.
2. Record the color, odor (sour, putrid), and consistency (coagulation slight, moderate, chunky) for the milk in each flask.
3. Perform Gram Staining as outlined below. **Note: We will be using chemicals and open flames. Exercise caution when performing this portion of the lab.**

Materials

- Microscope slide
- Bunsen burner and tubing
- Crystal violet (primary stain)
- Iodine solution/Gram's Iodine (mordant that fixes crystal violet to cell wall)
- Decolorizer (e.g. ethanol)
- Safranin (secondary stain)
- Water (preferably in a squirt bottle)

Procedure

1. Make a slide with your milk sample to be stained. Heat fix the sample to the slide by carefully passing the slide with a drop or small piece of sample on it through a Bunsen burner three times.
2. Add the primary stain (crystal violet) to the sample/slide and let sit for 15 seconds. Rinse slide with a gentle stream of water for a maximum of 5 seconds to remove unbound crystal violet.
3. Add Gram's iodine for 15 seconds – this is an agent that fixes the crystal violet to the bacterial cell wall.
4. Rinse sample/slide with acetone or alcohol for ~3 seconds and **rinse with a gentle stream of water**. The alcohol will decolorize the sample if it is Gram negative, removing the crystal violet. However, **if the alcohol remains on the sample for too long, it may also decolorize Gram positive cells**.
5. Add the secondary stain, safranin, to the slide and incubate for 15 seconds. Wash with a gentle stream of water for a maximum of 5 seconds. If the bacteria is Gram positive, it will retain the primary stain (crystal violet) and not take the secondary stain (safranin), causing it to look violet/purple under a microscope. If the bacteria is Gram negative, it will lose the primary stain and take the secondary stain, causing it to appear red when viewed under a microscope.

QUESTIONS

1. Describe the changing sequence of organisms and corresponding environmental changes during succession in the milk samples. Which bacteria are in each of your milk samples?
2. Describe the changing sequence of organisms and corresponding environmental changes during succession in chocolate milk. Do the results of your investigation match your hypothesis?
3. Compare succession in one or more types milk. Propose reasons for differences.
4. Propose another experiment to test the environmental factors and/or organisms changing in your proposed scenario for milk succession.
5. How could you improve your test of the hypotheses? Be specific!
6. Identify what happened to the pH of the milk as time passed.
7. Infer what the change in pH means about the populations of microorganisms in the milk.

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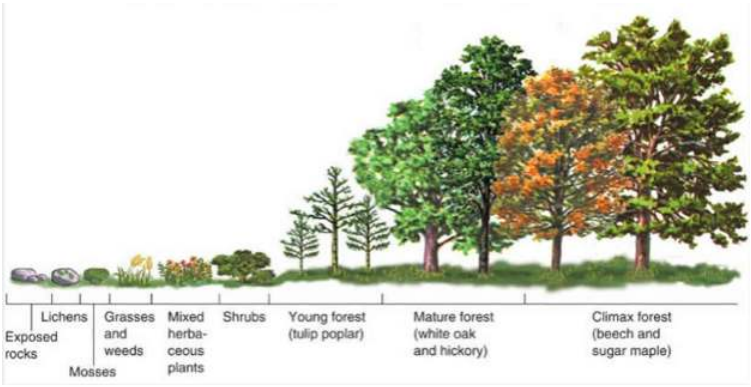
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22.3: Lab Report

1. What types of events could “reset” a succession process? Name at least one biotic and one abiotic disturbance.
2. Explain how this retreat will result in different communities along the glacier’s retreat, though in similar environments. Hint: what is the difference between exposed soil at point A and point B.



1. What are some advantages to using bacteria as opposed to plants in this experiment?
2. What factors might speed or delay a successional process? Apply your example to succession in milk.
3. What hypotheses are you testing? List both here.
4. What information led you to ask these hypotheses?
5. Make predictions about your hypotheses. i.e. How will you know if the data supports or refutes your hypotheses?
6. Be sure to identify the variables you will *test*, and those you will *control* for each experiment.
7. Prepare a table for data collection. You will be recording the pH, smell, consistency, and bacteria shapes and colors present in your milk samples.
8. Describe the changing sequence of organisms and corresponding environmental changes during succession in the milk samples. Which bacteria are in each of your milk samples?
9. Describe the changing sequence of organisms and corresponding environmental changes during succession in chocolate milk. Do the results of your investigation match your hypothesis?
10. Compare succession in one or more types milk. Propose reasons for differences.
11. Propose another experiment to test the environmental factors and/or organisms changing in your proposed scenario for milk succession.
12. How could you improve your test of the hypotheses? Be specific!
13. Identify what happened to the pH of the milk as time passed.
14. Infer what the change in pH means about the populations of microorganisms in the milk.

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CHAPTER OVERVIEW

23: Protist Diversity

Learning Objectives

Content Objectives

- Examine the complex evolutionary relationships of Eukaryotic organisms.
- Identify and describe the 8 major characteristics of Eukaryotes.
- Identify and classify various protist representatives of the proposed Eukaryotic Supergroups groups.

Skill Objectives

- Compare and contrast select representatives of protists within the Eukaryotic Supergroups.
- Prepare wet mounts of select living protists, and draw, label, and describe each.
- Examine prepared slides of protist representatives, and draw, label, and describe each.

23.1: Introduction
23.2: Exercise
23.3: Lab Report

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23.1: Introduction

Organisms are classified into three domains: Archaea, Bacteria, and Eukarya. The first two lineages comprise all prokaryotic cells, and the third contains all eukaryotes. A very sparse fossil record prevents us from determining what the first members of each of these lineages looked like, so it is possible that all the events that led to the last common ancestor of extant eukaryotes will remain unknown. However, comparative biology of extant (living) organisms and the limited fossil record provide some insight into the evolution of Eukarya.

The earliest fossils found appear to be those of domain Bacteria, most likely cyanobacteria. They are about 3.5 to 3.8 billion years old and are recognizable because of their relatively complex structure and, for prokaryotes, relatively large cells. Most other prokaryotes have small cells, 1 or 2 µm in size, and would be difficult to pick out as fossils. Fossil stromatolites suggest that at least some prokaryotes lived in interactive communities, and evidence from the structure of living eukaryotic cells suggests that it was similar ancestral interactions that gave rise to the eukaryotes. Most living eukaryotes have cells measuring 10 µm or greater. Structures this size, which might be fossilized remains of early eukaryotes, appear in the geological record in deposits dating to about 2.1 billion years ago.

Characteristics of Eukaryotes

Data from these fossils, as well as from the study of living genomes, have led comparative biologists to conclude that living eukaryotes are all descendants of a single common ancestor. Mapping the characteristics found in all major groups of eukaryotes reveals that the following characteristics are present in at least some of the members of each major lineage, or during some part of their life cycle, and therefore must have been present in the *last common ancestor*.

1. **Cells with nuclei surrounded by a nuclear envelope with nuclear pores**: This is the single characteristic that is both necessary and sufficient to define an organism as a eukaryote. All extant eukaryotes have cells with nuclei.
2. **Mitochondria**: Most extant eukaryotes have "typical" mitochondria, although some eukaryotes have very reduced mitochondrial “remnants” and a few lack detectable mitochondria.
3. **Cytoskeleton of microtubules and microfilaments**: Eukaryotic cells possess the structural and motility components called *actin microfilaments and microtubules*. All extant eukaryotes have these cytoskeletal elements.
4. **Flagella and cilia**: Organelles associated with cell motility. Some extant eukaryotes lack flagella and/or cilia, but their presence in related lineages suggests that they are descended from ancestors that possessed these organelles.
5. **Chromosomes organized by histones**: Each eukaryotic chromosome consists of a linear DNA molecule coiled around basic (alkaline) proteins called histones. The few eukaryotes with chromosomes lacking histones clearly evolved from ancestors that had them.
6. **Mitosis**: A process of nuclear division in which replicated chromosomes are divided and separated using elements of the cytoskeleton. Mitosis is universally present in eukaryotes.
7. **Sexual reproduction**: A meiotic process of nuclear division and genetic recombination unique to eukaryotes. During this process, diploid nuclei at one stage of the life cycle undergo meiosis to yield haploid nuclei, which subsequently fuse together (karyogamy) to create a diploid zygote nucleus.
8. **Cell walls**: It might be reasonable to conclude that the last common ancestor could make cell walls during some stage of its life cycle, simply because cell walls were present in their prokaryote precursors. However, not enough is known about eukaryotes’ cell walls and their development to know how much homology exists between those of prokaryotes and eukaryotes. If the last common ancestor could make cell walls, it is clear that this ability must have been lost in many groups.

In the span of several decades, the Kingdom Protista has been disassembled because sequence analyses have revealed new genetic (and therefore evolutionary) relationships among these eukaryotes. Moreover, protists that exhibit similar morphological features may have evolved analogous structures because of similar selective pressures—rather than because of recent common ancestry. This phenomenon, called convergent evolution, is one reason why protist classification is so challenging. The emerging classification scheme groups the entire domain Eukarya into six “supergroups” that contain all of the protists as well as animals, plants, and fungi that evolved from a common ancestor (Figure 23.1.1). Each of the supergroups is believed to be monophyletic, meaning that all organisms within each supergroup are believed to have evolved from a single common ancestor, and thus all members are most closely related to each other than to organisms outside that group. There is still evidence lacking for the monophyly of some groups. Each supergroup can be viewed as representing one of many variants on eukaryotic cell structure. In

each group, one or more of the defining characters of the eukaryotic cell—the nucleus, the cytoskeleton, and the endosymbiotic organelles—may have diverged from the "typical" pattern.

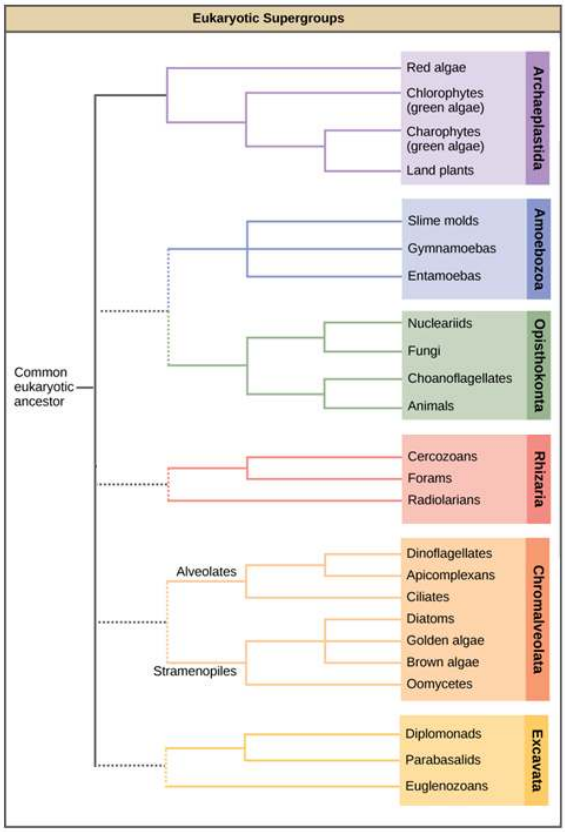


Figure 23.1.1: Eukaryotic supergroups. This diagram shows a proposed classification of the domain Eukarya. Currently, the domain Eukarya is divided into six supergroups. Within each supergroup are multiple kingdoms. Although each supergroup is believed to be monophyletic, the dotted lines suggest evolutionary relationships among the supergroups that continue to be debated.

Keep in mind that the classification scheme presented here represents just one of several hypotheses, and the true evolutionary relationships are still to be determined. The six supergroups may be modified or replaced by a more appropriate hierarchy as genetic, morphological, and ecological data accumulate. When learning about protists, it is helpful to focus less on the nomenclature and more on the commonalities and differences that illustrate how each group has exploited the possibilities of eukaryotic life.

Archaeplastida

Molecular evidence supports the hypothesis that all Archaeplastida are descendents of an endosymbiotic relationship between a heterotrophic protist and a cyanobacterium. The protist members of the group include the red algae and green algae. It was from a common ancestor of these protists that the land plants evolved, since their closest relatives are found in this group. The red and green algae include unicellular, multicellular, and colonial forms. A variety of algal life cycles exists, but the most complex is alternation of generations, in which both haploid and diploid stages are multicellular. A diploid sporophyte contains cells that undergo meiosis to produce haploid spores. The spores germinate and grow into a haploid gametophyte, which then makes gametes by mitosis. The gametes fuse to form a zygote that grows into a diploid sporophyte. Alternation of generations is seen in some species of Archaeplastid algae, as well as some species of Stramenopiles. In some species, the gametophyte and sporophyte look quite different, while in others they are nearly indistinguishable. We will cover the non-protist Archaeplastids (land plants) in a later lab.

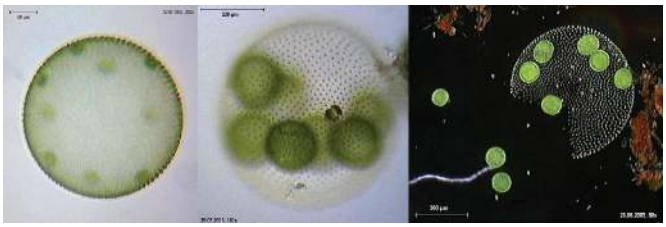


Figure 23.1.2: Volvox. *Volvox aureus* is a green alga in the supergroup Archaeplastida. This species exists as a colony, consisting of cells immersed in a gel-like matrix and intertwined with each other via hair-like cytoplasmic extensions. (credit: Dr. Ralf Wagner)

Amoebozoa

Like the Archaeplastida, the Amoebozoa include species with single cells, species with large multinucleated cells, and species that have multicellular phases. Amoebozoan cells characteristically exhibit pseudopodia that extend like tubes or flat lobes. These pseudopods project outward from anywhere on the cell surface and can anchor to a substrate. The protist then transports its cytoplasm into the pseudopod, thereby moving the entire cell. This type of motion is similar to the cytoplasmic streaming used to move organelles in the Archaeplastida, and is also used by other protists as a means of locomotion or as a method to distribute nutrients and oxygen. The Amoebozoa include both free-living and parasitic species.



Figure 23.1.3: Cellular Slime Mold. The image shows several stages in the life cycle of *Dictyostelium discoideum*, including aggregated cells, mobile slugs and their transformation into fruiting bodies with a cluster of spores supported by a stalk. (credit: By Usman Bashir (Own work) [CC BY-SA 4.0 ([creativecommons.org])], via Wikimedia Commons)

Opisthokonta

The Opisthokonts are named for the single posterior flagellum seen in flagellated cells of the group. The flagella of other protists are anterior and their movement pulls the cells along, while the opisthokonts are pushed. Protist members of the opisthokonts include the animal-like choanoflagellates, which are believed to resemble the common ancestor of sponges and perhaps, all animals. Choanoflagellates include unicellular and colonial forms (Figure 23.1.4), and number about 244 described species. In these organisms, the single, apical flagellum is surrounded by a contractile collar composed of microvilli. The collar is used to filter and collect bacteria for ingestion by the protist. A similar feeding mechanism is seen in the collar cells of sponges, which suggests a possible connection between choanoflagellates and animals.

The Mesomycetozoa form a small group of parasites, primarily of fish, and at least one form that can parasitize humans. Their life cycles are poorly understood. These organisms are of special interest, because they appear to be so closely related to animals. In the past, they were grouped with fungi and other protists based on their morphology. We will cover the non-protist Opisthokonts (animals and fungi) in later labs.

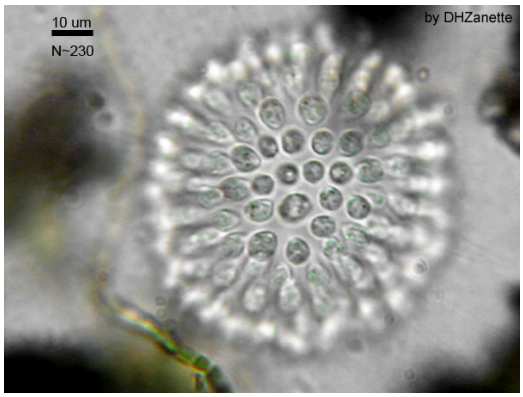


Figure 23.1.4: A Colonial Choanoflagellate. (credit: By Dhzanette ([en.Wikipedia.org]) [Public domain], via Wikimedia Commons)

The previous supergroups are all the products of primary endosymbiontic events and their organelles—nucleus, mitochondria, and chloroplasts—are what would be considered "typical," i.e., matching the diagrams you would find in an introductory biology book. The next three supergroups all contain at least some photosynthetic members whose chloroplasts were derived by secondary endosymbiosis. They also show some interesting variations in nuclear structure, and modification of mitochondria or chloroplasts.

Rhizaria

The Rhizaria supergroup includes many of the amoebas with thin threadlike, needle-like or root-like pseudopodia (Figure 23.1.5), rather than the broader lobed pseudopodia of the Amoebozoa. Many rhizarians make elaborate and beautiful tests—armor-like coverings for the body of the cell—composed of calcium carbonate, silicon, or strontium salts. Rhizarians have important roles in both carbon and nitrogen cycles. When rhizarians die, and their tests sink into deep water, the carbonates are out of reach of most decomposers, locking carbon dioxide away from the atmosphere. In general, this process by which carbon is transported deep into the ocean is described as the biological carbon pump, because carbon is “pumped” to the ocean depths where it is inaccessible to the atmosphere as carbon dioxide. The biological carbon pump is a crucial component of the carbon cycle that maintains lower atmospheric carbon dioxide levels. Foraminiferans are unusual in that they are the only eukaryotes known to participate in the nitrogen cycle by denitrification, an activity usually served only by prokaryotes.

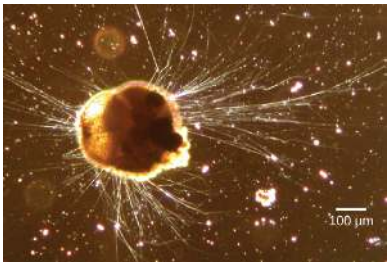


Figure 23.1.5: Rhizaria. *Ammonia tepida*, a Rhizaria species viewed here using phase contrast light microscopy, exhibits many threadlike pseudopodia. It also has a chambered calcium carbonate shell or test. (credit: modification of work by Scott Fay, UC Berkeley; scale-bar data from Matt Russell)

Foraminiferans

Foraminiferans, or forams, are unicellular heterotrophic protists, ranging from approximately 20 micrometers to several centimeters in length, and occasionally resembling tiny snails (Figure 23.1.6). As a group, the forams exhibit porous shells, called tests that are built from various organic materials and typically hardened with calcium carbonate. The tests may house photosynthetic algae, which the forams can harvest for nutrition. Foram pseudopodia extend through the pores and allow the forams to move, feed, and gather additional building materials. Typically, forams are associated with sand or other particles in marine or freshwater habitats. Foraminiferans are also useful as indicators of pollution and changes in global weather patterns.

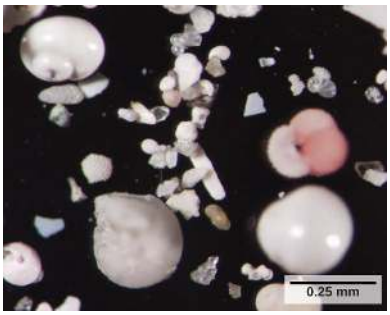


Figure 23.1.6: Foraminiferan Tests. These shells from foraminifera sank to the sea floor. (credit: Deep East 2001, NOAA/OER)

Radiolarians

A second subtype of Rhizaria, the radiolarians, exhibit intricate exteriors of glassy silica with radial or bilateral symmetry (Figure 23.1.7). Needle-like pseudopods supported by microtubules radiate outward from the cell bodies of these protists and function to catch food particles. The shells of dead radiolarians sink to the ocean floor, where they may accumulate in 100 meter-thick depths. Preserved, sedimented radiolarians are very common in the fossil record.

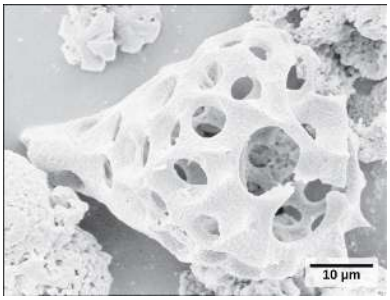


Figure 23.1.7: Radiolarian shell. This fossilized radiolarian shell was imaged using a scanning electron microscope. (credit: modification of work by Hannes Grobe, Alfred Wegener Institute; scale-bar data from Matt Russell)

Chromalveolata

Current evidence suggests that species classified as chromalveolates are derived from a common ancestor that engulfed a photosynthetic red algal cell, which itself had already evolved chloroplasts from an endosymbiotic relationship with a photosynthetic prokaryote. Therefore, the ancestor of chromalveolates is believed to have resulted from a secondary endosymbiotic event. However, some chromalveolates appear to have lost red alga-derived plastid organelles or lack plastid genes altogether. Therefore, this supergroup should be considered a hypothesis-based working group that is subject to change. Chromalveolates include very important photosynthetic organisms, such as diatoms, brown algae, and significant disease agents in animals and plants. The chromalveolates can be subdivided into alveolates and stramenopiles.



Figure 23.1.8: Dinoflagellate bioluminescence. Bioluminescence is emitted from dinoflagellates in a breaking wave, as seen from the New Jersey coast. (credit: “catalano82”/Flickr)

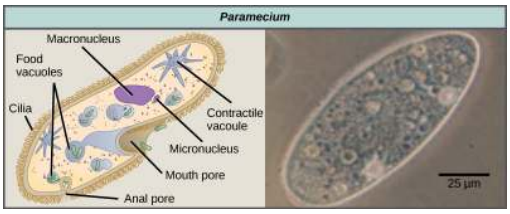


Figure 23.1.9: Paramecium. *Paramecium* has a primitive mouth (called an oral groove) to ingest food, and an anal pore to eliminate waste. Contractile vacuoles allow the organism to excrete excess water. Cilia enable the organism to move. (credit "paramecium micrograph": modification of work by NIH; scale-bar data from Matt Russell)

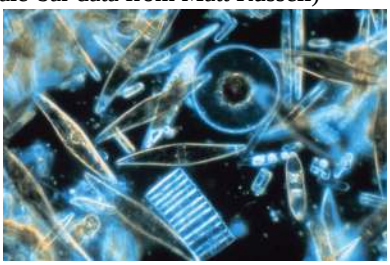


Figure 23.1.10: Diatoms. Assorted diatoms, visualized here using light microscopy, live among annual sea ice in McMurdo Sound, Antarctica. Diatoms range in size from 2 to 200 µm. (credit: Prof. Gordon T. Taylor, Stony Brook University, NSF, NOAA)

Excavata

Many of the protist species classified into the supergroup Excavata are asymmetrical, single-celled organisms with a feeding groove "excavated" from one side. This supergroup includes heterotrophic predators, photosynthetic species, and parasites. Its subgroups are the diplomonads, parabasalids, and euglenozoans. The group includes a variety of modified mitochondria, as well as chloroplasts derived from green algae by secondary endosymbiosis. Many of the euglenozoans are free-living, but most diplomonads and parabasalids are symbionts or parasites.



Figure 23.1.11: Giardia. The mammalian intestinal parasite *Giardia lamblia*, visualized here using scanning electron microscopy, is a waterborne protist that causes severe diarrhea when ingested. (credit: modification of work by Janice Carr, CDC; scale-bar data from Matt Russell)

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23.2: Exercise

Procedure

Live Specimens

Make wet mount slides of the live specimens we have in lab. You may want to make one slide at a time and view each one, so you can see the moving organisms.

1. Amoeba
2. Paramecium
3. Euglena
4. Pond water (this will contain many organisms, some of which are protists and some are not)

Fill out the table below.

Organism, Supergroup	Characteristics	Mode of motility	Drawing

Preserved Specimens and Slides

View the available specimens in lab, and fill out the table below.

Organism	Supergroup	Characteristics	Drawing

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23.3: Lab Report

1. Fill out the table below.

Organism, Supergroup	Characteristics	Mode of motility	Drawing

Preserved Specimens and Slides

View the available specimens in lab, and fill out the table below.

Organism	Supergroup	Characteristics	Drawing

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CHAPTER OVERVIEW

24: Fungal Diversity

Learning Objectives

Content Objectives

- Learn the traits used to distinguish between true fungi and fungus-like organisms
- Compare feeding strategies and ecology of different fungal groups
- Learn the differences between groups of macrofungi
- Understand the relationships between fungi and other organisms

Skill Objectives

- Identify important structures in the haplontic *Rhizopus* life cycle. Know the function and ploidy of these structures.
- Interpret a phylogeny to draw conclusions about the relatedness of organismal groups
- Use stains to view microscopic features more effectively
- Identify features of fungi both microscopically and macroscopically

24.1: Introduction
24.2: Exercise
24.3: Lab Report

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24.1: Introduction

There are many groups of unrelated organisms that are referred to as ‘fungus’ or ‘mold’. Formerly, these groups were all believed to have been related, sharing the characteristics of being heterotrophic eukaryotes, generally with cell walls, usually decomposers or parasites, and often with the storage carbohydrate glycogen. These organisms were placed into groups ending with “-mycota”, meaning fungus. On closer inspection, we find that these groups differ on many important life history traits and very few of them actually belong to the true Fungi. However, if you take a mycology course, you will most likely study these unrelated organisms, as well. Similarly, fungi are still studied under the umbrella of botany as a relic of past classification, but they are more closely related to animals than they are to plants.

Fungus-Like Organisms

Myxogastria (formerly Myxomycota): Plasmodial Slime Molds

Myxomycetes (members of the Myxogastria) are fungus-like organisms called slime molds, but they are not members of Kingdom Fungi. In their feeding stage, myxomycetes form one large amoeba with many nuclei and no cell wall. This amoeba moves over damp, decaying material looking for bacteria to engulf and digest. When it dries out or runs out of food, it begins to make fruiting structures called **sporangia** (sporangium, singular). Inside these sporangia, the myxomycete will undergo meiosis, wall off individual nuclei and make **haploid spores** for aerial dispersal. Dispersal by spores, heterotrophism, and glycogen as a storage carbohydrate originally classified this group within Kingdom Fungi, but this is the end of the similarities. The spores have **cell walls made of cellulose**, like plants. When these spores land, they will germinate into haploid cells (called swarm cells) that will fuse together to form a diploid amoeba. This means that, unlike true Fungi, they have a diplontic life cycle.

> Observe the slime molds on display. If possible, make a wet mount of a small sample. If there are motile cells (cells that are actively moving around), look for the presence of 2 flagella. This also distinguishes myxomycetes from Kingdom Fungi, where only one flagellum is present. If a mature amoeba is present, can you see **cytoplasmic streaming** occurring?
>
> Draw what you see and make observations below. Label the name, function, and ploidy of any identifying structures.

Other Slime Molds: Dictyostelia and Protostelia

Quite on theme for fungal classification, there are several different groups of unrelated organisms that are called slime molds. The two mentioned above, Dictyostelia and Protostelia, represent cellular slime molds and are relatively closely related to the plasmodial slime molds. Unlike the plasmodial slime molds, these groups exist as individual amoebae until it is time to leave the area. In the Dictyostelia, the amoebae assemble together to form elaborate fruiting structures where only some of the individuals will become spores and the rest will die. They are studied for this altruistic behavior.

Oomycota -- The Water Molds

Oomycetes are also fungus-like organisms with **cell walls made of cellulose**. Similar to myxomycetes, they have motile spores with 2 flagella. However, one of these flagella is "normal"-looking (called a whiplash flagellum) and the other is ornamented. This strange characteristic puts organisms into a group called the heterokonts (meaning "different flagella"). Like us, true Fungi are part of the opisthokonts (opisth- meaning rear, -kont meaning flagellum).

> Also similar to myxomycetes, oomycetes have a **diplontic life cycle**. What does this mean?

Kingdom Fungi - The True Fungi

Though many heterotrophic decomposers and parasites are fungus-like, an organism must be in Kingdom Fungi to be considered a true fungus. In addition to genetic relationships, organisms are classified into Kingdom Fungi based on the following traits:

- Eukaryotic
- Heterotrophic by absorption
- Morphology: Unicellular or a thallus composed of **hyphae**. If present, motile cells have only a single, whiplash flagellum (like us).
- Cell walls: **Chitin**
- Storage carbohydrate: **Glycogen**

- Life cycle: Haplontic (though this one gets a bit weird)
- Ecology: Many fungi are decomposers, primarily in terrestrial ecosystems. Other groups of fungi live in symbiosis with other organisms as parasites, mutualists, or commensalists.

Determining the ancestry and relatedness of groups of fungi is surprisingly difficult. DNA sequencing has led to continual rearrangements of the fungal tree of life and, at the time of writing, there is no one agreed upon picture of the history of fungi that the author is aware of. However, there are a few major groups that Kingdom Fungi is commonly broken into, and these will be discussed in the following section.

The image below is from an open-access publication (doi.org/10.1128/ mBio.01739-16) and presents one possible hypothesis for the relationships between groups of fungi. This hypothesis is called a phylogeny and is based on genetics, as well as physiological and morphological features. No single phylogeny is currently accepted by all mycologists.

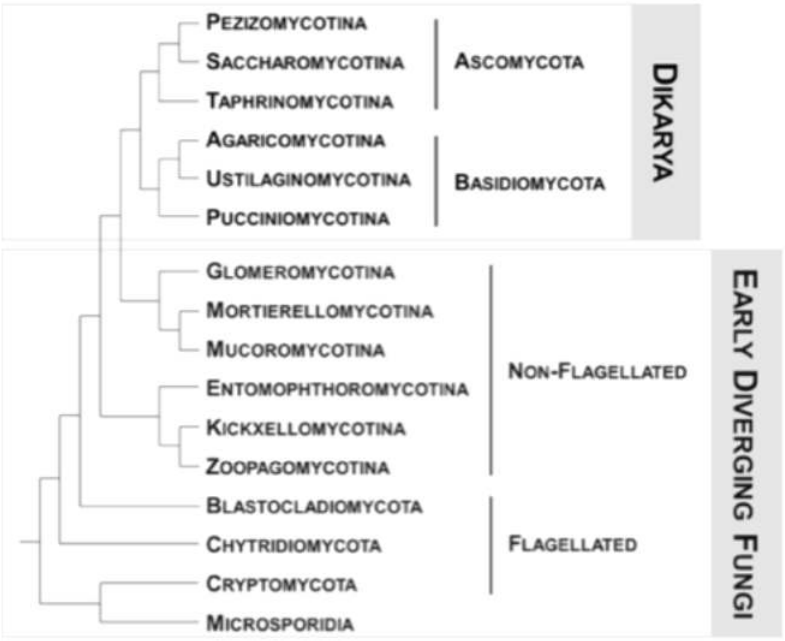


Figure 24.1.1: Fungal Phylogeny

Chytrids: includes Chytridiomycota and Blastocladiomycota from the above phylogeny

Chytrids comprise the oldest lineages of fungi. Unlike any other group within this kingdom, they are **aquatic** and have **swimming spores** (zoospores) with a single flagellum. Though many in this group are harmless decomposers, the most famous of the chytrids is *Batrachochytrium dendrobatidis*, a fungus that infects the skin of amphibians. This chytrid is contributing to a worldwide decline in many amphibian species (though there are numerous other contributing factors), particularly frogs.

If available view specimens of chytrids under the dissecting and compound microscopes. What features can you find?

Zygomycetes: includes all of the non-flagellated, early diverging fungi above, except Glomeromycotina

The zygomycetes are composed of at least two distinct lineages of fungi that all share a common structure during sexual reproduction, the **zygosporangium**. This is a large, ornamented, orange-to-brown structure where both fertilization and meiosis occur. Zygomycetes have no septations in their hyphae, which is referred to as being **coenocytic**. These fungi are commonly found on high-sugar substrates, such as rotting fruits or molding bread, or as insect parasites. Zygomycetes can reproduce asexually by producing **mitosporangia** (shown below), making haploid spores by mitosis.

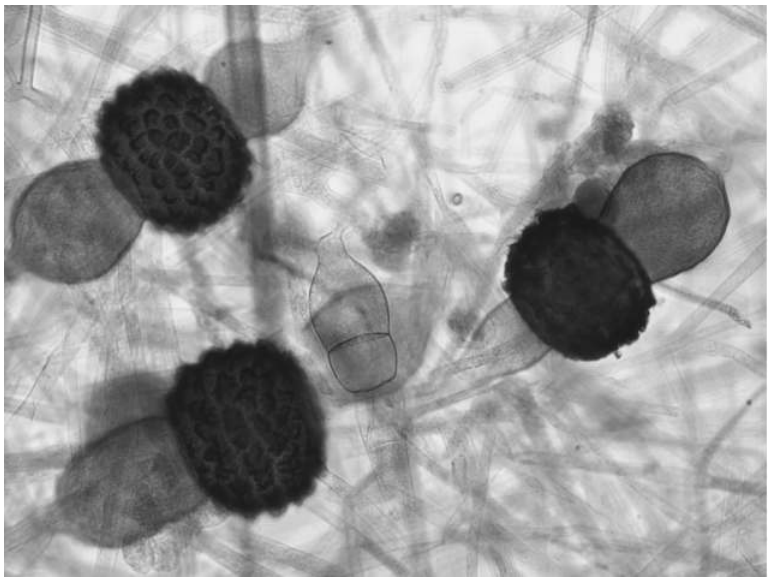


Figure 24.1.2: Zygosporangia

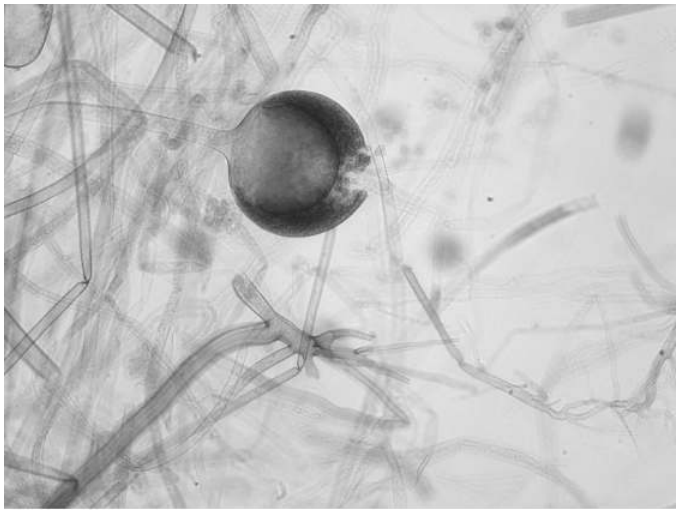


Figure 24.1.3: Mitosporangium24.6.6.3

Above are three mature zygosporangia produced during sexual reproduction of *Rhizopus stolonifer*. In the center of the image, two compatible mycelia (+ and -) have connected together and are currently forming gametangia. They will flood these gametangia with haploid nuclei, which will fuse within the zygosporangium to create diploid zygotes. Each of these will immediately undergo meiosis to produce haploid spores.

> View specimens of zygomycetes available in lab and record your observations below. Which features would help you identify this group?

Glomeromycota: listed as Glomeromycotina in the fungal phylogeny

This single lineage within Kingdom Fungi forms relationships with the roots of almost all land plant species and thalli of the earliest plant lineages, who evolved before roots. This **mycorrhizal** (myco- meaning fungus, rhiza meaning root) relationship has existed for 400 million years and was likely involved in the movement of plants onto land. Glomeromycetes are called **endomycorrhizal** because the fungal hyphae enter inside of plant cells. The fungus enters the plant tissue, usually through the roots, and penetrates the cell walls of the cortex cells in the root. However, they hyphae do not go through the plasma membrane. Instead, they form highly branched, tree-like structures called **arbuscles**. This provides a large amount of surface area for the plant and fungus to interact with each other. How are they interacting?

These images are from an open access paper (doi: 10.1038/srep29733) studying fungal colonization of plant cells. In the image on the right, the fungal tissue was stained with a fluorescent dye. In the lower two (B and C), fungal hyphae were stained dark and are forming arbuscules within the plant cell walls.

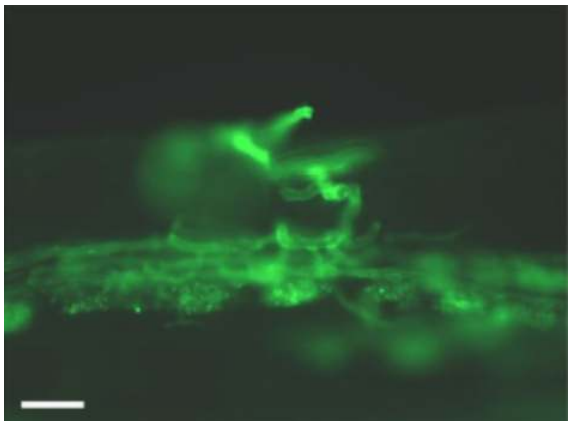


Figure 24.1.4: Endomycorrhizae

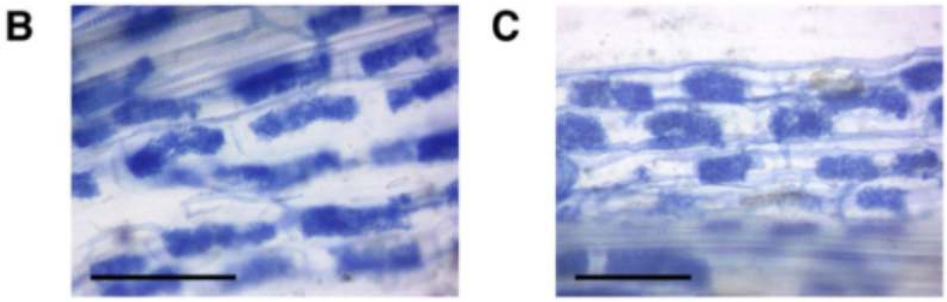


Figure 24.1.5:Endomycorrhizae B and C

Because it is a heterotroph, the fungus takes sugars from the plant. The fungal hyphae extend beyond the plant roots into the soil, where it can absorb water and nutrients that are transferred to the plant. Each partner gains a benefit from this relationship, so this is called a mutualistic symbiosis, or **mutualism**.

> A symbiotic relationship refers to a shared relationship between at least two organisms of different species. This relationship can benefit both parties, as above, only benefit one, potentially causing harm to the other. What would you call this last type of symbiosis?

> View a mycorrhizal root tip under the compound microscope, either as a prepared slide or from a fresh sample. If from a fresh sample, use 5% KOH + Phloxine B or Cotton Blue to stain the fungal tissue. Draw what you see below and label any distinguishing features of both the plant and fungus.

The Dikarya: Ascomycota and Basidiomycota

These two groups of fungi are referred to as the dikarya because some portion of their life cycle is **dikaryotic**. When two compatible mating types meet, they fuse together and begin the process of fertilization with plasmogamy (fusion of the cytoplasm). However, the nuclei do not fuse. Instead, a new mycelium is formed with two different haploid nuclei in each cell, making the ploidy n + n (as opposed to diploid, 2n). This state is called dikaryotic. Karyogamy (fusion of the nuclei) does not occur until the fungus is about to make spores. The two nuclei fuse and the zygote immediately undergoes meiosis, forming haploid spores.

> Which type of life cycle would you classify this as and why?

Though there are microscopic species and life stages in each group (including the yeast you saw earlier), members of the Ascomycota and Basidiomycota both form macroscopic fruiting bodies.

Fungi make a diversity of fruiting structures, structures in which meiosis occurs to form spores. In the microfungi, you saw zygomycetes making their fruiting structures: zygosporangia. In the macrofungi, you will learn three different types of ascocarps

and the anatomy of a basidiocarp, otherwise known as a mushroom. These structures are analogous to fruits produced on a tree. The rest of the fungal body (or, the rest of the tree, in this analogy) is the mycelium, buried within the substrate and busily acquiring food. Note: The mycelium is not the equivalent of the roots of the tree, but of the entire tree, including the roots, stems, and branches. Mushrooms are the ephemeral fruits of this structure, emerging for sexual reproduction.

Ascomycota -- The Sac Fungi

This group of fungi is when the life cycle starts to get particularly strange and the fungus forms something called a **dikaryon** (di- meaning two, karyo- meaning nucleus). When two different hyphal bodies fuse, they do not fuse their nuclei, only the cytoplasm. The result is a single fungal body with two separate types of nuclei floating around inside it. Because it is neither haploid (n), nor truly diploid (2n), this condition is called being dikaryotic (n+n).

Ascomycota are distinguished from other groups of fungi by the following characteristics:

- Production of **ascospores** (usually 8) within an **ascus**, a normally elongate, sac-like structure
- Hyphae with **simple septations**
- The mycelium is primarily haploid. When two haploid (n) mycelia of the correct mating types meet, they can form a dikaryotic (n+n) fruiting body called an **ascocarp**.

There are three different types of ascocarps:

1. **Apothecium** - a (usually) cup-shaped fruiting body with asci lining the interior of the cup. Two exceptions are Helvella and Morchella (morels), where the cup has been inverted, no longer looking like a cup, and the asci cover the now-exterior surface.
2. **Perithecium** - a round fruiting body with a long neck, much like a bottle, with the asci inside the bottom of the bottle. Microscopic and quite similar to a *Fucus* conceptacle.
3. **Cleistothecium** - a spherical, enclosed fruiting body packed with rounded asci. These are also microscopic and can be beautifully ornamented.

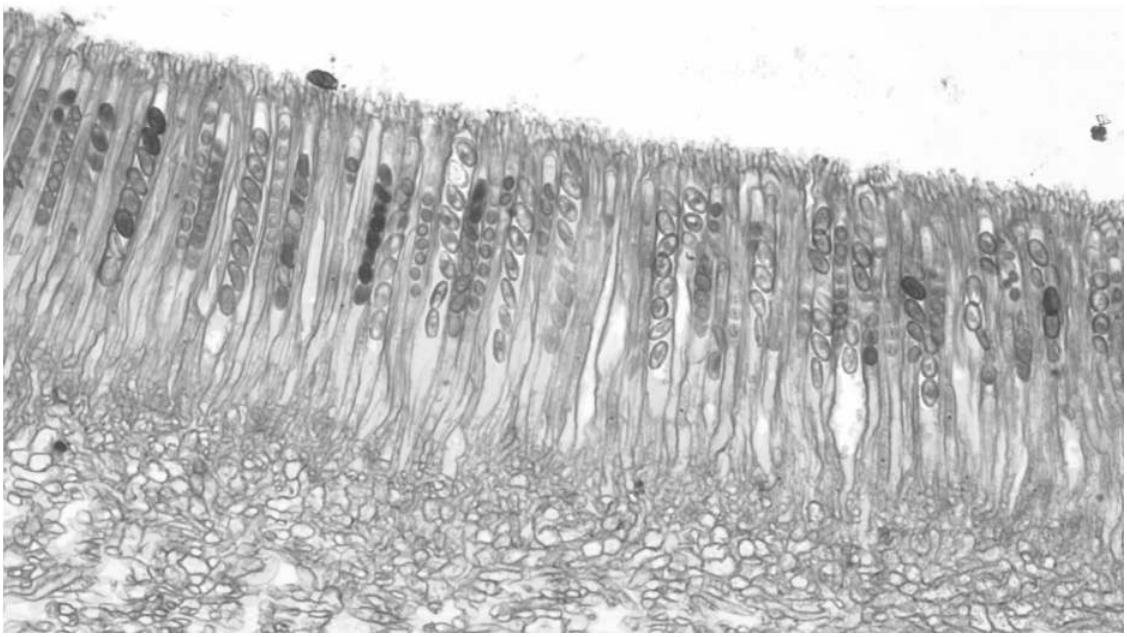


Figure 24.1.6:Peziza Asci

Above is the spore producing surface of an apothecium. The long asci each contain 8 ascospores.

Basidiomycota -- The Club Fungi

Basidiomycota are the other group of dikaryotic fungi. This group includes the mushroom-forming species of fungi, as well as two groups of (mostly) plant **parasites**, delightfully referred to as the rusts and the smuts.

Basidiomycota are distinguished from other groups of fungi by the following characteristics:

- **Basidiospores** (usually 4) produced from a **basidium**--a normally squat, roundish structure with prongs called **sterigmata** that spores are produced on.
- Hyphae with **complex septations.** These specialized septations look like they are surrounded by parentheses and help the mycelium maintain its dikaryotic state.
- **Clamp connections** can sometimes be seen, though are only obvious in certain genera.
- The mycelium is primarily dikaryotic. The haploid spores germinate and must find another germinating spore of the correct mating type to form a dikaryotic mycelium. This can then produce fruiting bodies called **basidiocarps**, or as we commonly call them, mushrooms.

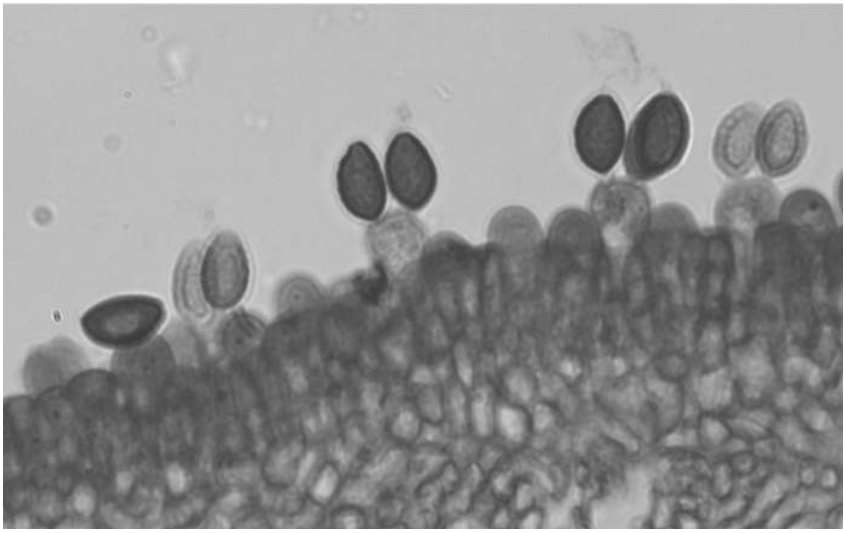


Figure 24.1.7:Coprinus Basidia

The image above shows the spore producing surface of a basidiomycete, *Coprinus*. The dark football-shaped structures are the haploid **basidiospores**. They sit atop small projections called sterigmata (**sterigma**, singular) that emerge from the top of the **basidium**. In most mushrooms, each basidium produces four basidiospores.

Contributors and Attributions

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24.2: Exercise

A. Microscopic Fungi

Unicellular fungi are called **yeasts**. Yeasts reproduce asexually by **budding**, where the cell produces an outgrowth that enlarges and is eventually pinched off, creating an identical copy of the parent cell. The parent cell is left with a small circular scar (called a **bud scar**) that, at the right angle, refracts the light of the microscope and appears to glow a brighter white.

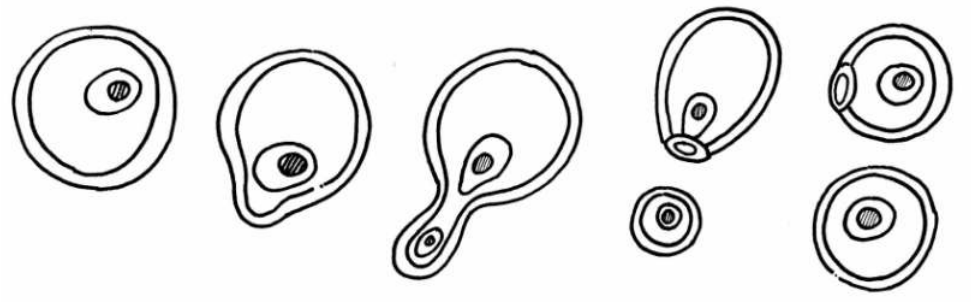


Figure 24.2.1: Yeast budding

Place a small drop of the yeast culture on a slide, dilute it with a drop or two of water, and add a cover slip. Observe your slide under the microscope at 400x (or 1000x, if possible) and look for yeast reproducing asexually. Can you see bud scars? (A1)

When fungi produce a larger vegetative body, it is called a **thallus** (a body plan that is not differentiated into tissues). The thallus is composed of microscopic strands called **hyphae** (pronounced high-fay). As a collective, these hyphae are called a **mycelium** (pronounced my-seal-ee-um).

> **Safety Note**
>
> Congo Red is carcinogenic, do not contact with skin. Do not wash slides with Phloxine B in the sink, as this compound is harmful to aquatic life. See your institution's MSDS for safety information on these compounds before use.

Obtain a sample of mycelium, make a wet mount with 5% KOH and stain it with Phloxine B and/or Congo Red. The stains are optional, but usually the fungal mycelium lacks pigment. The stains are picked up by the hyphae and allow them to stand out from the background. Phloxine B stains only the contents of the hyphae, while Congo Red stains the contents and walls. You can combine these two stains together or use separately.

In your prepared slide, can you see divisions of the hyphae into compartments? These dividing walls are called **septa** (septum singular) and are only present in certain groups of fungi. (A2)

Molds

M**olds** are the asexual form of a fungus. Before the advent of DNA sequencing, we thought molds were fungi that only reproduced asexually and they were placed in their own group, the Fungi Imperfecti (also sometimes called the Deuteromycota). As we are sequencing different mold species, we are discovering that they are identical to sexually reproducing fungi that we have already identified. This is causing a lot of trouble for taxonomy, as now we have one species with two names.



Figure 24.2.2: Mold Conidiophore

Asexually reproducing fungi produce spores called **conidia.** Much like the yeasts, many conidia are formed from budding off of another cell. Make a wet mount of a mold by adding a drop of 5% KOH to your slide, scraping a small amount of mold onto a razor blade or other tool, and gently depositing it onto the droplet. GENTLY lay down a cover slip. You may also consider trying to view molds as a dry mount, as they tend to be extremely hydrophobic. (A3)

B. Macroscopic Fungi and Lichens

Ascomycota

Observe the ascomycetes on display. What type of ascocarps do you see? (B1)

Make a thin section slide of Xylaria using KOH for the wet mount. (B2)

Make a thin section slide of an apothecium. (B3)

If available, view a cleistothecium from the Erysiphales (powdery mildews) under both the dissecting and compound microscope. (B4)

Basidiomycota

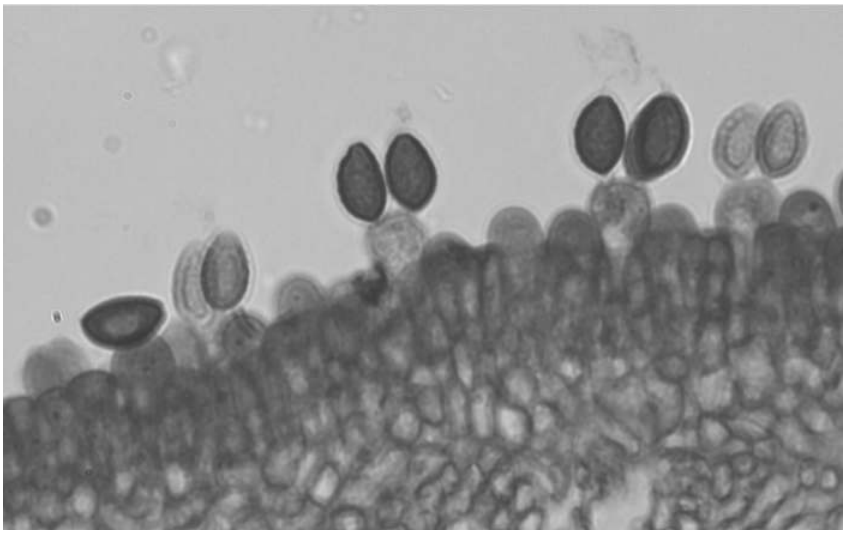


Figure 24.2.3: Coprinus Basidia

The image above shows the spore producing surface of a basidiomycete, *Coprinus*. The dark football-shaped structures are the haploid **basidiospores**. They sit atop small projections called sterigmata (**sterigma**, singular) that emerge from the top of the **basidium**. In most mushrooms, each basidium produces four basidiospores.

Based on the presence of four haploid spores, which type of cell division do you think formed these spores and why?

How could you explain the presence of 8 spores in ascomycetes?

Not all basidiocarps look like *Amanita muscaria*. Observe the diversity of basidiomycetes on display. (B4)

Make a thin section of a gilled mushroom, using 5% KOH for the wet mount. Try to find basidia and basidiospores. Can you see clamp connections? It may help to stain with Phloxine B. (B5)

The phylum Basidiomycota is composed of three major groups. All of the fungi above are from the subphylum Agaricomycotina. The two other groups are composed of primarily parasitic fungi, rusts (Pucciniomycotina) and smuts (Ustilaginomycotina). (B6)

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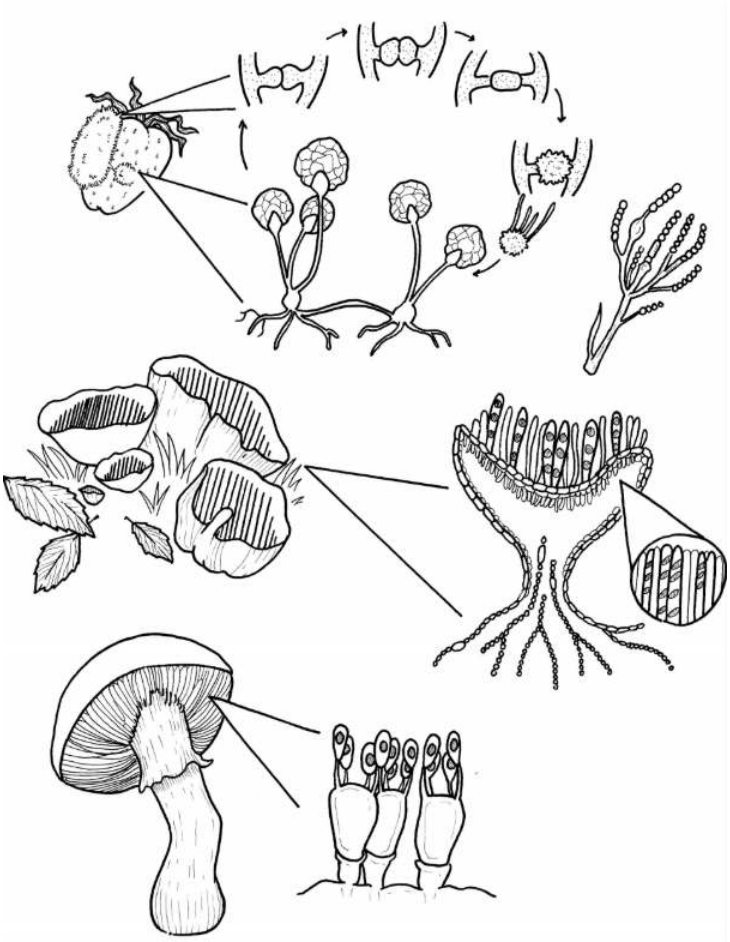
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24.3: Lab Report

A1. Draw what you see in the space below and label any important structures.

A2. Draw a strand of the hyphae (also sometimes called a hyphal thread or filament), label the **cell wall**, **septum** (if present), **plasma membrane**, and any other features you see.

A3. Look for **conidia** and **conidiophores**, structures where the conidia are produced. Draw what you find and label any identifiable structures.



https://bio.libretexts.org/@go/page/106102

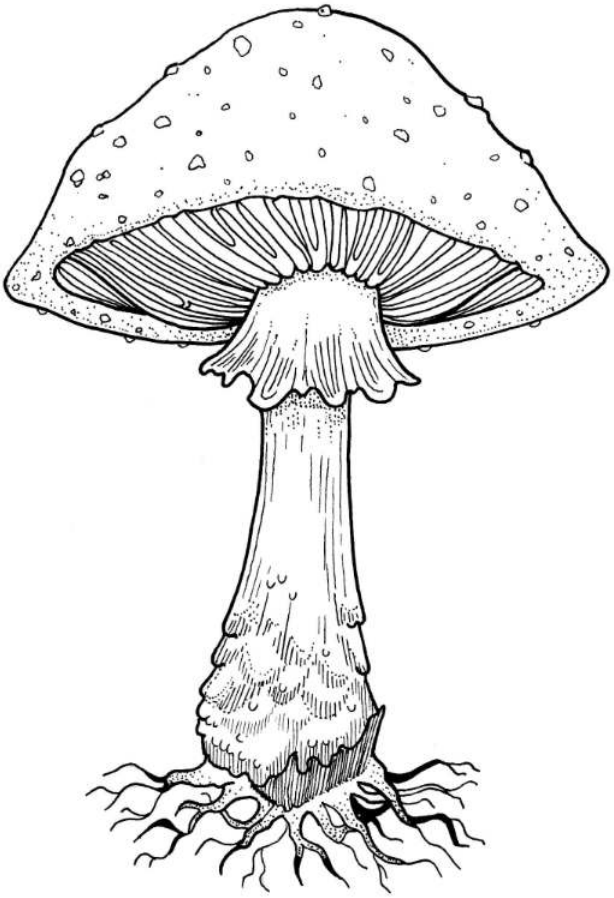


Figure 24.3.1: Label the structures in the image above.B1. Draw and label 2 ascomycetes on display, indicating where the asci would be found.24.6.5.3B2. Draw a perithecium. Label an ascus and ascospores.B3. Draw your slide of an apothecium, labelling an ascus and ascospores. Can you find septations in the hyphae?B4. If available, view and draw a cleistothecium from the Erysiphales (powdery mildews), labelling an ascus and ascospores.Label the anatomy of an Amanita muscaria basidiocarp below, including the cap (pileus), universal veil scales, gills (lamellae), annulus, stipe, volva, and mycelium.Figure 24.3.2: Amanita muscaria mushroom24.6.3.3B4. Draw and label a jelly mushroom, a coral mushroom, a bracket, and a puffball.B5. Draw and label a slice of a gilled mushroom.B6. View the available Basidiomycota prepared slides and draw what you see below. Can you find basidia and basidiospores? Compare and contrast what you see with the Agaricomycetes.Contributors and Attributions Maria Morrow (College of the Redwoods)

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CHAPTER OVERVIEW

25: Plant Diversity

Learning Objectives

- **Content Objectives**
- Examine seedless and seed plants. Compare and contrast the characteristics that distinguish them.
- Review the major characteristics of plants and their evolution from algae.

- **Skill Objectives**
- Draw and label various examples from the plant phyla, using prepared slides, wet mounts, and/or preserved specimens.
- Be able to identify major categories of plants based on characteristics

25.1: Introduction
25.2: Exercise
25.3: Lab Report

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25.1: Introduction

Plants (Kingdom Plantae) are a monophyletic group of eukaryotes that are multicelluar, photosynthetic, and living on land. The most recent evidence suggests that early land plants were most similar to multicellular green algaes (specifically charophytes). As they moved onto land, plants faced a different environment - the ocean environment differs from the land environment in features such as temperature, water availability, sunlight (and UV radiation), buoyancy, and more. With this new environment came challenges that resulted in new selective pressures and different adaptations in those that survived.

Major adaptations of land plants that also define the kingdom include

- Similar life cycles (including alternation of generations)
- Multicellular embryos
- Specialized multicellular structures for protection of gametes, spores, and/or seeds

In this lab you will investigate the diversity of plants on Earth, with a focus on two major innovations of land plants: vascular tissue and seeds. We will group plants into three major categories

1. Nonvascular plants (also called bryophytes). Examples: Mosses, liverworts, hornworts.
2. Vascular seedless plants. Examples: Club mosses, ferns, horsetails.
3. Vascular seed plants. Examples: Conifers, ginkgo, flowering plants

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Nonvascular plants (Bryophytes)

Bryophytes arose in a period of Earth's history before soils had formed. The terrestrial surface was rocky and consisted primarily of crusts (microbial mats) composed of assemblages of prokaryotes. The exposure to sunlight would have been intense relative to the buffer provided by water. In addition, being surrounded by water would provide regulation of surrounding temperature and structural support. As green algae began to colonize the terrestrial surface, at least one of these lineages accumulated adaptations that were favorable to living on land--a waxy **cuticle** to prevent water loss, desiccation-resistant dispersal propagules called **spores**, and retention and feeding of the developing zygote. This lineage of green algae evolved into the ancestor of the bryophytes. This evolutionary group includes liverworts, mosses, and hornworts. These plants do not have true roots to absorb water, nor do they have vascular tissue to transport that water to other regions of the plant. Because of this, bryophytes tend to grow prostrate (close to the surface they are growing on) and stay quite small. They also tend to grow in moist areas where there is access to water and are reliant on water for the dispersal of gametes and fertilization. There are approximately 23,000 known extant species.

The evolutionary relationships between bryophyte lineages are currently unresolved.* Members of this group have the following characteristics:

- Morphology: Multicellular, can be leafy or thalloid. Complex tissues, including an exterior protective layer. Root-like structures called rhizoids provide anchorage.
- Cell wall composition: Cellulose
- Chloroplasts: 2 membranes, pigments are chlorophyll a, chlorophyll b, and carotenoids
- Storage carbohydrate: Starch
- Life cycle: Alternation of generations. Gametophyte dominant: sporophytes grow from and are nourished by the female gametophyte.
- Ecology: Terrestrial, gametes are dispersed in water

*Note: As of 2019, much is unresolved on the early lineages of plants and who was first on land. Recent genetic analyses interpret bryophytes as being monophyletic, all deriving from a common ancestor that branched from the main line of plants. Read this open-access paper for further information: https://doi.org/10.1073/pnas.1323926111

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As bryophytes began to colonize the terrestrial surface, they produced organic acids during metabolism that aided in the breakdown of the rocky substrate. When they died, their organic matter mixed with the weathered rock, forming the Earth's earliest soils.

Formerly abundant to the first photosynthesizers to become terrestrial, access to sunlight became competitive as bryophytes expanded. This led to selection for individuals that could lift themselves higher and transport water throughout their tissues. Eventually, this selection resulted in the evolution of **vascular tissue** -- pipes that could bring water up from the ground so that parts of the plant could be raised upward, and those parts raised upward could transport their photosynthates down to the lower parts of the plant. The cells in the **xylem** (water-transporting vascular tissue) contained **lignin**, the tough, decay-resistant compound that wood is made out of. This rigid molecule in the vascular tissue allowed for structural support, allowing plants to grow taller -- some over 100 feet! The vascular system also allowed for the specialization of organs: **roots** for water absorption, **leaves** for photosynthesis, and **stems** for structural support.

Seedless vascular plants (SVPs) also began to rely more on the sporophyte stage. The sporophyte became the larger, nutritionally independent stage of the life cycle. **Branching sporophytes** offered more sites for meiosis to occur, resulting in increased opportunities for variation, which could be interpreted as more options in an increasingly competitive environment. There are approximately 20,000 known extant species, most of which are ferns.

SVPs are considered to be a paraphyletic group of organisms, forming two distinct lineages: Ferns and Lycophytes. These two lineages share the following characteristics:

- **Morphology:** Sporophytes develop complex tissues, including **lignified vascular tissue**, true roots, stems, and leaves. Sporophytes are branched, producing many sporangia. Gametophytes are reduced and thalloid. In some groups, the gametophyte is subterranean and parasitizes mycorrhizal fungi for sugars.
- **Life cycle:** Alternation of generations. **Sporophyte dominant**: sporophytes still grow from the gametophyte, but are now photosynthetic and the larger, longer-lived phase of the life cycle.
- **Ecology:** Gametes are still dispersed in water, so moisture is still required for fertilization.

Selection Pressures and Drivers

1. **Competition for sunlight**. To get access to sunlight, SVPs needed to grow taller than bryophytes. However, this presents a problem of distributing water around the plant body to prevent drying out. Seedless vascular plants solved this problem with the adaptation of lignified vascular tissue. The lignin in the secondary walls of sclerenchyma cells allowed SVPs the structural support to grow taller.
2. **The initial forming of soils**. Before the bryophytes, terrestrial surfaces were primarily rocky. The first land plants would have contributed to the chemical weathering of these rocks by producing acids during metabolism. After death, these plants add their organic matter to these weathering rocks, beginning to form Earth's early soils. Fungi were likely involved in this process, as there are genes in the bryophytes for mycorrhizal relationships.

Gymnosperms

Toward the end of the carboniferous period, major changes in the climate occurred. The current day European and North American continents slammed together, forming the Appalachian mountains (which were taller, at that time, than the present-day Himalayas). Fossil and geologic records show a tendency toward a drier climate, with evidence of glaciation and lowered sea levels. Inland seas were increasingly diverted into distinct river channels as woody debris channelled the movement of waterways. In short, the terrestrial surface began to dry out and there was much more of it. The ancestors of birds, reptiles and mammals were adapting eggs that could survive outside of the water -- plants were working toward a similar strategy. Around this time, a group of animals likely took flight for the first time -- the insects! The presence of flying insects allowed for another option for distributing pollen, as well as a large source of potential herbivory.

The plants that would become the gymnosperms evolved **xerophytic leaves** (see lab 7) to prevent desiccation in the dry air. Some would have the ability to grow wider (and thus taller) via the production of a new layer of secondary xylem (**wood**) each year. These plants could also produce exterior layers of dead cells, unlike the living epidermis, called **bark**. Together, the production of bark and wood are part

of a process called **secondary growth**. To increase the chances of fertilization in the absence of water, gametes began to be dispersed aerially via **pollen**. Perhaps most importantly, the zygote and female gametophyte were surrounded in a protective coating and dispersed as **seeds**. Both seeds and pollen develop within structures called **cones**.

The fossil record shows gymnosperms diversifying in a dry period called the Permian that followed the swampy Carboniferous period. Extant groups of gymnosperms include conifers, cycads (somewhat similar in appearance to palms), gnetophytes, and a single species from the ginkgophytes, *Ginkgo biloba*. Of the approximately 1,000 species of gymnosperms alive today, about 600 of these are conifers, 58 of which can be found in California. In fact, some of the oldest (bristlecone pine), tallest (coast redwood), and most massive (giant sequoia) organisms on the planet are conifers and all are native to California.

See this open-access paper for recent genetic work on the evolutionary relationships between gymnosperms: http://dx.doi.org/10.1098/rspb.2018.1012

Selection Pressures and Drivers

1. **Competition for sunlight**. Seedless vascular plants were able to reach heights up to 100 feet tall. In the lineage leading to the gymnosperms and angiosperms, some plants developed the ability to grow wider as they grew taller. This secondary growth allowed for increased stability and, eventually, to reach heights over 300 feet.
2. **Drought**. Dry conditions would have selected for plants with thicker cuticles, leaves with less surface area to evaporate from, and propagules that could disperse without water and survive through dry periods to germinate when water was available.
3. **Herbivory**. In addition to leaves that could resist drought, the presence of insects would have driven selection for plants that could defend against herbivory. The thick cuticle and tough texture of xerophytic leaves made them difficult to eat, while resin canals in both leaves and stems provided another line of defense.

Angiosperms

The exact timing of the emergence of angiosperms is unknown, so it is difficult to relate their evolution to specific climatic conditions or other circumstances. However, there is relatively new fossil evidence of flowering plants as early as the Jurassic period, 174 mya. This was the age of the dinosaurs and coincides with the emergence of the first feathered dinosaurs -- birds! Angiosperms represent a single origin of related organisms, the phylum Anthophyta, that experienced an exceptional radiation in species. As of 2019, there are approximately 370,000 known extant species. Most of the plants that you see, eat, and otherwise interact with in your daily life are likely to be in this group.

Angiosperms can be distinguished from other plant groups by the production of **flowers**. These collections of modified leaves allowed angiosperms to attract pollinators and increase the chances of successful fertilization. Over time, angiosperms evolved different flower morphologies, smells, and colors that corresponded to their particular pollinators. These sets of characteristics, called **pollination syndromes**, allow scientists to predict the pollinators for different plants.

In the xylem, this group of plants evolved large diameter conducting cells for rapid water uptake called **vessel elements**, though this made them vulnerable to freezing conditions. In the phloem, sieve cells evolved into **sieve tube elements** with their associated **companion cells**, increasingly specialized for transportation of photosynthates.

Selection Pressures and Drivers

1. **Competition for space**. Present day gymnosperms include the tallest, most massive, and some of the oldest organisms on the planet. With this in mind, you can imagine that they would be difficult to compete with. Angiosperms needed to evolve more

efficient methods of transporting water and photosynthates, fertilization, and survival of offspring.

2. **Insects, birds and mammals**. The primary response to insects that we see in gymnosperms is prevention of herbivory. While herbivory is still a driver of selection for angiosperms, insects also served as a more efficient method of pollen delivery. Insects and birds could be lured in with sugary nectar or scents and colors that mimicked other resources, then dusted with pollen as they investigated. If the lures were specialized enough, they would continue seeking the same resource, leading them to another plant of the same species. These scents, colors, and nectar resources were produced by structures that also produced pollen and ovules -- the flower. The diversity of animals present on Earth when angiosperms evolved would have resulted in increased seed predation. Fruits allowed for the dual purpose of protecting seeds and co-opting animals as dispersal agents, whether by ingestion or attachment. Some fruits evolved production of sugary tissues and bright colors to attract animals, while others evolved hairs or spines to latch onto their bodies.

The term angiosperm means “seed vessel” and refers to the production of fruits. Every flower becomes a fruit, though these fruits might not always fit with our cultural perception of what it means to be a fruit. Once pollinated, the fertilized seeds are encased in a protective ovary whose structure can be specialized for different methods of dispersal, such as animal ingestion, animal attachment, flotation, or wind dispersal. This protective ovary and the encased seed or seeds are more commonly called a **fruit**. Inside the developing seeds, angiosperms provide an additional food source to the developing zygote, the **endosperm**. The endosperm is produced by a process called **double fertilization** where one sperm fertilizes the egg and another fertilizes a pair of haploid nuclei, which makes the endosperm triploid (3n).

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25.2: Exercise

- Gametophyte morphology
  - **Leafy liverworts** have leaves arranged in a flat plane with a set of smaller underleaves
  - **Thalloid liverworts** have no leaves
  - Liverwort cells have multiple chloroplasts
  - Simple pores allow for gas exchange (no guard cells, meaning pores are permanently open)
  - Asexual clones, called **gemmae**, are sometimes produced
- Sporophyte morphology
  - Leafy liverworts produce single sporangium at the end of a seta (often fragile, transparent)
  - Marchantia, a thalloid liverwort, develops complex structures called where **gametangia** are produced

Leafy Liverworts

Cut off the end of a leafy liverwort and make a wet mount or obtain a prepared slide to view under the compound microscope. Draw the specimen below and indicate the three different rows of leaves (two in one plane, and a row of smaller underleaves running beneath the stem).

Still under the compound microscope, observe one of the leaf cells. Can you tell how many chloroplasts are in it? Draw this cell in the space below and label the chloroplasts and any other features you recognize.

Thalloid Liverworts: Marchantia

If available, observe a Marchantia polymorpha gametophyte under the dissecting scope. Look for **simple pores, rhizoids, archegoniophores, antheridiophores**, and **gemmae cups** containing asexual clones of the gametophyte, called **gemmae**. Label the bolded features in the life cycle diagram.

Marchantia life cycle:

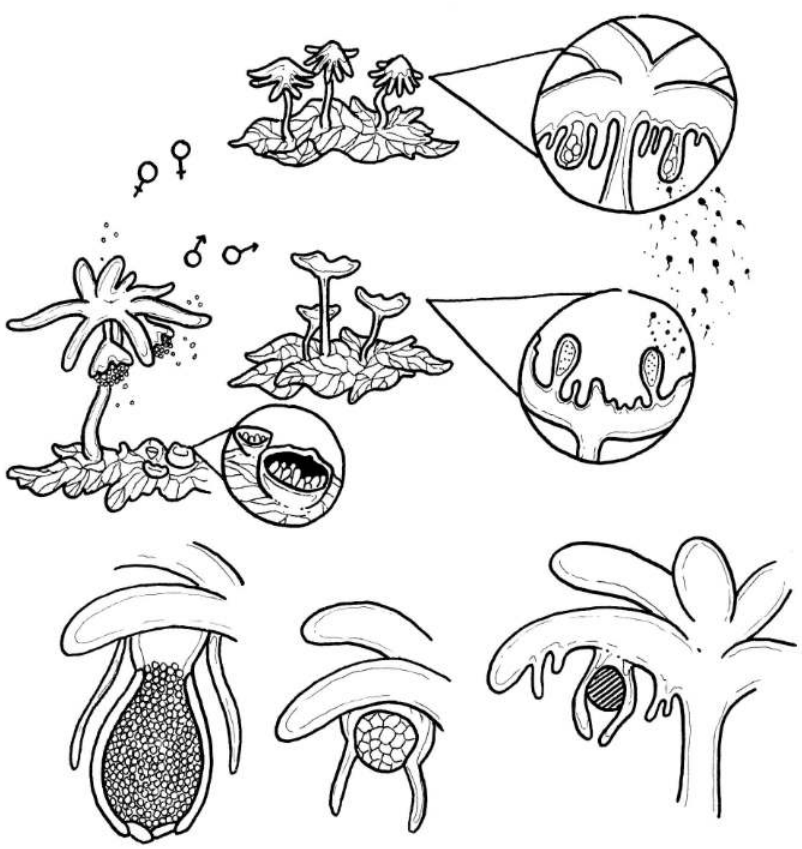


Figure 25.2.1: Marchantia life cycle diagram

In the diagram above, indicate where meiosis and fertilization occur. Color the haploid and diploid tissue differently, and draw arrows to show when mitosis is happening.

Obtain a prepared slide of a Marchantia ***antheridiophore***. The male gametangia, **antheridia**, are produced on the top of this structure. Each antheridium produces haploid, swimming **sperm** by mitosis. Label the bolded features in the life cycle diagram.

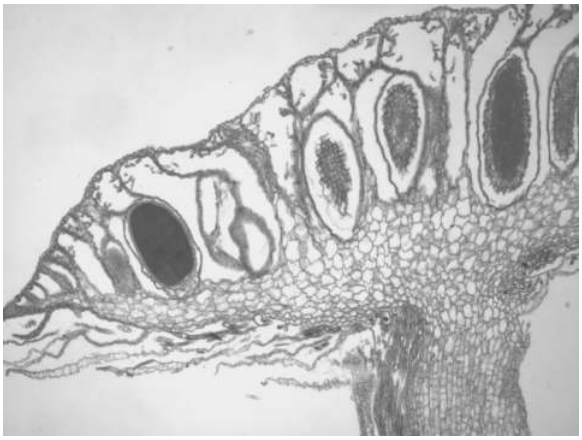


Figure 25.2.2: Marchantia antheridiophore

Obtain a prepared slide of an unfertilized Marchantia ***archegoniophore***. This is the structure that produces the female gametangia, **archegonia**. Each archegonium produces a single haploid egg by mitosis.



Figure 25.2.3: Marchantia archegniophore

A sperm will be transported by water to the archegoniophore, travel down the venter of the archegonium, and fertilize the **egg**. This forms a diploid **zygote**. Label the bolded features in the life cycle diagram.

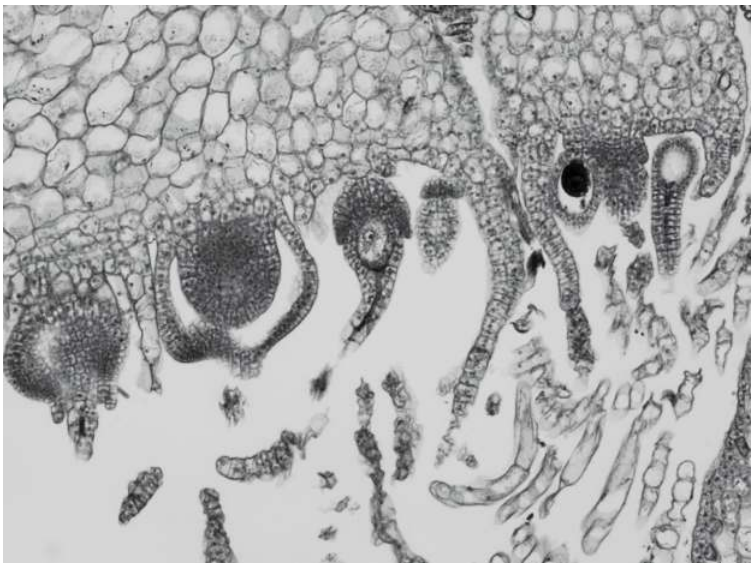


Figure 25.2.4: Archegonia close up

Obtain a prepared slide of a fertilized Marchantia archegoniophore with sporophytes. The zygote will be retained within the archegonium and nourished through the **placenta**, an area of gametophyte tissue adjacent to the **foot** of the sporophyte.

The mature sporophyte produces haploid spores via meiosis, which will grow into gametophytes. The sporophyte has a **seta** (the stalk), **sporangium** (also called a **capsule**), **spores**, and **elaters**, which aid in spore dispersal. Label the bolded features in the life cycle diagram.

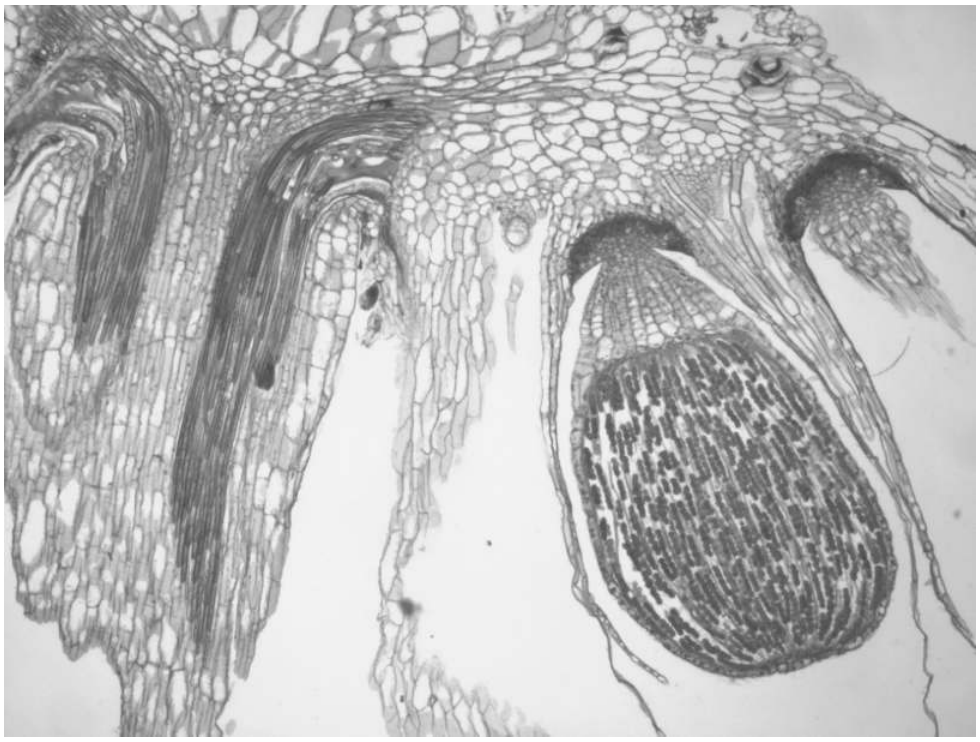


Figure 25.2.5: Marchantia sporophyte

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25.3: Lab Report

A1. View the bryophytes on display. Compare and contrast the overall morphology of bryophytes to the plants you can see outside. Why do you think the bryophytes all share such a similar growth form?

A2. Which part of the sporangium is the oldest? How do you know?

Make a wet mount of a small piece of hornwort thallus or obtain a prepared slide. Can you tell how many chloroplasts are in it? Draw this cell in the space below and label the chloroplasts and any other features you recognize.

C1. Pollination is essential for producing offspring. Because of this, it serves as a strong driver of selection in angiosperms. Flowering plants have evolved to utilize different pollinators, such as wind or birds, to transport their pollen to other flowers of the same species. Some of these pollinators, such as wind, are not selective and rely on producing large quantities of pollen. Others, like hummingbirds, require large quantities of nectar and are attracted by particular colors. Use the table of pollination syndromes below to determine the likely pollinator for the flowers available in your lab.

Table

: Pollination Syndromes (adapted from US Forest Service)

	Color	Structure	Scent	Nectar or Pollen
Wind	Dull, perianth often absent or reduced	Large feathery stigmas, large anthers	None	No nectar, large amounts of pollen
Birds	Reds and pinks	Often tubular or cupped	None	Lots of hidden nectar, moderate pollen
Bees	Purples, blues, yellows, white, UV	Flat and shallow or tubular, with landing area	Sweet, fresh, mild	Pollen often sticky and scented, nectar usually present
Bats	White, dull green, or purple	Often bowl-shaped or pendant, anthers protruding	Musty or fruity, strong, emitted at night	Lots of hidden nectar
Moths	White, pale pink or purple	Often tubular or cupped, no landing pad	Strong and sweet, emitted at night	Lots of hidden nectar, limited pollen
Butterflies	Bright colors	Tubular, with wide landing pad	Faint, fresh	Lots of hidden nectar, limited pollen
Flies	Dark red, purple, brown	Shallow, funnel, or trap-like	Putrid, rotting	No nectar, moderate pollen

Who is the pollinator?

Record the most likely pollinator and the trait(s) in the flower that lead you to this conclusion:

1.

2.

3.

4.

5.

C2. Different fruits have evolved in response to different **dispersal agents**. The fruits that we buy at the grocery store are tasty because they have evolved for animal ingestion. The burrs that get stuck to your socks when you walk through a field have evolved for animal attachment. How can we use characteristics of fruits to predict the dispersal agent?

Observe the fruits available and use the table below to predict which characteristics are related to each of the following dispersal agents.

Table

: Predictive Characteristics for Fruit Dispersal Agents

Dispersal Agent	Expected Characteristics
Animal ingestion	
Animal attachment	
Wind	
Water	
Ballistic (explosive, projectile)	

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CHAPTER OVERVIEW

26: Animal Diversity

Learning Objectives

Content Objectives

- Examine select phyla from the animal kingdom. Compare and contrast the characteristics that distinguish them.
- Review the current phylogeny of animals.

Skill Objectives

- Draw and label various examples from the animal phyla, using prepared slides, wet mounts, and/or preserved specimens.
- Dissect and identify crayfish (Phylum Arthropoda) internal and external anatomy.
- Be able to identify chordates based on their 4 key characteristics.

26.1: Introduction
26.2: Exercise
26.3: Lab Report

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26.1: Introduction

Several different groups within the Domain Eukaryota have produced complex multicellular organisms: The plants arose within the Archaeplastida, whereas the animals (and their close relatives, the fungi) arose within the Opisthokonta. However, plants and animals not only have different life styles, they also have different cellular histories as eukaryotes. The opisthokonts share the possession of a single posterior flagellum in flagellated cells, e.g., sperm cells.

Most animals also share other features that distinguish them from organisms in other kingdoms.

Features of animals include:

- Multicellular
- Eukaryotic
- Heterotrophic: eating other organisms, living or dead
- Ability to move (usually with muscle fibers)
- Complex tissue structure
- Specific reproduction and development patterns

Biologists strive to understand the evolutionary history and relationships of members of the animal kingdom, and all of life, for that matter. The study of *phylogeny* (the branching sequence of evolution) aims to determine the evolutionary relationships between phyla. Currently, most biologists divide the animal kingdom into 35 to 40 phyla. Scientists develop phylogenetic trees, which serve as *hypotheses* about which species have evolved from which ancestors.

Recall that until recently, only morphological characteristics and the fossil record were used to determine phylogenetic relationships among animals. Scientific understanding of the distinctions and hierarchies between anatomical characteristics provided much of this knowledge. Used alone, however, this information can be misleading. Morphological characteristics (such as skin color, body shape, etc.) may evolve multiple times, and independently, through evolutionary history. Analogous characteristics may appear similar between animals, but their underlying evolution may be very different. With the advancement of *molecular technologies*, modern phylogenetics is now informed by genetic and molecular analyses, in addition to traditional morphological and fossil data. With a growing understanding of genetics, the animal evolutionary tree has changed substantially and continues to change as new DNA and RNA analyses are performed on additional animal species.

Constructing an Animal Phylogenetic Tree

The current understanding of evolutionary relationships among animal, or Metazoa, phyla begins with the distinction between animals with *true differentiated tissues*, called Eumetazoa, and animal phyla that do not have true differentiated tissues, such as the sponges (Porifera) and the Placozoa. Similarities between the feeding cells of sponges (choanocytes) and choanoflagellate protists (Figure 26.1.1) have been used to suggest that Metazoa evolved from a common ancestral organism that resembled the modern colonial choanoflagellates.

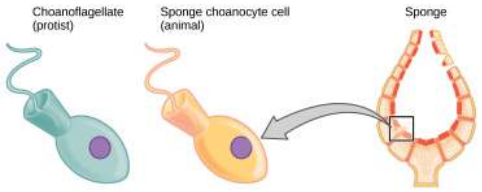


Figure 26.1.1: Choanoflagellates and choanocytes. Cells of the protist choanoflagellate clade closely resemble sponge choanocyte cells. Beating of choanocyte flagella draws water through the sponge so that nutrients can be extracted and waste removed.

Eumetazoa are subdivided into radially symmetrical animals and bilaterally symmetrical animals, and are thus classified into the clades Bilateria and Radiata, respectively. As mentioned earlier, the cnidarians and ctenophores are animal phyla with true radial, biradial, or rotational symmetry. All other Eumetazoa are members of the Bilateria clade. The bilaterally symmetrical animals are further divided into deuterostomes (including chordates and echinoderms) and two distinct clades of protostomes (including ecdysozoans and lophotrochozoans) (Figure 26.1.2a,b). Ecdysozoa includes nematodes and arthropods; they are so named for a commonly found characteristic among the group: the physiological process of exoskeletal *molting* followed by the “stripping” of the outer cuticular layer, called *ecdysis*. Lophotrochozoa is named for two structural features, each common to certain phyla within

the clade. Some lophotrochozoan phyla are characterized by a larval stage called *trochophore larvae*, and other phyla are characterized by the presence of a feeding structure called a *lophophore* (thus, the shorter term, “lopho-trocho-zoa”).



Figure 26.1.2: Ecdysozoa. Animals that molt their exoskeletons, such as these (a) Madagascar hissing cockroaches, are in the clade Ecdysozoa. (b) Phoronids are in the clade Lophotrochozoa. The tentacles are part of a feeding structure called a lophophore. (credit a: modification of work by Whitney Cranshaw, Colorado State University, Bugwood.org; credit b: modification of work by NOAA)

Nucleic acid and protein analyses have greatly modified and refined the modern phylogenetic animal tree. These data come from a variety of molecular sources, such as mitochondrial DNA, nuclear DNA, ribosomal RNA (rRNA), and certain cellular proteins. Many evolutionary relationships in the modern tree have only recently been determined from the molecular evidence. For example, a previously classified group of animals called lophophorates, which included brachiopods and bryozoans, were long-thought to be primitive deuterostomes. Extensive molecular analysis using rRNA data found these animals are actually protostomes, more closely related to annelids and mollusks. This discovery allowed for the distinction of the protostome clade Lophotrochozoa. Molecular data have also shed light on some differences within the lophotrochozoan group, and the placement of the Platyhelminthes is particularly problematic. Some scientists believe that the phyla Platyhelminthes and Rotifera should actually belong to their own clade of protostomes termed Platyzoa.

Scientists have developed a classification scheme that categorizes all members of the animal kingdom, although there are exceptions to most “rules” governing animal classification (Figure 26.1.3). Animals have been traditionally classified according to two characteristics: body plan and developmental pathway. The major feature of the body plan is its symmetry: how the body parts are distributed along the major body axis. Symmetrical animals can be divided into roughly equivalent halves along at least one axis. Developmental characteristics include the number of germ tissue layers formed during development, the origin of the mouth and anus, the presence or absence of an internal body cavity, and other features of embryological development, such as larval types or whether or not periods of growth are interspersed with molting.

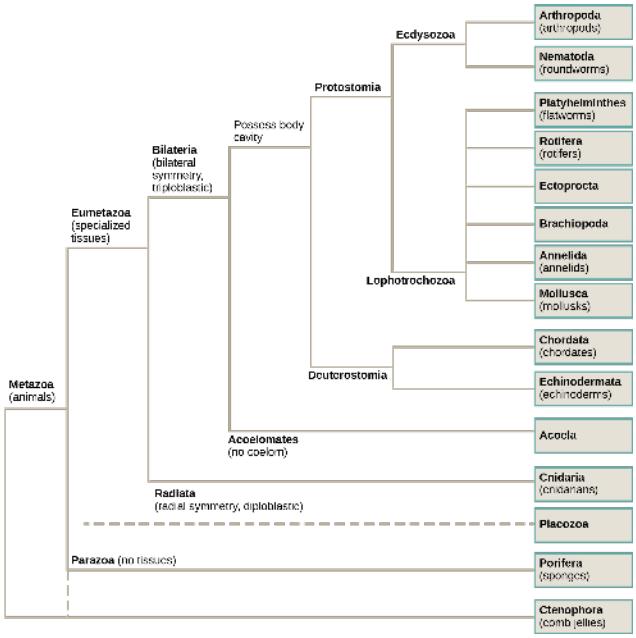


Figure 26.1.3: Animal phylogeny. The phylogenetic tree of animals is based on morphological, fossil, and genetic evidence. The Ctenophora and Porifera are both considered to be basal because of the absence of Hox genes in this group, but how they are related to the “Parahoxozoa” (Placozoa + Eumetazoa) or to each other, continues to be a matter of debate.

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26.2: Exercise

Sponges (Phylum Porifera)

Sponges have the most simplified body plan of animals, with no true tissues (cell-level organization), no symmetry, and structural components called **spicules**. The spicules can be made of calcium carbonate or silica.

Procedure

View the preserved specimens and slides on display.

Questions

1. The preserved sponge specimens will be on display, but may differ from the ones directly mentioned in the lab handout. Please make observations on the available specimens and fill in the chart below.

Name of Specimen	Physical Description	Sponge Structures Visible (osculum, other spores, spicules)

    1. What type of symmetry is displayed in the sponge specimens?
2. View the *Grantia* slides. There will not be slides available of the spicules but view the pictures in the lab materials.
    1. What is the function of spicules?
    2. Do sponges contain true tissues?
    3. Can you find any collar cells in the slide?
    4. What is the function of the collar cells?
    5. Can you find any epidermal cells in the sponge slide?
    6. What is the function of the epidermal cells?

Cnidarians (Phylum Cnidaria)

Cnidarians have **radial symmetry**. The body parts of a radially symmetrical animal are arranged around a central axis so that each part extends from the center. The animal can be cut along the axis in more than one plane to produce identical halves. Animals that exhibit radial symmetry tend to be **sessile** (immobile). Radial symmetry allows them to reach out in all directions.

Cnidarians have two tissue layers. The outer layer is the **epidermis**. It is formed from ectoderm. The inner layer, the **gastrodermis**, secretes digestive juices into the inner space called the **gastrovascular cavity**. The gastrodermis is formed from endoderm.

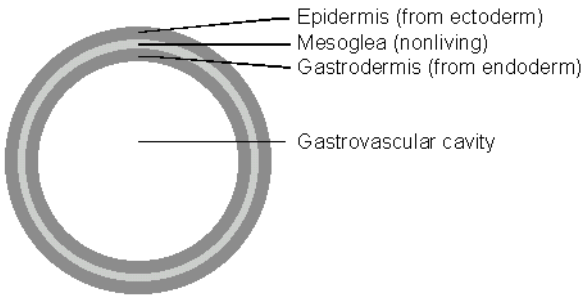


Figure 26.2.1: Cnidarian tissue layers. Cnidarians do not have mesoderm and therefore do not have organs.

A nonliving gelatinous material called **mesoglea** separates the two tissue layers. A nerve net is located between the epidermis and mesoglea. The body contains long structures called **tentacles** that can be moved to capture prey. The tentacles contain stinging cells called **cnidocytes** and within each one is a capsule called a nematocyst, which discharges to either trap or sting the prey. Contractile

(muscle-like) fibers are found in both the epidermis and the gastrodermis. Their movements are not complex because they do not have a brain.

Cnidarians have a **hydrostatic skeleton**. The contractile fibers act against the fluid-filled gastrovascular cavity. The movements are like a balloon; the animal can be short and thick or long and thin. Cnidarians have a saclike gut and extracellular digestion.

Two body forms are found among the Cnidarians, a **polyp** and a **medusa**. A polyp is attached and has the tentacles and mouth directed upward. A medusa is free-floating and has the mouth and tentacles on the ventral surface. It resembles an upside-down polyp. Some species have both a polyp and a medusa in their life cycle, others have one or the other form dominant.

Procedure

Hydra

1. Use a dropper to place a live *Hydra* on a slide. Examine the *Hydra* using a dissection microscope.

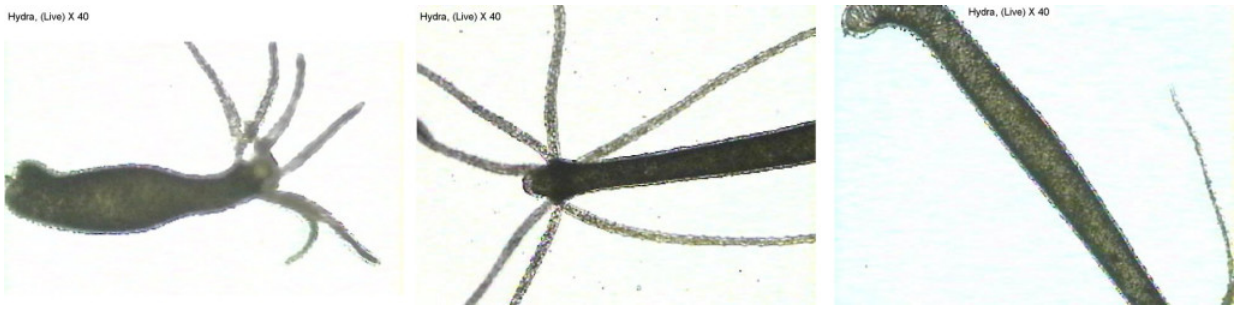


Figure 26.2.2: Three images of live Genus *Hydra* 40x magnification.

2. Hydra reproduce both sexually and asexually by budding. Try to find a live *Hydra* with buds. If you cannot find a live *Hydra* budding, look for budding in a prepared slide of *Hydra*.
3. Add a drop of vinegar to the slide containing *Hydra*. Describe what happened to the cnidocytes.



Figure 26.2.3: Hydra cnidocytes discharge their nematocysts when exposed to a 5% vinegar solution.

4. Examine microscope slides of hydra l.s. and hydra c.s. Look for the presence of two tissue layers. Identify stinging cells (Cnidocytes) in a slide of the whole animal.

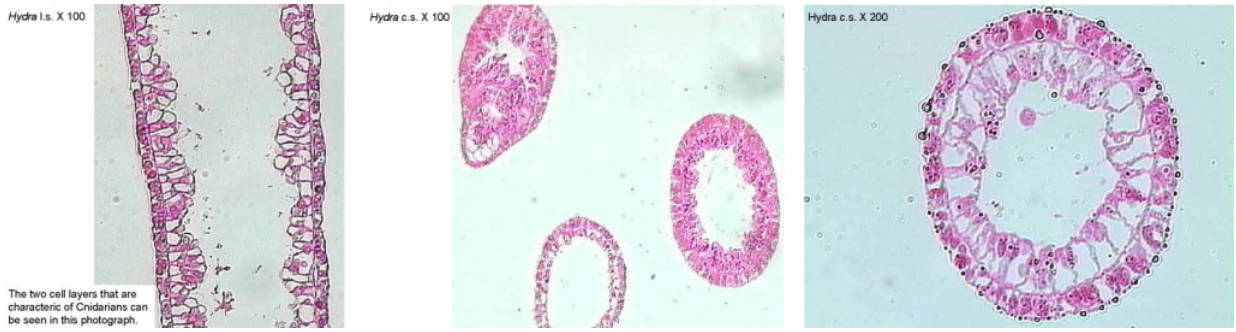


Figure 26.2.4: Hydra l.s. 100x, c.s. 100x, and c.s. 200x showing the two tissue layers.

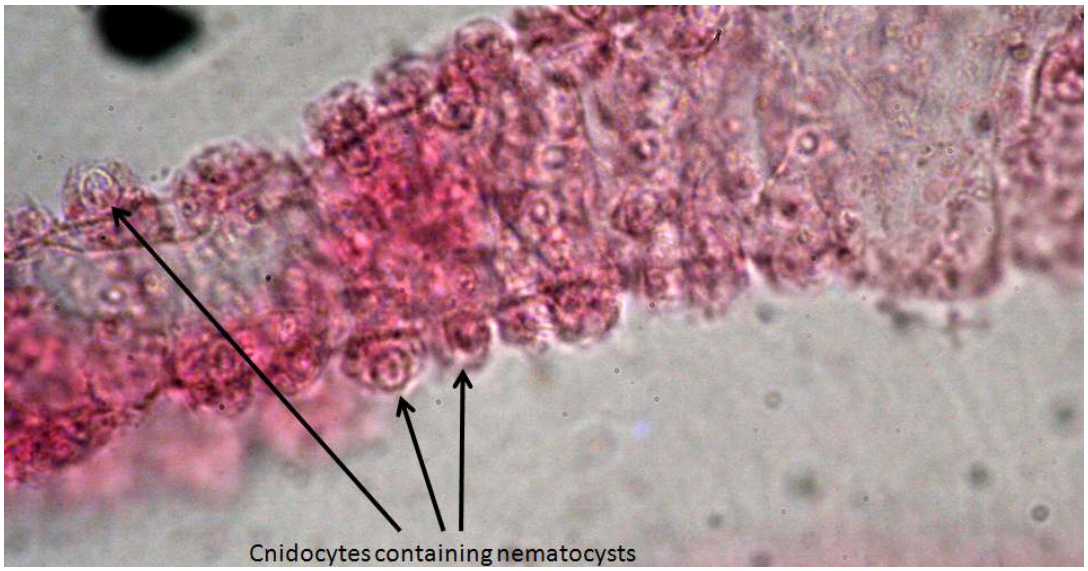


Figure 26.2.5: Portion of a *Hydra* tentacle showing cnidocytes

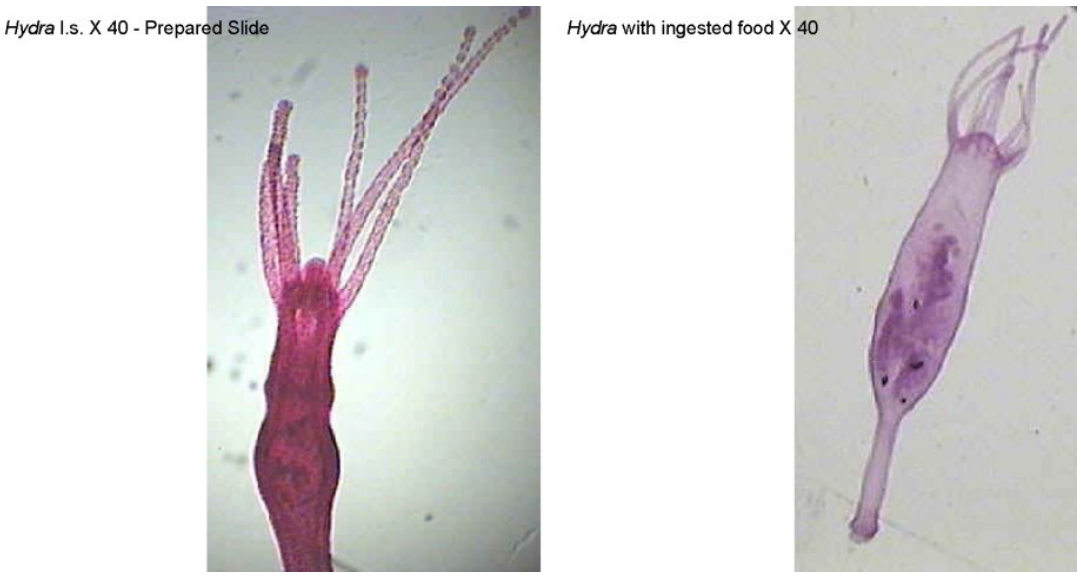


Figure 26.2.6: Left: *Hydra* l.s. X 40. Right: Hydra l.s. with ingested food X 40

View other preserved specimens and slides on display (anthozoans, scyphozoans).

Questions

1. Does the hydra illustrate the polyp or the medusa stage?
2. How many germ layers does the hydra contain?
3. What type of symmetry is seen in the hydra?
4. Can you find the hydra tentacles? How many tentacles does your hydra specimen contain?
5. Name the stinging cells present on the tentacles that are unique to cnidarians.
6. Explain the movement of the hydra if live specimens are available. If they are not, review the video on hydra movement.
7. If there are live hydra specimens do not add the vinegar as indicated on the lab website. Vinegar causes them to expel the cnidocytes. Review the nematocysts video, which shows a jellyfish discharging the nematocyst cells.

8. View the preserved cnidarian specimens will be on display. They may differ from the ones directly mentioned in the lab website but there should be some medusa cnidarians as well as corals and sea anemones. Please make observations on the available specimens. And fill in the chart below.

Name of Specimen	Physical Description	Polyp or Medusa Stage?

Lophotrochozoa

Flatworms (Phylum Platyhelminthes)

Procedure

View specimens and slides on display.

Questions

1. Observe the live planaria, if present, under the dissecting scope. If there are no live specimens, review the planarian video.
    1. What type of symmetry does the planaria display?
    2. Does the planaria exhibit cephalization?
    3. Can you locate the planaria eyespots? What do the eyespots sense?
2. View the large planaria model. Make sure you can identify the pharynx, the eyespots, and the flame cells.
    1. Does the planaria have a complete or incomplete digestive system?
    2. What is the function of the flame cell?
    3. Are planaria hermaphrodites?
    4. Scientists say planaria have ladder like organs. Why?

View the preserved liver fluke specimens. Liver flukes are an example of a parasitic flatworm.

Questions

1. Where does the adult liver fluke live?
2. When the liver fluke egg hatches, what organism does it infect first?
3. Can humans become infected?

View the preserved tapeworm and the slides of the tapeworm scolex (head) and proglottids (reproductive bodies). This is another example of a parasitic flatworm.

Questions

1. What structures are located on the scolex to help the tapeworm attach to the host?
2. Are tapeworms hermaphrodites?
3. Name two livestock that can be infected by tapeworms.
4. If a human is infected, where does the tapeworm live?

Molluscs (Phylum Mollusca)

Molluscs include bivalves (clams, scallops), gastropods (snails, slugs), cephalopods (octopus, squid), and polyplacophorans (chitons). Molluscs have a body divided into the head, visceral mass, mantle, and muscular foot. In many members of this group the mantle layer secretes a calcium carbonate shell for protection. Many molluscs have a **radula**, which is a toothed tongue-like structure they can use to scrape food particles off a surface.

Procedure

View the preserved specimens and slides on display.

Please make observations on the available specimens and fill in the chart below.

Name of Specimen	Physical Description	Gastropod, chiton, bi-valve or cephalopod

Questions

1. What is the function of the foot?
2. What is the function of the gills?
3. What does the mantle secrete for the clam?
4. What is contained within the visceral mass?

Segmented Worms (Phylum Annelida)

Procedure

View the preserved specimens and slides on display.

Questions

1. What type of symmetry does the earthworm display?
2. Does the earthworm exhibit cephalization?
3. Does the earthworm exhibit segmentation?

The preserved annelida specimens will be on display, but may differ from the ones directly mentioned in the lab handout. Please make observations on the available specimens and fill in the table below.

Name of Specimen	Physical Description	Leech, Earthworm, or Marine Worm

Ecdysozoa

Roundworms (Phylum Nematoda)

Procedure

View the preserved specimens and slides on display.

1. View the specimens and preserved slides of *Ascaris*
    1. What type of symmetry is seen in the roundworm?
    2. Does it exhibit cephalization?
    3. *Ascaris* is a parasite that swims constantly in human intestines. What structure protects the nematode from being digested?
2. View the *Trichinella* slide. *Trichinella* is also a parasite. It can infect humans as well as other mammals like pigs, bears, and rodents. If untreated, it can lead to death.

1. What mammal tissue does this roundworm infect?
2. Draw a picture of the *Trichinella* as viewed under the microscope.

3. If there are live vinegar eel specimens available, view them. If there are no live specimens available, review the vinegar eels video.
    1. Describe the movement of the vinegar eels.
    2. Do they have a complete or incomplete digestive system?

Arthropods (Phylum Arthropoda)

Arthopods include the following

- Chelicerates
    - Arachnids—(Spiders, Scorpions, Harvestmen, Ticks and Mites)
    - Horseshoe Crabs
- Myriapods
    - Millipedes
    - Centipedes
- Pancrustaceans
    - Crustaceans
        - Decopods
        - Isopods
        - Krill
        - Copepods
        - Barnacle
    - Insects

Arthropods have an **exoskeleton** that cannot enlarge as the animal grows. As a result, it must be shed from time to time to allow growth.

The young of some arthropods look like the adults. The change from young to adult that these species undergo is called **incomplete metamorphosis**.

In many species, the **egg** hatches to produce a **larva** (pl. larvae) that does not look like the **adult**. At some point in the maturation process, the larva will produce a **pupa** (pl. pupae). In this phase its tissues will become reorganized into the adult form. This type of development is called **complete metamorphosis**.

Procedure

View preserved specimens and slides on display.

Questions

1. View the preserved arthropod specimens available. There will be at least one example of each lineage group discussed on the website but not all of the specimens may be available. Use the table below to organize your observations.

Name of Specimen	Lineage	Exoskeleton?	Jointed Appendages?	Specialized Segments

2. What type of symmetry is displayed in the arthropods?
3. Do the arthropods exhibit cephalization?

Crayfish Dissection

Procedure

Obtain a crayfish and identify the structures shown in the photograph below. You should familiarize yourself with the names of the external structures shown in the photograph below.

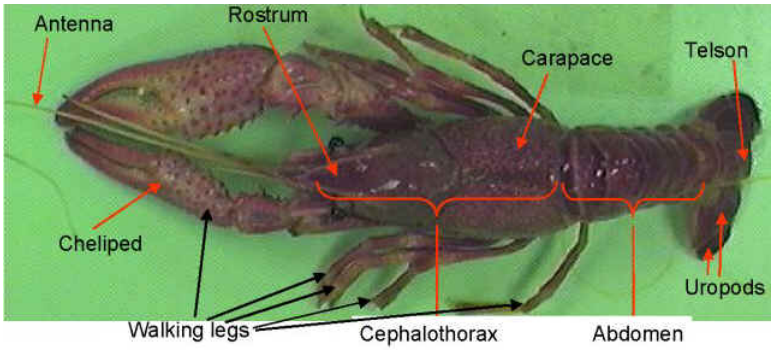


Figure 26.2.7: Crayfish external anatomy.

The photographs below show the ventral surface of male and female crayfish. Click on the photographs to view them. Identify the sex of your crayfish. Find somebody in class that has the opposite sex and view the ventral surface of their crayfish. Notice that crayfish have five pairs of swimmerets. In males, the anterior two pairs are large and less flexible than those of females. They are used to transfer sperm to the female.

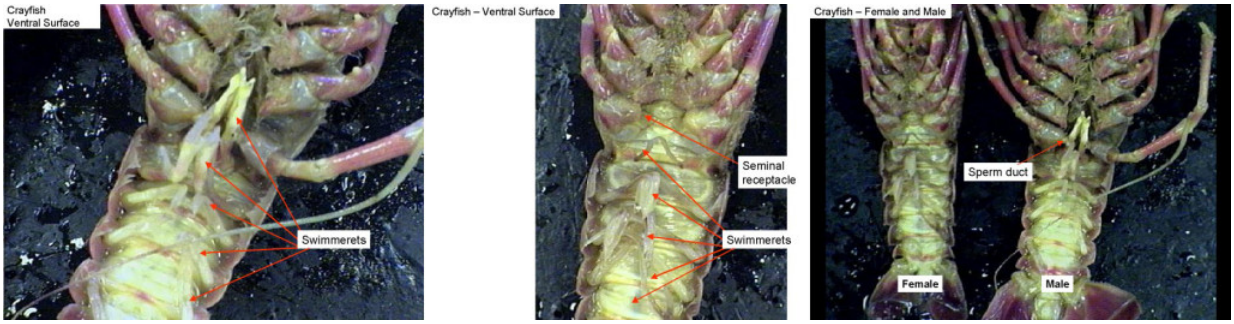


Figure 26.2.8: ventral surface showing swimmerettes, and ventral surface of male and female crayfish.

Find the seminal receptacles located between the third and fourth walking legs. These structures function to receive sperm from the male during mating.

Observe the sperm duct in males located at the base of the fifth walking leg.

Begin your dissection by cutting along the midline of the carapace from the posterior edge to an area just behind the two eyes (see the photographs below). Next, cut laterally just behind the eye until you reach the ventral edge. Carefully remove the piece of carapace that you have cut to expose the gills underneath. As you peel this piece of carapace away, it may be necessary to reach underneath with the point of a scissors or needle to brush away and detach any tissue from the interior of the animal that is attached to the carapace.

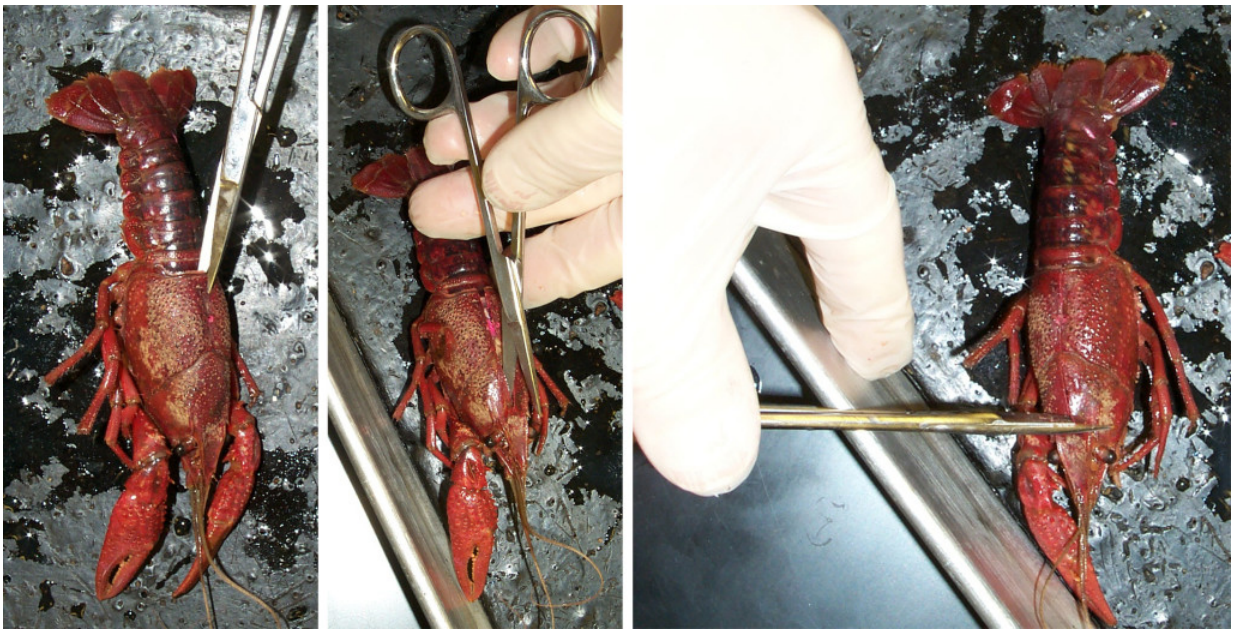


Figure 26.2.9: Crayfish dissection; carapace removal.

Your crayfish should look like the first photograph below. Next, remove the carapace on the other side.

Using a needle, carefully separate the first row of gills and notice that there is another row underneath. Remove one of the legs and observe how the gills are attached to the walking legs.

The first two photographs below show the gills on the left side of a crayfish. The third photograph is a crayfish with the entire carapace removed. The first row of gills on each side has been moved aside to expose a row underneath it.

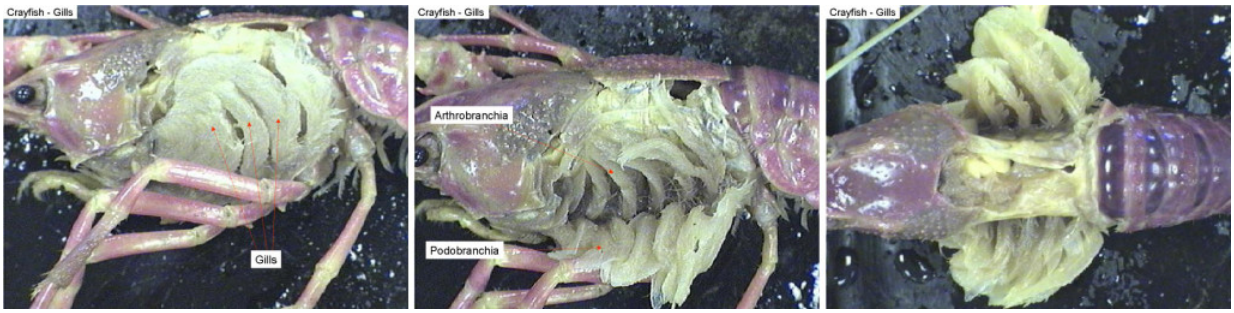


Figure 26.2.10:Crayfish with carapace removed.

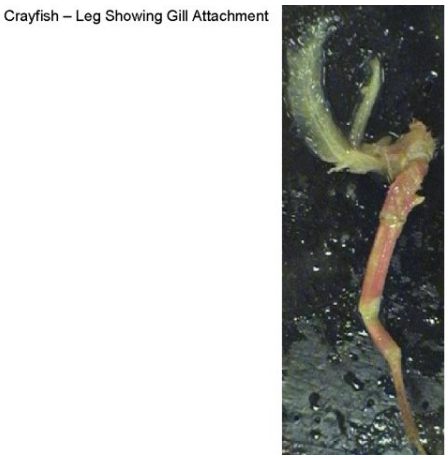


Figure 26.2.11: Crayfish leg showing gill attachment

Brush all of the gills to the side and then carefully cut the membrane (epidermis) that covers the internal organs.

The heart is a small diamond-shaped structure located below where the posterior edge of the carapace was. It may be difficult to see the blood vessels attached to the heart. Openings (ostia) in the side of the heart should be visible. These allow blood to enter the circulatory system.

Large white digestive glands can be seen on each side of the stomach. They produce digestive enzymes.

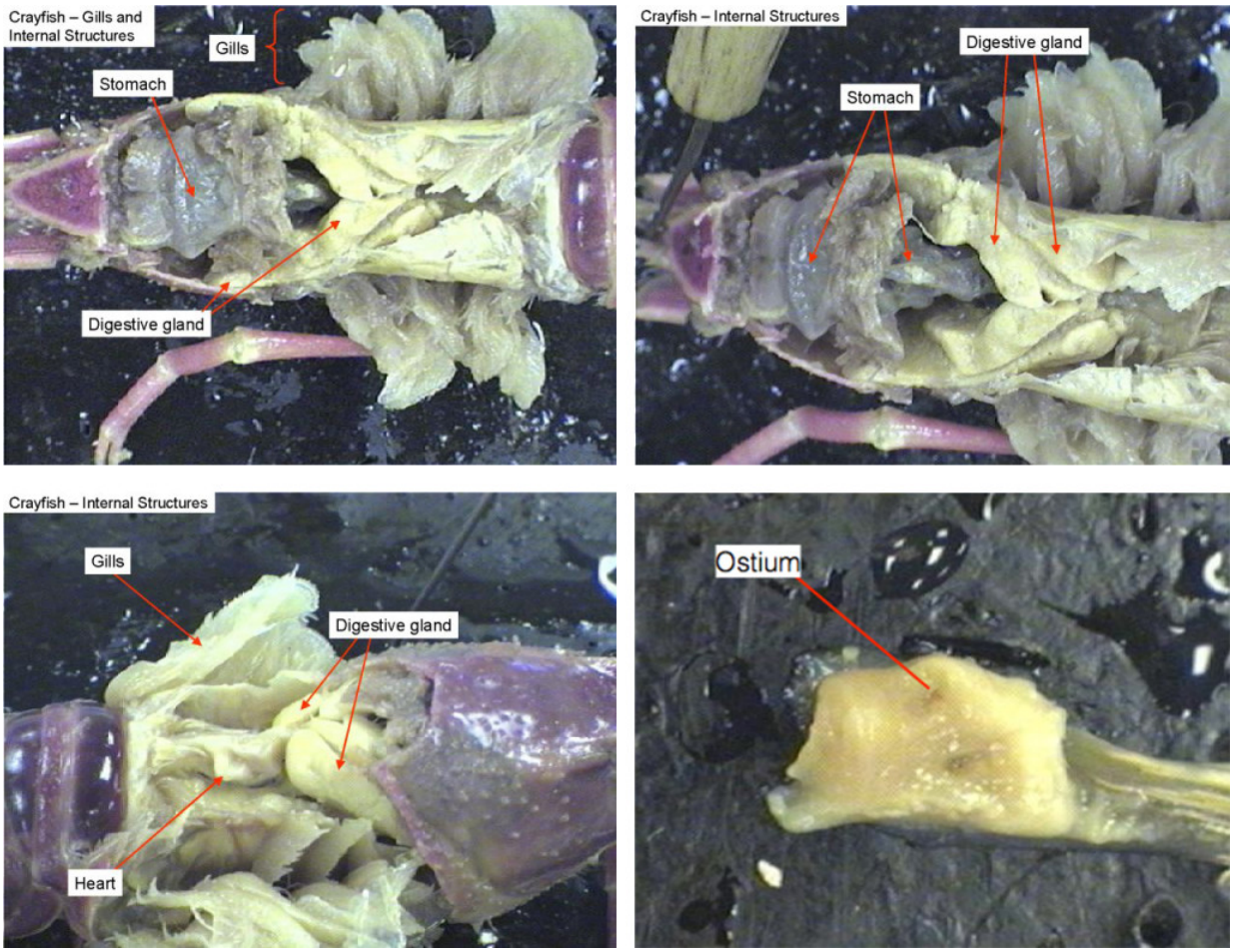


Figure 26.2.12: Crayfish internal anatomy, showing stomach, digestive glands, gills, heart, and ostium.

Remove the digestive glands and then carefully remove the stomach. Notice how the stomach is attached to the mouth. Cut open the stomach and observe the tooth-like structures at the anterior end for grinding food. This is called the gastric mill.

The green glands are positioned ventrally near the anterior end of the body cavity. They are spherical and will appear to be embedded within the surface. Their function is excretion. Find the openings for the green glands on the outer surface near the base of each antenna.

Find the brain near the anterior end and the two ventral nerve cords attached to the brain.

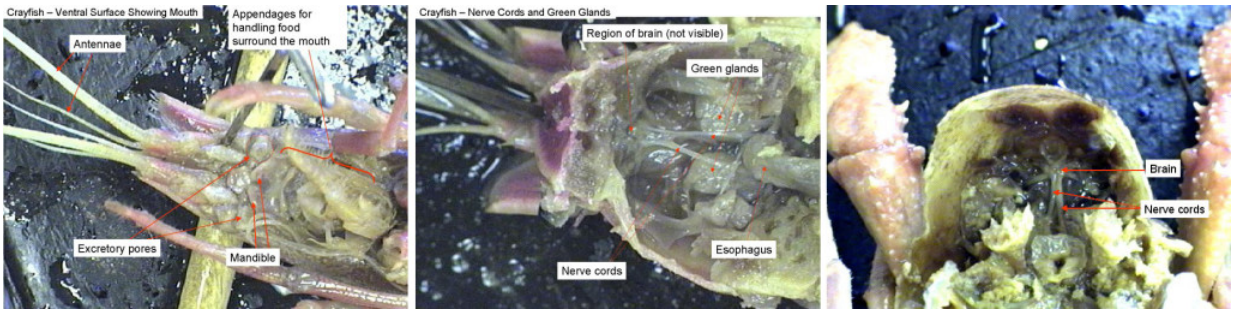


Figure 26.2.13: Left: Crayfish ventral surface. Middle: Crayfish viewed from above. The stomach has been removed revealing the nerve cords and green glands underneath. Right: Crayfish viewed from above looking into the head region. The stomach has been removed. The brain can be seen.

Questions

1. Make sure you can identify the following external structures: antenna, chiliped, cephalothorax, abdomen, and walking legs
2. Do you have a male or female crayfish?
3. How many swimmerets does your crayfish have?
4. How many rows of gills does the crayfish have?
5. Where do the gills attach?
6. Can you find the stomach and the digestive glands?
7. What does the stomach attach to directly?
8. Try to locate the green glands. What is the function of this structure?

Review Questions

Answer the review questions below. The phyla we viewed today were the porifera, the cnidaria, the nematoda and the arthropoda.

1. Which phyla exhibited bilateral symmetry?
2. Which phyla had no true tissues?
3. Which phyla contained parasitic organisms?
4. Which phyla were coelomates?
5. Which phyla exhibited cephaliziation?
6. Which phyla that you viewed today contained specialized appendages?
7. Which phyla exhibited radial symmetry?
8. Which phyla were pseudocoelomates?
9. Which phyla had a complete digestive system?
10. Which phyla were multicellular?
11. Which phyla were asymmetrical?
12. Which phyla were acoelomates?

Deuterostomia

Echinoderms (Phylum Echinodermata)

Echinoderms are coelomate, and deuterostomes.

Echinoderms include sea stars (starfishes), sea urchins, sand dollars, sea cucumbers, and sea lilies. There are 6,000 species of echinoderms; they are all marine.

Although echinoderm adults have radial symmetry, they evolved from ancestors that were bilaterally symmetrical. They have free-swimming, bilateral larvae that metamorphose (change as they mature) into adults with radial symmetry.

The adult body usually has five-part organization.

They possess an internal skeleton (**endoskeleton**) composed of calcium carbonate plates just beneath the surface of the skin. The plates often bear spines that protrude through the skin.

Echinoderms have numerous **tube feet** underneath each arm. The tube feet are connected to a system of pipes referred to as the **water vascular system**. Water enters the system by a sieve plate on the **aboral** surface. Each tube foot has a fleshy bulb or **ampulla** attached so that the entire structure looks like an medicine dropper or pipette. When muscles surrounding the ampulla contract, fluid inside the bulb moves down into the tube foot, extending it.

Large digestive glands produce enzymes necessary for digestion. Sexes are separate and gametes are shed into the water. The gonads are large due to the necessity of releasing large numbers of gametes into the marine environment. Coelomic fluid circulates substances and carries amoeboid cells that clean up particulate wastes. Gas exchange is done with numerous tiny gills that extend from the surface of the skin.

The nervous system consists of a central nerve ring with nerve branches extending into the arms. They do not have a brain.

Identify the blastopore. What structure in the adult does this give rise to? Notice that the embryo shows bilateral symmetry yet the adult is radial. The ancestral echinoderms were bilateral and bilateral symmetry is maintained in the larval stage.

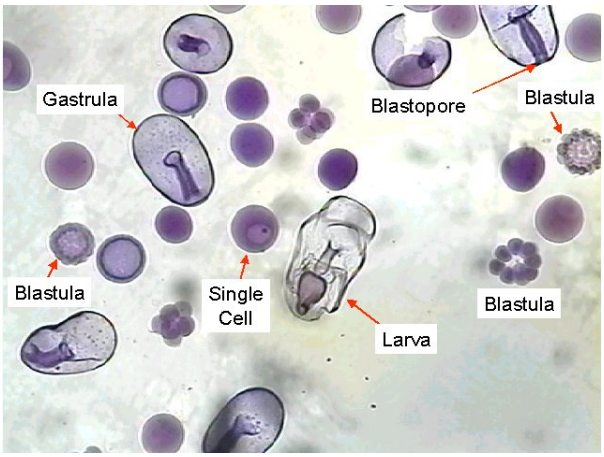


Figure 26.2.14: Echinoderm development (various stages)

Procedure

View the preserved echinoderm specimens will be on display. Please make observations on the available specimens and fill in the chart below.

Name of Specimen	**Physical Description**

Chordates (Phylum Chordata)

Phylum Chordata includes two groups of invertebrate chordates (the cephalochordates and the urochordates) and the subphylum Vertebrata (vertebrates). We will be dissecting several vertebrates soon.

The diagram below shows evolutionary relationships among the major clades of chordates but avoids classification into subphylum and classes. For example, the close relationship between Myxini and the rest of the vertebrates can be seen even though there is uncertainty in the classification of Myxini.

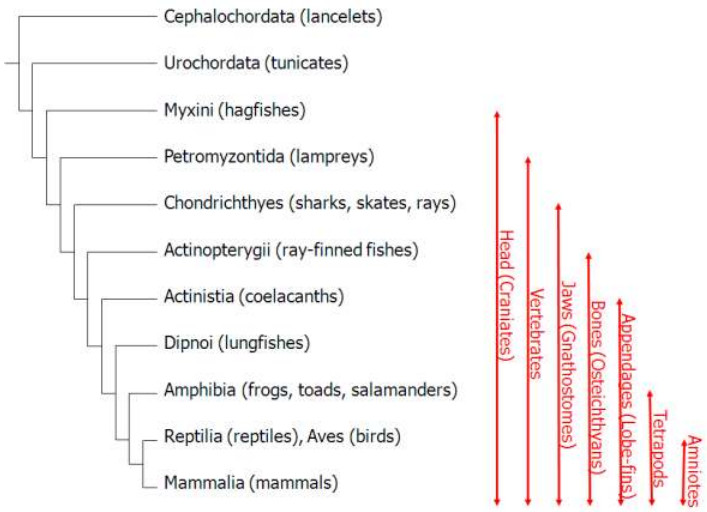


Figure 26.2.15: Evolutionary relationships among the major clades of chordates.

Chordates exhibit bilateral symmetry.

Chordates have the following characteristics at some point in their life history:

1. a dorsal, hollow nerve cord.
2. a dorsal supporting rod called a **notochord**. This is replaced by a vertebral column in vertebrates.
3. pharyngeal clefts (pouches). These develop into openings to the exterior (gill slits) in some chordates. Gill slits functioned as a mechanism for filter-feeding in primitive vertebrates. The gills of fish function in gas exchange.
4. a postanal tail. In most other kinds of animals, the digestive tract extends the entire length of the animal.

Procedure

View specimens and slides of the three major groups of chordates on display

Questions

1. View the lancelet slide. The lancelet is an example of a cephalochordate. It contains all four chordate characteristics. List the four chordate characteristics below.
2. View the lancelet model. Make sure you can identify all four chordate characteristics on the model.
3. Chooses several vertebrates on display and fill in the chart below. Can you identify all four chordate characteristics on each specimen? Why or why not?

Name of Specimen	**Physical Description**

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26.3: Lab Report

Sponges (Phylum Porifera)

Questions

1. The preserved sponge specimens will be on display, but may differ from the ones directly mentioned in the lab handout. Please make observations on the available specimens and fill in the chart below.

Name of Specimen	Physical Description	Sponge Structures Visible (osculum, other spores, spicules)

    1. What type of symmetry is displayed in the sponge specimens?

2. *Grantia* slides.
    1. What is the function of spicules?
    2. Do sponges contain true tissues?
    3. Can you find any collar cells in the slide?
    4. What is the function of the collar cells?
    5. Can you find any epidermal cells in the sponge slide?
    6. What is the function of the epidermal cells?

Cnidarians (Phylum Cnidaria)

Questions

1. Does the hydra illustrate the polyp or the medusa stage?
2. How many germ layers does the hydra contain?
3. What type of symmetry is seen in the hydra?
4. Can you find the hydra tentacles? How many tentacles does your hydra specimen contain?
5. Name the stinging cells present on the tentacles that are unique to cnidarians.
6. Explain the movement of the hydra if live specimens are available. If they are not, review the video on hydra movement.

7. Please make observations on the available specimens. And fill in the chart below.

8.

Name of Specimen	Physical Description	Polyp or Medusa Stage?

Lophotrochozoa

Flatworms (Phylum Platyhelminthes)

Questions

1. Observe the live planaria, if present, under the dissecting scope. If there are no live specimens, review the planarian video.
    1. What type of symmetry does the planaria display?
    2. Does the planaria exhibit cephalization?
    3. Can you locate the planaria eyespots? What do the eyespots sense?
2. View the large planaria model. Make sure you can identify the pharynx, the eyespots, and the flame cells.
    1. Does the planaria have a complete or incomplete digestive system?
    2. What is the function of the flame cell?
    3. Are planaria hermaphrodites?
    4. Scientists say planaria have ladder like organs. Why?
3. View the preserved liver fluke specimens.
    1. Where does the adult liver fluke live?
    2. When the liver fluke egg hatches, what organism does it infect first?
    3. Can humans become infected?
4. View the preserved tapeworm and the slides of the tapeworm scolex (head) and proglottids (reproductive bodies).
    1. What structures are located on the scolex to help the tapeworm attach to the host?
    2. Are tapeworms hermaphrodites?
    3. Name two livestock that can be infected by tapeworms.
    4. If a human is infected, where does the tapeworm live?

Molluscs (Phylum Mollusca)

Please make observations on the available specimens and fill in the chart below.

Name of Specimen	Physical Description	Gastropod, chiton, bi-valve or cephalopod

Questions

1. What is the function of the foot?

2. What is the function of the gills?

3. What does the mantle secrete for the clam?

4. What is contained within the visceral mass?

Segmented Worms (Phylum Annelida)

Questions

1. What type of symmetry does the earthworm display?

2. Does the earthworm exhibit cephalization?

3. Does the earthworm exhibit segmentation?

The preserved annelida specimens will be on display, but may differ from the ones directly mentioned in the lab handout. Please make observations on the available specimens and fill in the table below.

Name of Specimen	Physical Description	Leech, Earthworm, or Marine Worm

Ecdysozoa

Roundworms (Phylum Nematoda)

Questions

1. View the specimens and preserved slides of *Ascaris.*
    1. What type of symmetry is seen in the roundworm?

    2. Does it exhibit cephalization?

    3. *Ascaris* is a parasite that swims constantly in human intestines. What structure protects the nematode from being digested?

2. View the *Trichinella* slide.
    1. What mammal tissue does this roundworm infect?

    2. Draw a picture of the *Trichinella* as viewed under the microscope.

3. If there are live vinegar eel specimens available, view them. If there are no live specimens available, review the vinegar eels video.
    1. Describe the movement of the vinegar eels.

    2. Do they have a complete or incomplete digestive system?

Arthropods (Phylum Arthropoda)

Questions

1. View the preserved arthropod specimens available. There will be at least one example of each lineage group discussed on the website but not all of the specimens may be available. Use the table below to organize your observations.

Name of Specimen	Lineage	Exoskeleton?	Jointed Appendages?	Specialized Segments

2. What type of symmetry is displayed in the arthropods?

3. Do the arthropods exhibit cephalization?

Crayfish Dissection

Questions

1. Make sure you can identify the following external structures: antenna, chiliped, cephalothorax, abdomen, and walking legs.

2. Do you have a male or female crayfish?
3. How many swimmerets does your crayfish have?

4. How many rows of gills does the crayfish have?

5. Where do the gills attach?

6. Can you find the stomach and the digestive glands?

7. What does the stomach attach to directly?

8. Try to locate the green glands. What is the function of this structure?

Review Questions

Answer the review questions below. The phyla we viewed today were the porifera, the cnidaria, the nematoda and the arthropoda.

1. Which phyla exhibited bilateral symmetry?

2. Which phyla had no true tissues?

3. Which phyla contained parasitic organisms?

4. Which phyla were coelomates?

5. Which phyla exhibited cephaliziation?

6. Which phyla that you viewed today contained specialized appendages?

7. Which phyla exhibited radial symmetry?

8. Which phyla were pseudocoelomates?

9. Which phyla had a complete digestive system?

10. Which phyla were multicellular?

11. Which phyla were asymmetrical?

12. Which phyla were acoelomates?

Deuterostomia

Echinoderms (Phylum Echinodermata)

1. View the preserved echinoderm specimens will be on display. Please make observations on the available specimens and fill in the chart below.

Name of Specimen	**Physical Description**

Chordates (Phylum Chordata)

Questions

1. View the lancelet slide. The lancelet is an example of a cephalochordate. It contains all four chordate characteristics. List the four chordate characteristics.

2. View the lancelet model. Make sure you can identify all four chordate characteristics on the model.
3. Chooses several vertebrates on display and fill in the chart below. Can you identify all four chordate characteristics on each specimen? Why or why not?

Name of Specimen	**Physical Description**

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CHAPTER OVERVIEW

27: Plant Anatomy and Transport

Learning Objectives

Content Objectives

- Identify the three types of plant cells.
- Study the three types of plant organs; roots, stems, and leaves.

Skill Objectives

- Prepare slides to examine the three types of plant cells.
- Identify the different types of specialized cells found in leaves.
- Examine and label plant vascular tissue.
- Observe plant transpiration in action.

27.1: Introduction
27.2: Exercise
27.3: Lab Report

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27.1: Introduction

Plant Cells

Plant cells can be classified into three major categories based on their cell wall structure. Most plant cells are **parenchyma cells** (par- meaning equal), with an evenly-thickened primary cell wall. Some areas of a plant, particularly in young shoots, will require more flexibility, allowing them to bend without breaking. In these regions, you will often find **collenchyma cells** (coll- meaning glue), cells with strange, unevenly-thickened primary walls. Both of these cell types are alive at maturity. A third cell type, **sclerenchyma cells** (scler- meaning hard), develops a rigid **secondary wall** that is composed of lignin. This secondary wall provides structural support, but it also cuts off the exchange of water and other molecules permitted by the plasma membrane. Ultimately, this results in the death of the cell at maturity and loss of internal cell components.

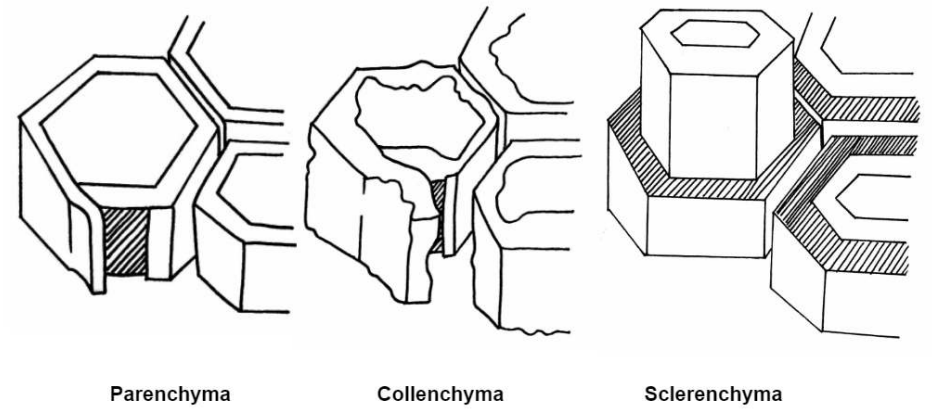


Figure 27.1.1: Collenchyma cell

Plant Tissues: Parenchyma, Collenchyma, Sclerenchyma

Plant cells performing a similar function can be assembled into tissues. This lab will introduce you to the three primary cell types found in plants, the tissues where you can find these cell types, and specialized cells that they can differentiate into. Specialized cells covered in this lab will be either parenchyma, collenchyma, or sclerenchyma (referred to as the “cell type”) and will have evolved for a specific function, such as conducting water. At the end of the lab, you will complete a table with the cell types, specialized cells, and functions of the major tissues within a leaf.

Plant Organs: Roots, Stems, Leaves

Plants are nature’s great water filters. They absorb water from the soil through their roots (if they have roots), use this water to maintain homeostasis, and whatever is left evaporates from open stomata across the epidermis of the plant. Each water molecule that leaves the plant is electrically charged and, due to these charges, tugs on the molecule behind it. The vast majority of water absorbed by most plants will exit via this process, creating a continuous vacuum of water through the plant, from the soil to the atmosphere. This evaporation of water from plant tissues is called **transpiration**. This process is highly dependent on the current environment, but to provide some perspective, an acre of corn transpires about 3,500 gallons of water per day.

Why do plants transpire if they are losing so much water everyday? Why not close their stomata to prevent water from escaping? There are many reasons why plants have evolved to transpire instead of retaining water.

1. Transpiration drives the flow of water and dissolved nutrients through the plant. If plants stop releasing water through the stomata, they will stop pulling in the nutrients dissolved in that water essential for plant function.
2. Transpiration provides evaporative cooling. As water leaves the plant tissues into the atmosphere, it takes energy with it in the form of heat. Much like when we sweat, this allows the plant to cool and maintain homeostasis. This is particularly valuable in hot environments.

3. Leaving stomata open is required for photosynthesis (except in certain plants, more on this in Lab 10: Photosynthesis). Carbon dioxide enters the plant through the stomata and oxygen is released as a waste product. If the stomata are closed, the plant cannot form sugars.

When water evaporates from plant tissues, it is called **transpiration**. Ninety percent of water that evaporates from terrestrial surfaces occurs via transpiration--plants are the world's greatest water filters! Water is absorbed by (most) plants through specialized organs called roots. The earliest plants, the bryophytes, don't have roots. Instead, these plants rely on the absorption of water across the entire plant body and dispersal of this water by osmosis. For this lab, we will focus on the later groups of plants--the **tracheophytes**--that have specialized tissues for water absorption and transportation throughout the plant.

Roots

An essential part of a plant's survival is obtaining access to water. Early plants did this by having small, creeping forms that grew in areas that would stay moist, never far from any surface. These plants did not have roots or the ability to transport water with xylem tissue. Instead, they absorbed and lost water across their tissues. As you can imagine, this would be a limiting state for plants and, soon after they moved onto land, plants evolved true roots with vascular tissue. Lignified tissues in roots would provide increased strength and stability for burrowing into the substrate (likely not yet a true soil) and access to water stored underground. Vascular tissue throughout the plant would allow water absorbed through the roots to be transported to other areas of the plant, meaning that tissues could elevate out of the water, getting increased access to sunlight.

In this lab, you will learn the general developmental pathway for tissues in the root, as well as the different anatomical organization of two groups of flowering plants: **monocots** and **eudicots**. Monocots, like corn and other grasses, germinate from seed with a single first leaf (called a cotyledon). Eudicots germinate with two leaves. Though this seems like a trivial distinction, these groups differ in many areas of growth and development.

Plants that have adapted to different environments might develop different root systems in response to the stressors in that environment. Observe the different root systems available in lab and try to classify them as one or more of the following:

Types of Root Systems

- ### Netted or Taproot System

In soils where water is readily available for most of the year, plants might develop a **netted root system** where many similar diameter roots capture as much water and nutrients as possible to outcompete their neighbors. In climates where there are droughts or freezes, plants might develop a **taproot system**, where a larger central root can burrow deeper into the soil profile, accessing water reserves that other plants cannot.

- ### Storage Roots

A larger diameter root can also store water and/or sugars for long periods. This type of root is called a **storage root**. A large central root, such as in the middle left on the following page, could be both a taproot and a storage root.

- ### Adventitious Roots

Adventitious roots emerge from stem tissue. This can happen when there is an underground stem, such as in the system at the top of the diagram on the following page, or to serve as a **prop root**, as in the center of the diagram.

Stems

The shoot of a plant is responsible for two major functions: photosynthesis and reproduction. In most plants, the **leaves** carry out photosynthesis, while the **stems** provide stability to elevate those leaves above potential competition. In annual plants, whose entire life cycle from germinate to death is completed in a single year, the epidermis on the stem is often photosynthetic, as well.

Tissues in the shoot are derived from the **shoot apical meristem** (SAM). Just like in the root, the SAM produces three primary meristems, which produce the primary tissues:

- Protoderm → Epidermis
- Ground meristem → Cortex and pith (simply **ground tissue** in monocots)
- Procambium → Primary xylem and primary phloem

These primary tissues will then either differentiate into specialized cells or, as is the case in many eudicots, become meristematic and produce secondary tissues.

Shoots and Leaves

Shoots are composed of nodes. Each **node** has a **leaf** and an **axillary bud**, which emerges from the **leaf axil**. The leaf is located just below the bud, which will become either a branch or a flower. The identity of each organ is determined by its location in the node.

In the diagram below, label the bolded features, as well as the photosynthetic part of the leaf (the **blade**) and the stem of the leaf (the **petiole**). Label the bolded features in the diagram below.

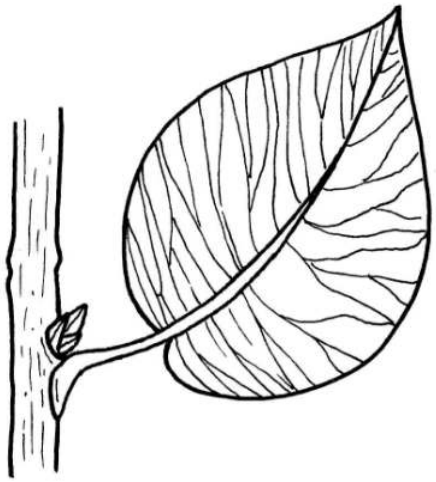


Figure 27.1.2: A node

Scars and Features of a Woody Shoot

When a leaf or bud falls off, it leaves a scar behind. **Leaf scars** are crescent shaped and have small, circular **bundle scars** within them where vascular bundles traversed the plant tissues. A leaf scar will be located below a bud, branch, or **bud scar**.

Plants that grow in temperate regions generally have a growing season. Each year, the newly emerging growth is within the **terminal bud** which is protected by **terminal bud scales**. When these scales fall off as the new growth emerges, they leave a region of **terminal bud scale scars** behind. Regions between terminal bud scale scars represent one year of growth.

On woody shoots, a layer of bark has replaced the epidermis (more on this in lab 8). To continue to exchange gases with the exterior environment, the bark layer develops small tears called **lenticels**. These can be small circular or elongated scars.

Label the bolded features in the diagram below. Draw in leaf scars where they are missing!

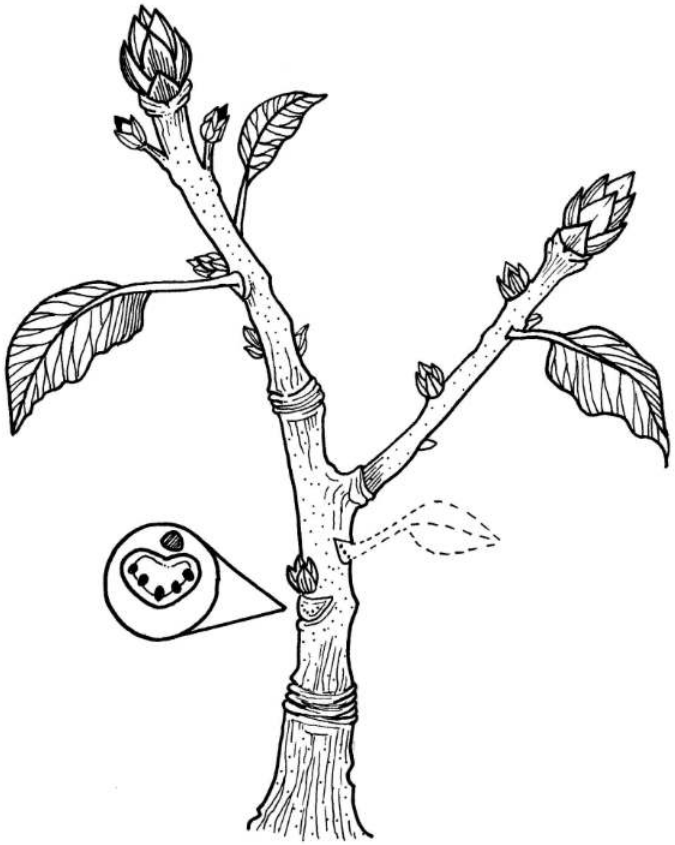


Figure 27.1.3: : Woody twig morphology

Leaf Arrangement

As shoots develop, the leaves are arranged in a particular order which can differ from plant to plant. In some plant species, one leaf emerges from the branch at a time (left) so leaves appear to alternate from side to side. This type of leaf arrangement is called **alternate**. In other plants, two leaves are formed on either side of the stem at the same time. This type of leaf arrangement is called **opposite** (middle). If three or more leaves are produced at the same region on the stem, the leaf arrangement is **whorled** (right).



Figure 27.1.4: Leaf arrangement

Leaf Anatomy

Leaves are specialized organs for performing photosynthesis. A leaf is often a relatively large, flat surface used to optimize sunlight capture. However, surfaces are areas that water can evaporate from, so a large amount of surface area exposed to sunlight results in increased transpiration. The anatomy of a leaf has everything to do with achieving the balance between photosynthesis and transpiration in the environment in which the plant grows. Plants that grow in moist areas can grow large, flat leaves to absorb sunlight like solar panels because sunlight is likely more limiting than water. Plants in dry areas must prevent water loss and adapt a variety of leaf shapes and orientations to accomplish the duel tasks of water retention and sunlight absorption. In general, leaves adapted to dry environments are small and thick with a much lower surface area to volume ratio.

Organ Modifications

Over the course of each different plant's evolutionary history, environmental pressures can lead to modifications of these features in predictable ways that we can then classify. For example, plants that frequently encounter drought will experience selection for the ability to access water when there is no water in the environment. The plants that evolve water-storage will have better survival in this environment and are more likely to successfully reproduce. However, animals in this dry environment might then seek out their water-filled tissues. Plants that either hide or protect these water reserves are more likely to survive and experience less herbivory. Over time, as the environment continues to select for these defenses, they improve. And thus, over long periods of evolutionary time, one lineage of plants evolves into cacti, while another related lineage evolves in a different environment to be carnations.

There are many selective pressures on plants. Climate has a strong selective effect on the anatomy and morphology of plants, particularly water availability and access to sunlight. Herbivory is another strong selective pressure, as plants cannot run away from their predators. Instead, they must evolve other deterrents while balancing the energy costs required for these adaptations with energy devoted to reproduction. A third strong selective pressure is nutrient availability, which is often determined by the soil environment. For example, serpentine soils have low levels of nitrogen and calcium. Plants in serpentine habitats often evolve the ability to trap and digest insects to absorb the missing nutrients. These "carnivorous" plants are not heterotrophic, because they do not use the trapped insects as an energy source.

Roots

Root tissue is derived from the root apical meristem. In some plants, environmental stressors will select for plants whose root tissues perform functions other than water absorption and anchorage. These organ modifications have specific names, depending on what function they serve.

1. **Storage roots**: In most roots, surface area is maximized for water absorption. In a storage root, the volume becomes more important. Cells in the cortex are enlarged and contain leucoplasts.

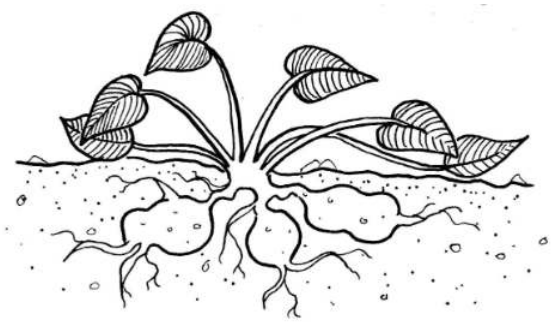


Figure 27.1.5: Storage Root

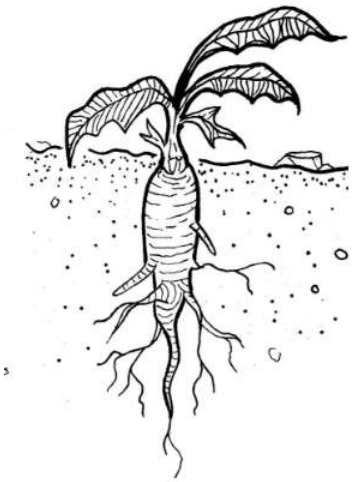


Figure 27.1.6: Taproot

2. **Pneumatophores**: Gases diffuse 10,000x more slowly in water than in air. In plants that grow in saturated soils, such as mangroves, roots cells need to access oxygen to perform cellular respiration at a rate that they cannot accomplish through water. Pneumatophores are roots that emerge above the surface of the saturated zone to “breathe” (pneumo- means lung) and exchange gases with the environment.
3. **Adventitious roots**: Unlike most roots, adventitious roots emerge from stem tissue. A root apical meristem is derived from tissues in the stem, then root tissues are formed from the RAM, as normal. Adventitious roots can be produced from nodes on horizontal or climbing stems or in response to environmental stressors.

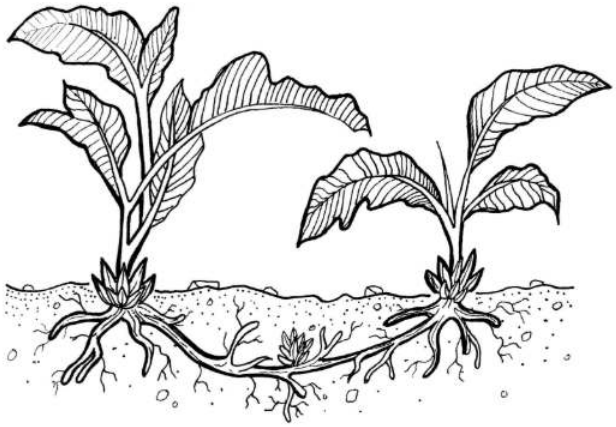


Figure 27.1.7: A rhizome

4. **Prop roots**: Prop roots are adventitious roots with the specific function of providing stability to a plant. This might happen in unstable soils, on climbing plants, or in plants that have a shallow root system.

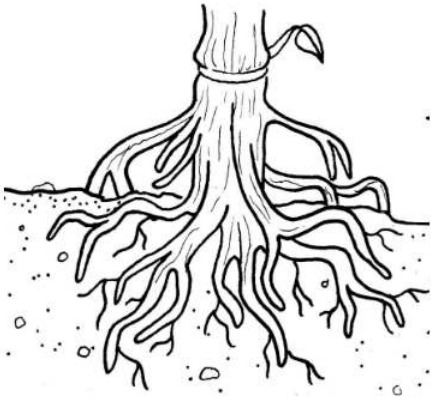


Figure 27.1.8: Prop Roots

Stems

Stem tissue is derived from the shoot apical meristem. In many plants, there is a central stem that lateral stems emerge from. You can distinguish stems from roots by the presence of nodes. You can distinguish lateral stems from leaves by location within the node: stems emerge above the leaf.

Just like with roots, stems can be adapted to a particular function in response to environmental stressors.

1. **Cladode**: A cladode is a stem that has increased surface area to perform photosynthesis. This is usually because the leaves have been modified to some other purpose and are no longer performing photosynthesis. In essence, the stem is imitating a leaf.
2. **Succulent**: In the case of succulence, it is the volume of the stem that increases. Stem tissues develop large, specialized cells called hydrenchyma to store extra water. The plant can access this water for metabolism in periods of drought.
3. **Tuber**: Some stems are modified for storage of starches instead of water. A tuber is an underground stem that can be identified by its nodes (often referred to as “eyes” on a potato tuber). Each node is capable of producing a new shoot.
4. **Corm**: A corm is also modified for storage of starches. A corm is swollen tissue at the base of the shoot with linear nodes travelling across it. From these nodes, papery leaves emerge.

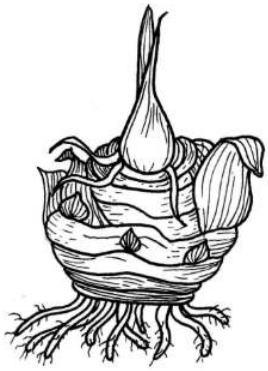


Figure 27.1.9: A Corm

5. **Rhizome**: Some plants produce horizontal stems that are used for asexual reproduction. A rhizome is a horizontal stem that is underground. Roots emerging from the nodes of rhizomes are adventitious.
6. **Stolon**: Similar to a rhizome, a stolon is a horizontal stem used for asexual reproduction. Unlike a rhizome, stolons are formed above the soil surface.
7. **Thorn**: A thorn is a lateral branch that has been modified to protect the plant, usually from herbivory. Thorns have a subtending leaf or leaf scar.



Figure 27.1.10: Thorns

8. **Stem tendril**: A tendril is a lateral branch that has been modified for climbing. A stem tendril will have a subtending leaf or leaf scar.

Leaves

Leaf tissue is derived from the shoot apical meristem. You can distinguish leaves from stem tissue by location within the node: leaves emerge below the axillary bud, lateral stem, or flower. Under normal conditions, the primary function of a leaf is photosynthesis. However, environmental stressors can select for the following modifications:

1. **Succulent**: Much like in stems, leaves can also be modified for water storage in environments where there is drought. You can distinguish a succulent leaf from a succulent stem because the stem will have nodes (the leaf will not).



Figure \(\PageIndex{11}\): A succulent

2. **Bulb**: Leaves can also be modified to store starch. A bulb, like an onion, is composed of fleshy leaves that surround a short, central stem.



Figure 27.1.12: A bulb

3. **Spine**: A leaf modified to be sharp for protection is called a spine. You can distinguish a spine from a thorn by the location within the node.
4. **Leaf tendril**: Sometimes leaves will be modified for climbing. You can distinguish a leaf tendril from a stem tendril by the location within the node.

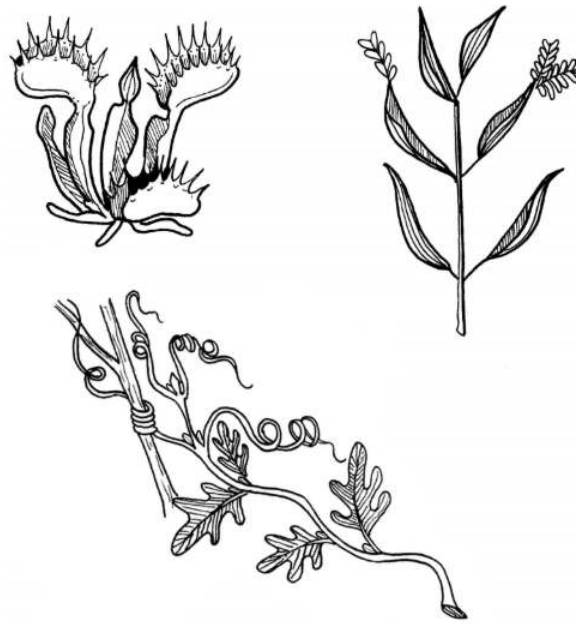


Figure 27.1.13: Leaf Modifications. Clockwise from top left: a trap and phyllode (venus fly trap), phyllode (Acacia), leaf tendril. A combination of all three modifications are shown in the pitcher plant below.

5. **Trap**: In environments where nutrients are low, some plants have evolved to capture insects for access to nitrogen, phosphorus, and calcium. There are a few ways plants can achieve this. One is to modify a leaf into some sort of trap.
6. **Phyllode**: Another instance of leaf imitation is when the petiole of the leaf becomes flat and leaf-like to perform photosynthesis. Similar to a cladode, this is usually in response to a modified leaf. The pitcher plant on the right shows a phyllode, leaf tendril, and trap all in one leaf.



Figure 27.1.14: Copy and Paste Caption here

7. **Stipular spines**: Another sharp armament against herbivory can be a replacement of a stipule with a spine. These can be distinguished from other sharp modifications, as they emerge in pairs at the base of a leaf.

Other Plant Adaptations

In addition to the major organs, plants can have adaptations to specific tissues, cells, or molecules produced by the plant.

1. **Prickles**: Similar to thorns and spines, prickles are a protective adaptation. In this case, it is a modification of the epidermal (and sometimes cortex) tissue. Prickles can emerge anywhere on the shoot, unlike spines and thorns, which are restricted to nodes.



Figure 27.1.15: Prickles

2. **Trichomes**: Trichomes are epidermal cells that have been modified as hairs. While they can still serve a protective function, they tend to be much smaller and less rigid than prickles.



Figure 27.1.16: Trichomes

3. **Raphides**: Some plants have molecular, internal sharp armaments. In plants like Tradescantia, calcium oxalate is crystallized into needle-like structures called raphides.

   Chop up the leaf or stem of a Tradescantia and make a squash mount to see raphides within the plant tissues.

4. **Latex**: About 10% of flowering plants have evolved latex production, a sticky substance exuded when plants are damaged. This latex can prevent infection of wounds. Many groups of plants have independently evolved the production of latex gum up the mouthparts of herbivorous insects. In some plants, the latex also includes toxic compounds to aid in defense. For example, in milkweeds (Asclepias) this latex contains neurotoxins.

Contributors and Attributions

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- Maria Morrow (College of the Redwoods)

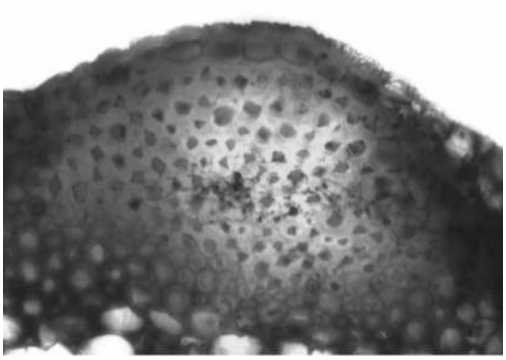
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27.2: Exercise

→ At the beginning of this lab as a class, set up and number 4 potted plants of similar size and type. On each plant, put a bag over one of the branches (or main stem) and tape it closed around the base. *Note: the bags should be the same type or the mass of each bag should be recorded beforehand.* Water plants 1, 2, and 3 until the soil is saturated. Put plant 1 under a light or next to a sunny window, plant 2 in the dark, and plants 3 and 4 somewhere away from a window but where light is still present. You will check on these later in the lab.

A. Cell Types

Make a thin section of a celery petiole or the main celery stalk. This needs to be very thin to see the features you are looking for, so make a few samples to look at! If you would like to stain your specimen, place the specimen on a slide and add a small drop of Toluidine Blue. Wait a few seconds for the dye to penetrate into the sample, then rinse by adding water to the slide and either soaking up or draining off the excess liquid. When the water is mostly clear, add another drop or two of water and a coverslip.



Figure

: A cluster of collenchyma cells in the celery petiole

View your specimen under the compound microscope.

You should be able to see several cell types in your specimen. Most of the cells will be parenchyma. A great place to look for textbook parenchyma cells is the outermost layer of the plant, the **epidermis**.

You will find collenchyma cells in dense clusters near the epidermis in a region called the **cortex**, forming the strings that you would find in your celery. They appear to have an almost checkerboard-like pattern, due to the unevenly thickened primary walls. In Toluidine Blue, primary walls stain purple.

Some specialized cells can be found in the **vascular tissue**, organized regions of cells that are transporting water, sugars, and other chemicals throughout the plant body. The **xylem** is the tissue responsible for conducting water. Specialized cells in the xylem tissue called **tracheids** and **vessel elements** have evolved specifically for this ability by forming hollow tubes with lignified secondary walls. Tracheids evolved first and are narrow with tapered ends. Vessel elements evolved in the most recent group of plants, the Angiosperms, and are usually much wider than tracheids. In Toluidine Blue, the lignin in the secondary wall stains bright aqua blue.



Figure

: Sclerenchyma cells from wood pulp

The image above shows three different types of cells with secondary walls found in wood pulp. A vessel element is shown in the center with a tracheid running parallel just above it. Note the pits in the walls of both of these cells and the large holes (**perforation plates**) on the ends of the vessel element only. Criss-crossing the rest of the slide are many thin fibers. All of these cells are dead at maturity and provide structural support due to the lignin in the cell walls.

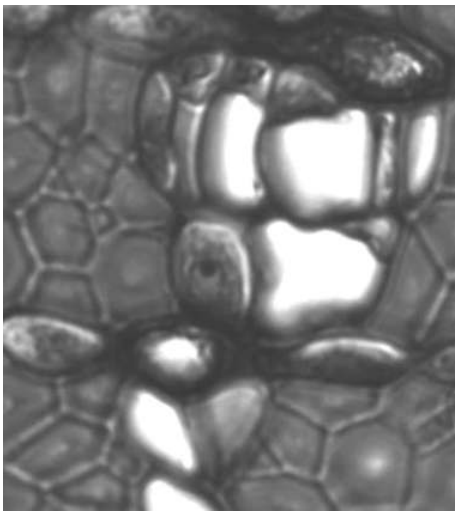


Figure

: Tracheid

In the image above, you can see the pits in the walls of a tracheid.

Unlike the xylem, conducting cells in the **phloem** tissue are alive so they may transport sugars and communication signals in any direction. These cells, **sieve tube elements** and **companion cells**, are more similar to parenchyma. The sieve tube elements conduct sugars and have specialized to do this by having reduced cytoplasm contents: sieve tube elements have no nucleus (or vacuole)! These cells are controlled by small, adjacent cells called companion cells. If you look closely, you can also see some sclerenchyma bunched together in the phloem. These are the **phloem fibers**. Their thick secondary walls should stain the same color as the tracheids and vessel elements.



Figure

: Phloem Tissue

In the image above, you can see clusters of thick walled fibers, large open sieve tube elements, and small companion cells containing nuclei. (A1, A2)

While collenchyma tissue tends to have one job--flexible support--parenchyma and sclerenchyma can fill a diverse set of roles. In a developing pear, there is a high density of a second type of sclerenchyma cells called **sclereids** (the first type of sclerenchyma cells were fibers)**.** These cells cause young pears to be tough and unpalatable, as the seeds inside are still developing. As the seeds mature, the pear ripens, making more parenchyma cells for storing large amounts of sugar, while the tough sclereids are slowly outnumbered by the larger, juicier cells. The grit that you feel when eating a pear are these remaining sclereids. (A3)

Make a squash mount of the flesh of a pear (not the skin) by scraping off a small amount with a razorblade. Do not take a slice or a chunk, just a tiny bit of pulp (consider chopping it up on the slide). Again, I recommend staining with Toluidine blue, as this should make the thick secondary walls of the sclereids appear a bright aqua blue. Sclereids tend to occur in clusters, surrounded by large parenchyma cells. You may need to gently squish your coverslip down a bit to help disperse these clumps. Coverslips are fragile, so ask your instructor what they recommend before doing anything that might result with glass in your fingers.

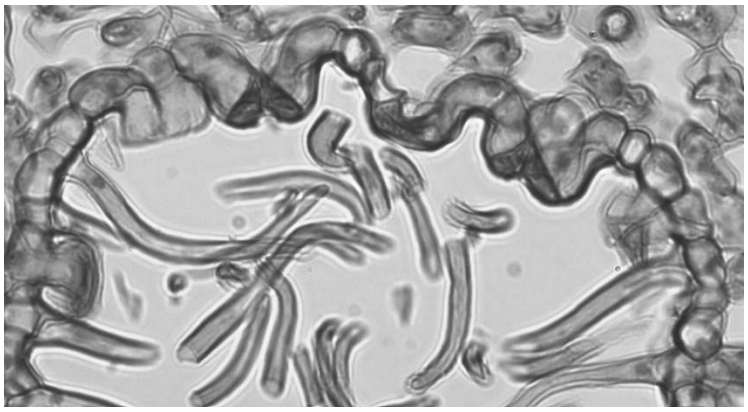
When you find a sclereid, you should see lines running through the secondary wall. These are channels where the plasmodesmata extended through to connect to other cells. After the cell dies, only the empty channels (called **pits)** remain.

> Draw a sclereid, located in the **ground tissue** of a pear. Label the **secondary wall**, **pits**, an adjacent **parenchyma cell**, and the **primary wall** of that parenchyma cell. (A4, A5)

B. Tissues in the Leaf

When cells of the same type work together to perform a collective function, the collection of cells is called a **tissue**. For example, the epidermis is a collection of parenchyma-like cells working together to separate the internal environment of the plant from the exterior. The epidermis also contains specialized cells. **Trichomes** are outgrowths from the epidermis that look like hairs. These can protect the plant from sun damage by being white and reflective, trap evaporating moisture on the plant's surface, secrete sticky substances, and be unpleasant for herbivores.

A second type of specialized cell in the epidermis is the guard cell. **Guard cells** are shaped like parentheses and flank small pores in the epidermis called **stomata** (sing. stoma). When the plant has adequate water, the guard cells inflate and the stoma is open, allowing water vapor to escape through transpiration. When the plant is low on water, the guard cells collapse, closing the stoma and trapping water inside. However, for the plant to perform photosynthesis, it must have access to carbon dioxide and be able to release oxygen. Both of these gases are exchanged through the stomata.



Figure

: Stomata in a stomatal crypt

The image above is from the lower epidermis of a Nerium leaf. These plants live in harsh, dry environments and have many adaptations to prevent water loss. This is a pocket on the lower side of the leaf where stomata are located. You can see three different sets of guard cells, currently closed, appearing slightly darker than the other epidermal cells. Surrounding these stomata and filling the pocket are trichomes. (B1, B2)

Peel off the lower epidermis of the leaf, similar to how you removed it from the onion. It may help to break the leaf slowly, hopefully getting a piece of the epidermis that you can peel off. It will look like a transparent layer of skin. Make a wet mount of the epidermis and view it under the compound microscope. Draw what you see below, labeling any specialized epidermal cells. (B3)

When multiple tissues work together to perform a collective function, this collection of tissues is called an **organ**. While we are familiar with the concept of organs in animals, it can sometimes be surprising to consider this aspect of plants.

An example of an organ in a plant is the leaf. A leaf is surrounded by epidermal tissue, protecting the interior environment, and allowing for the exchange of gases with the environment. The xylem tissue, found in the veins of the leaf, provides the water needed for specialized parenchyma, **mesophyll cells**, to carry out photosynthesis. Phloem tissue runs alongside the xylem tissue, transporting sugars made during photosynthesis to other areas of the plant for either immediate use or storage. Together, these tissues allow the leaf to function as an organ specialized for photosynthesis. (B4)

C. Plant Organs: Roots

The root system of a plant serves two primary functions: water absorption and anchorage. Roots with a higher surface area will be better adapted to absorbing water because they have more area to interact with the soil environment. Increased interaction with the soil environment can also contribute to increased anchorage, but there are always trade-offs. If roots become too fine, they will be easily broken and lose the anchorage function. Additionally, finer roots can also lose more water if the soil environment becomes dry.

Root Development

In plants, both roots and shoots grow from the tip or apex of the plant. New cells are produced in these growing tips by **meristems**, groups of undifferentiated cells whose function is to divide by mitosis to produce new cells. Root growth begins at the **root apical meristem** (RAM). This meristem divides in two directions, producing a **root cap** to the outside of the root to protect the growing tip and the primary meristems to the inside: the **protoderm**, **ground meristem**, and **procambium**.

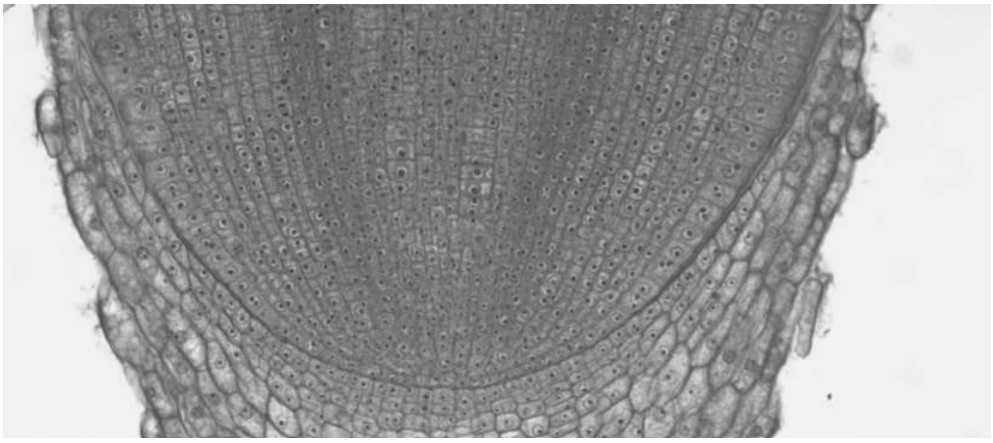
Primary meristems produce the primary tissues in the root:

- Protoderm → **Epidermis**
- Ground meristem → **Cortex** (and **pith** in monocots)
- Procambium → **Primary xylem** and **primary phloem**

These primary tissues will then either differentiate into specialized cells or, as is the case in many eudicots, become meristematic and produce secondary tissues.

Observe a long section of *Zea mays* (corn) root tip. This root tip can be divided into three regions based on what is occurring in that stage of development:

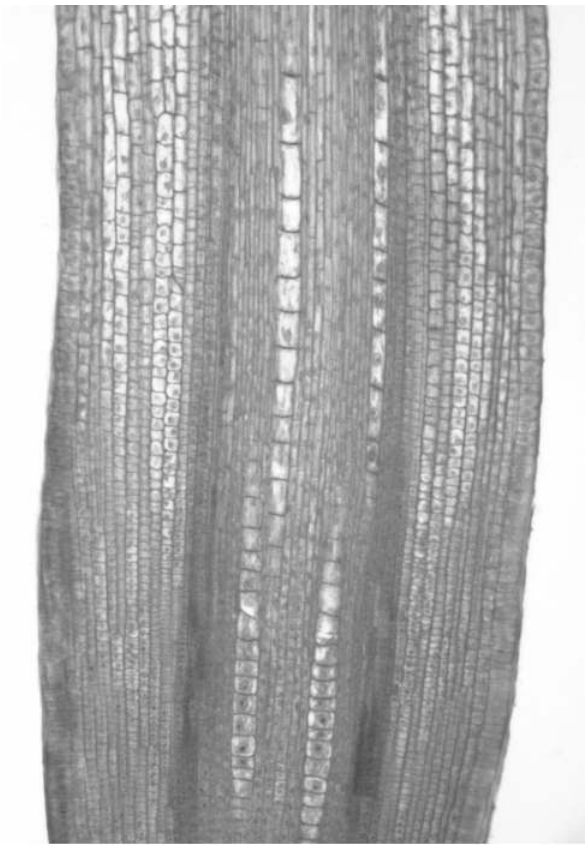
Locate the root cap, RAM, protoderm, ground meristem, and procambium. These can be found at the very tip of the root, where the cells are small and densely clustered. If you trace the columns of cells back to their origin, they will coalesce at the RAM. This area of meristematic activity is called the **zone of division**.



Figure

: Zone of division

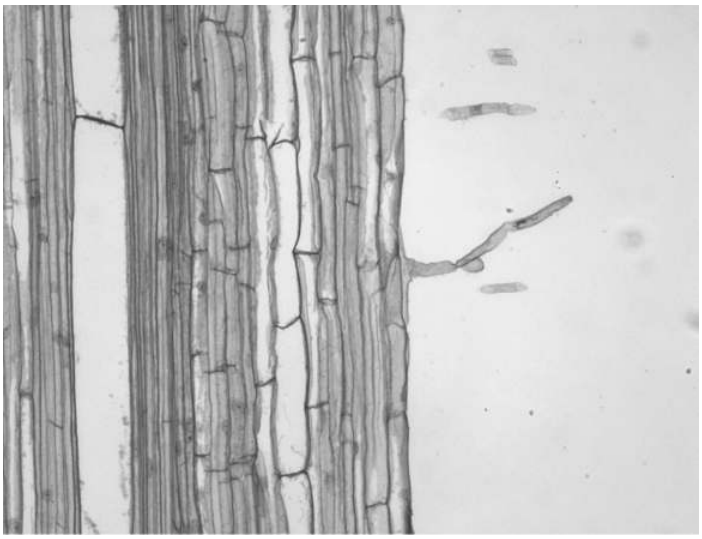
As you move up in the root, the cells begin to get larger, developing into primary tissues. This region is called the **zone of elongation**.



Figure

: Zone of elongation

Further up the root, the larger cells begin to differentiate into specialized cells. In the xylem and phloem, you can find sclerenchyma with secondary walls. In the epidermis, you can see elongated cells called **root hairs** projecting outward. This region is called the **zone of maturation**.



Figure

: Zone of maturation

Locate each of the zones described above and draw a root tip below. In the zone of division, label the **root cap** and the following meristems: **RAM, protoderm, ground meristem**, **procambium**. In the zone of elongation, label the following primary tissues: **epidermis, cortex, pith, primary phloem, primary xylem**. In the zone of maturation, label the following specialized cells: **root hair, sieve tube element, companion cell, vessel element**.

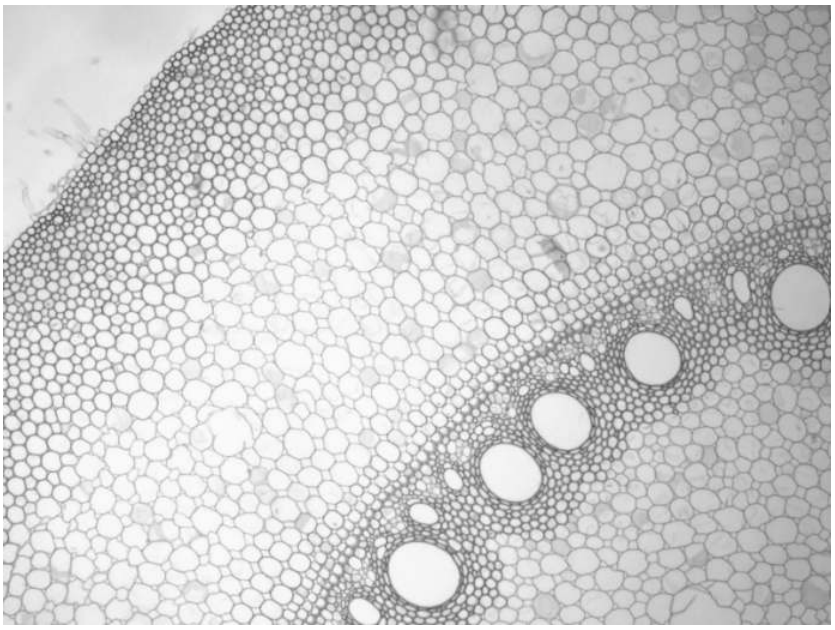
Root Development Flowchart

Below is a flowchart of eudicot root development in primary growth. There are two lines that will later lead to the secondary meristems. With this information and the filled in boxes, you should be able to determine which meristems and tissues go in the empty boxes. (C1, C2)

Root Anatomy

Monocots

Observe a cross section of the zone of maturation in a *Zea mays* root. Locate the primary tissues and specialized cells that you found in the long section and label them in the image below.



Figure

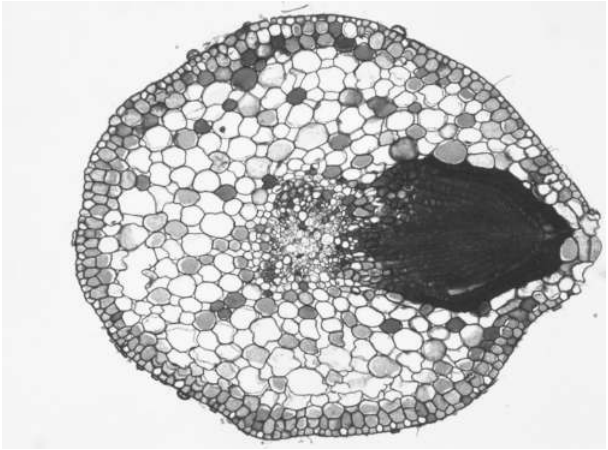
: Cross section of a Zea mays root

Eudicots

The development of roots in eudicots differs slightly from monocots. Monocots develop a ring of vascular tissue with ground tissue (pith) in the center. In eudicots, the vascular cylinder is closed. The primary xylem develops as an X (or sometimes Y) shape in the center with pockets of primary phloem in the spaces between the arms.

Encircling the conducting tissues is a meristematic layer called the **pericycle**. This single cell layer is responsible for producing lateral roots. Unlike root hairs which emerge on the outside of the root (**exogenous**, exo- meaning outside), lateral roots emerge from the internal tissues (**endogenous**, endo- meaning inside).

> Observe a cross section of a *Salix* (willow) root producing lateral roots. Locate the primary xylem, primary phloem, pericycle, cortex, and epidermis. Label these features in the image below:.

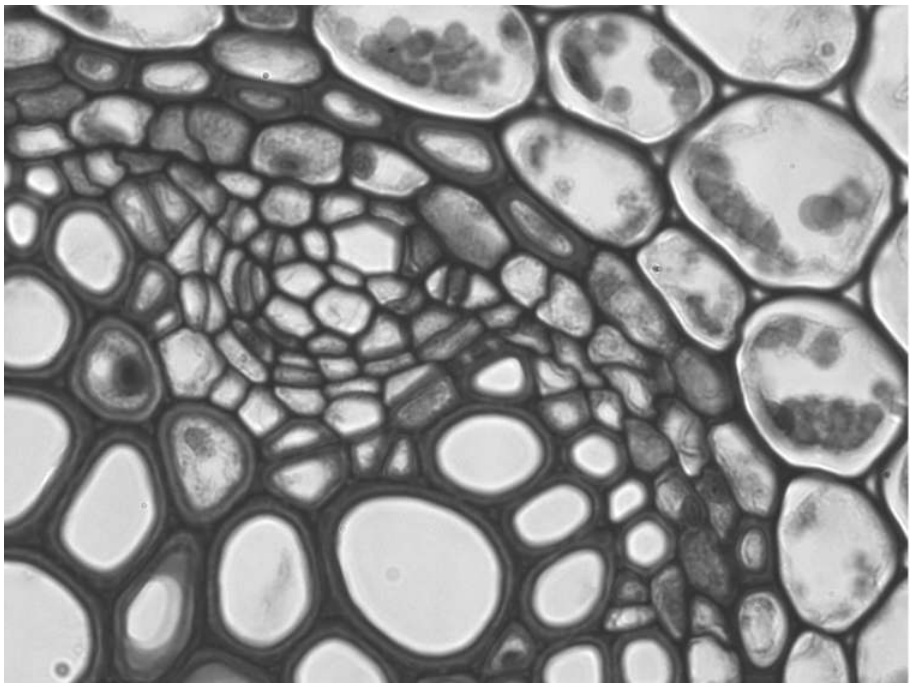


Figure

: Salix root with lateral root

Just outside the pericycle is the innermost layer of the cortex called the **endodermis**. The endodermis regulates what enters and exits the vascular cylinder. When the root is young, the epidermis contains many root hairs whose function is to absorb water. This water is transported through the cortex and into the xylem. As the root matures, the root hairs are lost from the epidermis. These areas of the root now function for anchorage and transportation of water. Because these areas are no longer taking up water, the

vascular cylinder is sealed off by a waxy layer of **suberin** that covers the endodermis called the **casparian strip**. The casparian strip forms in stages with the areas closest to the arms of the xylem forming last. These are the last areas to allow for the passage of water, giving them the name **passage cells**.



Figure

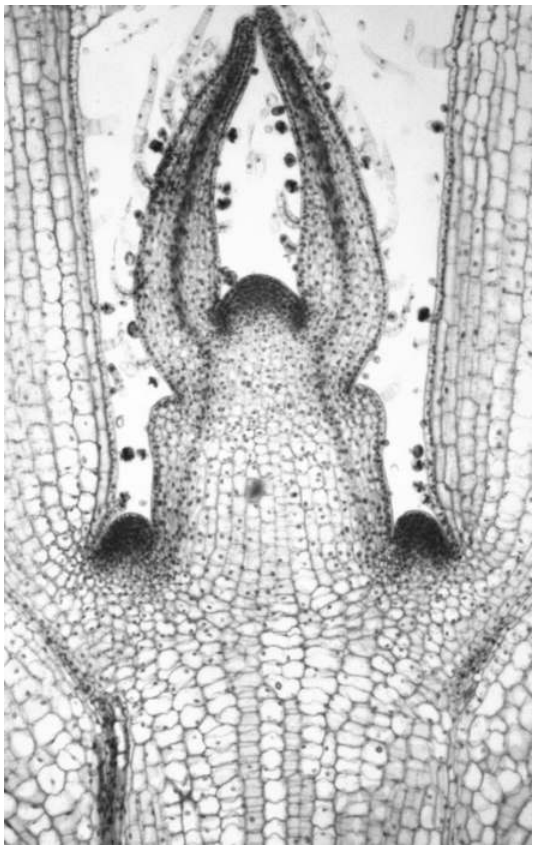
: Casparian strip and passage cells

Observe a cross section of a *Ranunculus* (buttercup) root. Locate the **pericycle, endodermis, casparian strip**, and **passage cells**. The suberin in the casparian strip often stains similarly to the secondary walls in the xylem. Draw and label the cross section in the space below.

Coleus Shoot Tip

Though it looks a bit alien, this is a section through a growing tip of a plant. In the center, where the alien's head might be, is a region of small, densely packed cells. This is the SAM of the apical bud. On either side of the SAM, like two upraised arms, are the **leaf primordia.** These are the early stages of developing leaves. Through the center of these leaf primordia is a darker region of small cells. This is the procambium, which will develop into the vascular tissue. Lining the outer edge of the SAM and the youngest portions of the leaf primordia is the protoderm. As the protoderm matures into the epidermis, it produces hair-like projections called **trichomes**. Between the protoderm and the procambium is the ground meristem.

On either side of the growing tip are two other darkened lumps of densely packed cells. These **bud primordia** will develop into **axillary buds**, producing either branches or flowers. Each bud primordium has its own SAM. (C6)



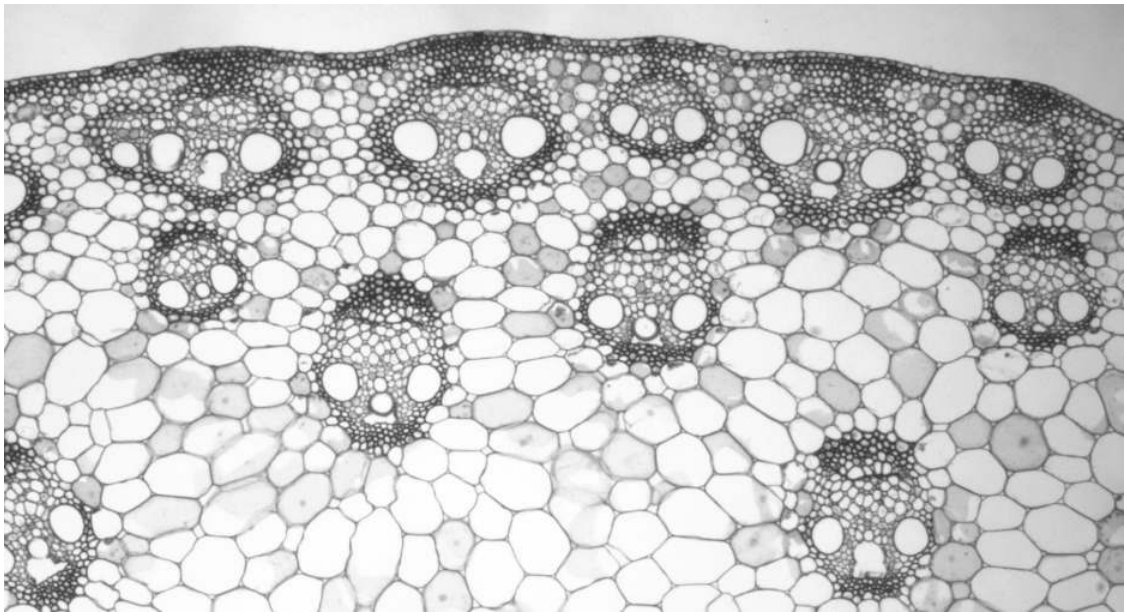
Figure

: Coleus shoot tip

Monocot Stem

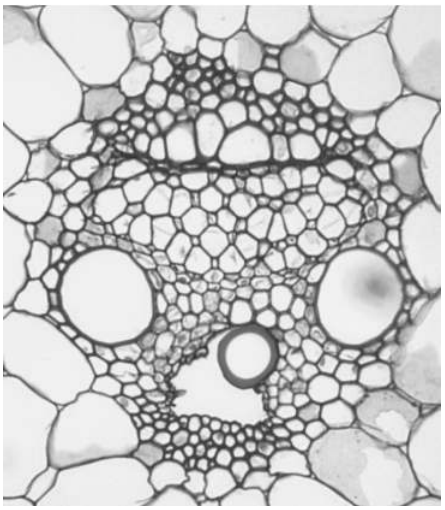
Monocots are a group of flowering plants that produce a single first leaf (**cotyledon**) as their seeds germinate. **Eudicots** (frequently referred to simply as dicots) produce two cotyledons. In addition to this feature, monocots and eudicots can be distinguished by several anatomical and morphological features. One of these features is the arrangement of tissues in the stem. In monocots, the vascular tissue is arranged in distinct bundles that are scattered throughout the stem. The xylem is located on the side of the vascular bundles that face the center of the stem and can be identified by the large, hollow **vessel elements** that stain differently due to their secondary walls. In the half of the bundle that faces the exterior is the phloem, which contains **sieve tube elements** and their accompanying **companion cells**. The phloem is capped by a clump of fibers called the **phloem fibers**. The vascular bundles are arranged throughout the **ground tissue** in concentric circles with xylem facing inward.

Vascular bundles in monocots are called **closed vascular bundles**, as they will not go on to form secondary tissues (no residual procambium).



Figure

: Monocot stem cross section



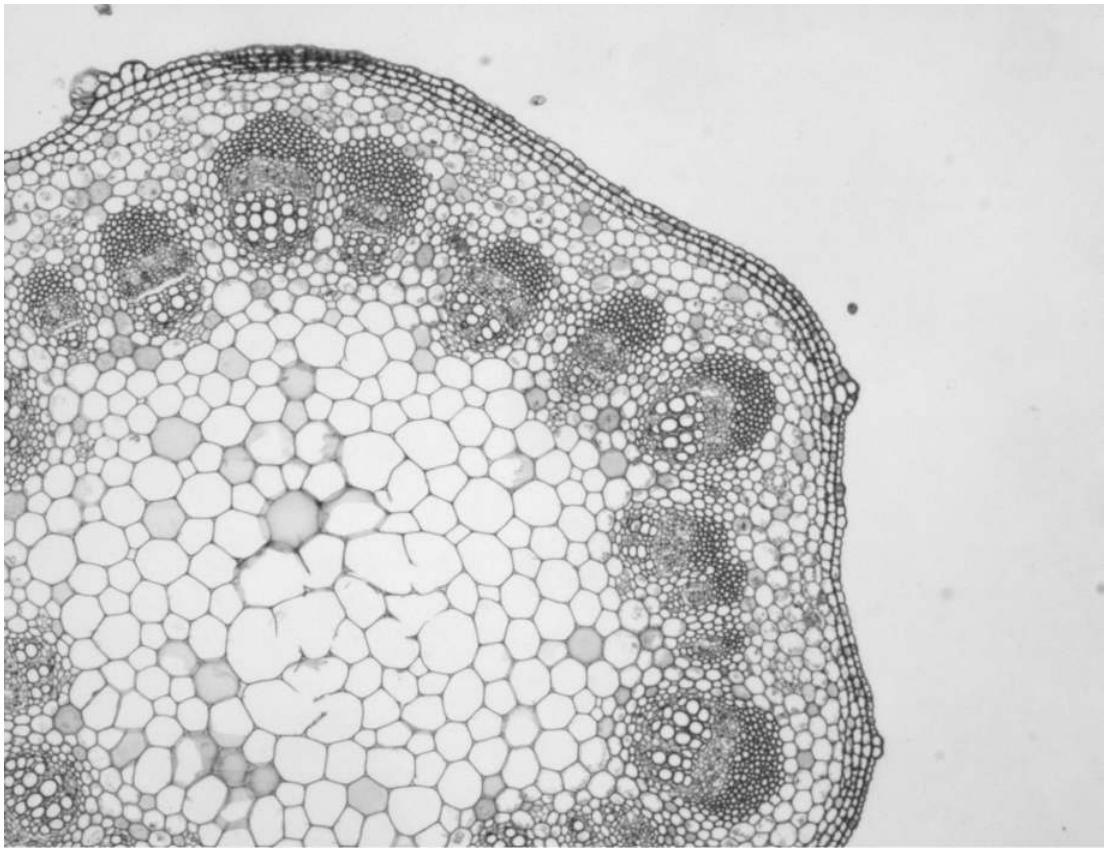
Figure

: Zea mays vascular bundle

Observe a cross section of a monocot stem. (C7)

Eudicot Stem

In eudicot stems, the vascular tissue is arranged into a ring (the **vascular cylinder**) that separates the ground tissue into two distinct regions. The region of ground tissue contained within the vascular cylinder is called the **pith**. The region of ground tissue that is between the vascular cylinder and the epidermis is called the **cortex**. Similar to the monocots, the primary xylem is located in the region of the vascular tissue facing the center of the stem, while the primary phloem is produced toward the exterior of the stem.



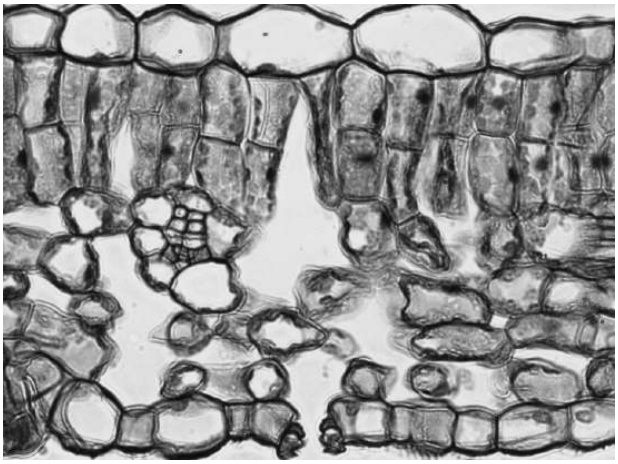
Figure

: Helianthus stem cross section

Observe a cross section of a young *Helianthus* (or other eudicot) stem. (C8)

Mesophytic Leaf Anatomy

View a prepared slide of a *Ranunculus* leaf. This small, herbaceous flowering plant is more commonly referred to as a buttercup. It grows in many environments, but tends to prefer shaded, cool spots with plenty of moisture. It is a good example of a "standard" leaf, not specially adapted to either wet or dry environments. This type of plant is called a **mesophyte** (meso- meaning middle, -phyte meaning plant), preferring moderate climatic conditions.



Figure

: Mesophytic Leaf

The outer layer of cells on both the upper and lower surface of the leaf is the **epidermis**. Can you find any pores (gaps) in the epidermis? These pores are called **stomata** and allow carbon dioxide (

) to enter the leaf for photosynthesis. Oxygen ( ), which is produced during photosynthesis as a waste product, is released through the stomata. A third gas, water vapor (

), also escapes through the stomata, though this has both beneficial and detrimental effects for the plant.

Look to either side of a **stoma** (this is the singular version of stomata) to see the flanking **guard cells**. These cells regulate the opening and closing of the stoma by either inflating and opening when there is high water content in the leaf, or collapsing and closing the stoma when water content in the leaf is low. This prevents water vapor from escaping the plant if it has too little water. However, it also prevents

from entering, stopping the formation of sugars in the plant and cutting of its source of energy. The flow of water vapor out of the leaf helps pull water up from the roots, see lab 5a for a full description of transpiration.

Beneath the upper epidermis is a layer of elongated cells full of chloroplasts. This is the **palisade mesophyll**, which has specialized for capturing incoming sunlight, rotating chloroplasts to the top of the leaf and then allowing them to regenerate by cycling them toward the leaf's center. Just below the palisade mesophyll is the **spongy mesophyll**. The spongy mesophyll is full of air pockets (hence the name spongy) that allow

to move into the leaf to the palisade mesophyll, as well as allowing oxygen to diffuse from the palisade mesophyll through the spongy mesophyll and out the stomata.

You may see circles of densely packed cells that both stain a different color than the mesophyll cells, as well as have a different organizational structure. These are veins of vascular tissue running through the leaf. The xylem is on the top, staining differently from the rest of the cells due to its lignified secondary walls. The phloem is on the bottom of the bundle, supported by a cluster of fibers (sclerenchyma) that increase structural support for the veins. The xylem is transporting water and dissolved minerals from the roots up to the leaf, while the phloem is transporting sugars made in the leaf to other regions of the plant. (C9)

Campus Scavenger Hunt

Obtain a list of plant anatomy and organ modifications and use it to explore the plants on campus. How many examples of those listed can you find?

Contributors and Attributions

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27.3: Lab Report

A1. Draw a cross section of the celery petiole, labeling **parenchyma** in the epidermis, **collenchyma** in the cortex, and **sclerenchyma** in the vascular tissue. Make notes about the differences in the cell wall for your future study.

A2. The central region of the celery petiole is called the **pith**. What type of cells are present in this region?

A3. What other cellular changes might occur to signal that a pear is ripe?

A4. Is this sclereid alive or dead? What about the parenchyma cells around it?

A5. What is the compound in the secondary wall that stains differently from the primary wall?

B1. How does the location of the trichomes relate to prevention of water loss?

B2. View a leaf under the dissecting scope. Can you find trichomes, guard cells, or other specialized epidermal cells?

B3. What cell type (-enchyma) are these cells most similar to?

B4. *View a prepared slide of a leaf cross section.* Draw what you see below. Identify and label as many tissues, cell types, and specialized cells as you can.

Summary Table of Cells and Tissues in the Leaf Organ

A **simple tissue** contains only a single cell type, while a **complex tissue** contains multiple cell types. The leaf organ is composed of both simple and complex tissues. In the table below under Tissue Type, try to identify whether it is a simple or complex tissue. If it is a simple tissue, identify which cell type it is composed of.

Tissue	Specialized Cells	Tissue Type	Function
Epidermis	1. Guard cell 2. Trichome		
Mesophyll	Mesophyll cells		
Xylem	Tracheids & vessel elements		
Phloem	1. Sieve tube elements 2. Companion cells 3. Phloem fibers		

Why didn't I include a stoma among the specialized cells in the epidermis?

The image below is a specialized cell called a tracheid. What tissue would you find this cell in? Is that tissue simple or complex?



Figure 27.3.1: A tracheid

Based on your knowledge of root words, what does the term tracheophytes mean?

Early plants have tracheids, while later groups of plants have an additional type of water conducting cell: vessel elements.

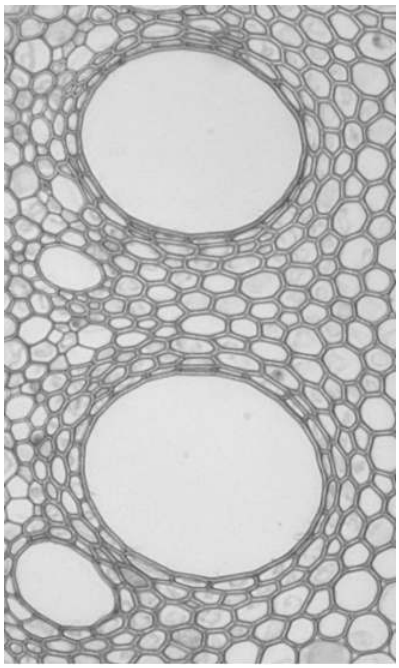


Figure 27.3.2: Vessel elements in corn root

The image above is a cross section through the xylem of a corn root. You can see large open areas (vessel elements) surrounded by smaller, more densely packed cells (tracheids).

How would these two cell types differ in the ability to take up and transport water?

Transpiration in Action

Water moves through the dead water-conducting cells in the xylem much like it moves through a tube. Transpiration acts like suction from the top of the tube, but as you saw in the previous experiment, other forces aid in the movement of the water: cohesion, adhesion, tension, and capillary action. All of these forces work to pull water into the plant through the root hairs, into the xylem, and out through the stomata.

You set up four plants at the start of lab. What were the conditions for each plant?

Plant 1 -

Plant 2 -

Plant 3 -

Plant 4 -

Check on the plants and, before doing anything, simply observe the appearance of the bags. Describe your observations below.

Turn each plant on its side and carefully remove the bags. Try not to let any condensation in the bag escape. Use a scale to obtain the mass of each bag. Note: if you used different types of bags, adjust your end mass measurements by subtracting the initial mass.

Mass of bag (adjusted)	1:	2:	3:	4:

C1. Fill in the diagram below with the following terms: **cortex, ground meristem, pericycle, primary phloem,** and **protoderm**. Choose a different color to represent meristems and tissues, then color the boxes accordingly.

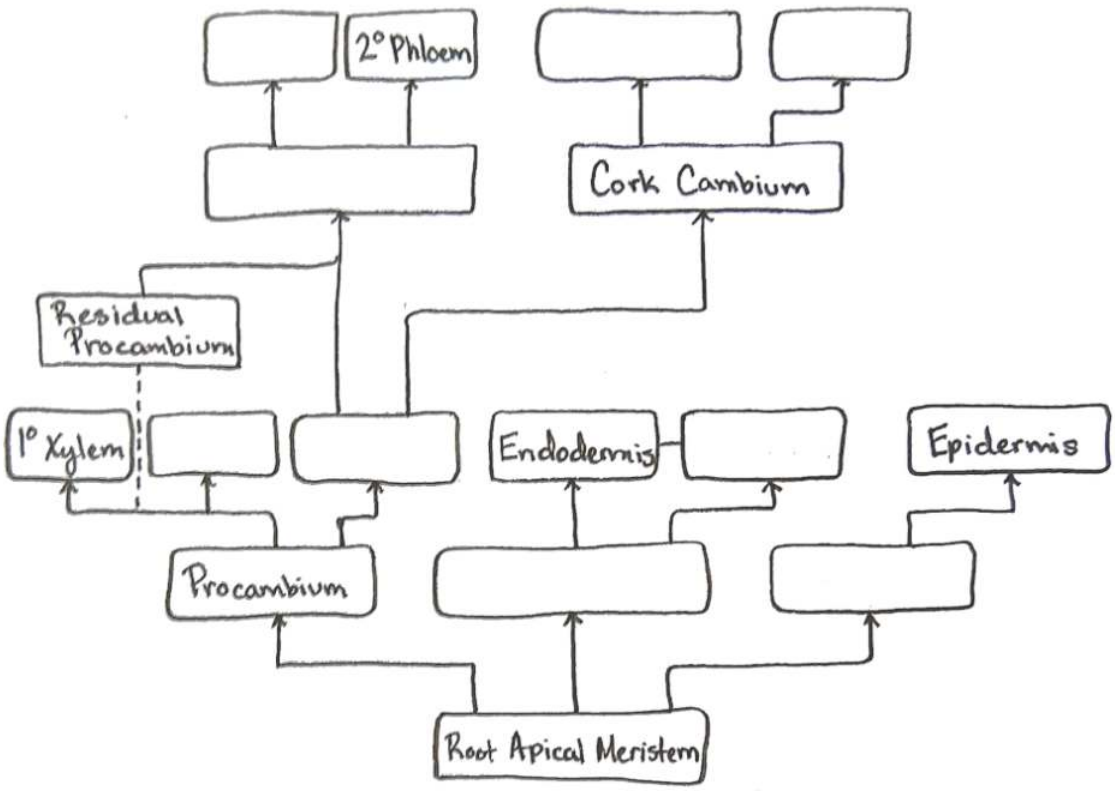


Figure 27.3.3: Flowchart of root development primary growth

C2. How would this flowchart be different in a monocot? Draw a flowchart of monocot root development in the space below.

C3. Observe a cross section of the zone of maturation in a *Zea mays* root. Draw and label your microscope slide.

C4. Observe a cross section of a *Salix* (willow) root producing lateral roots. Locate the primary xylem, primary phloem, pericycle, cortex, and epidermis. Draw and label these from your microscope slide.

C5. Observe a cross section of a *Ranunculus* (buttercup) root. Locate the **pericycle, endodermis, casparian strip**, and **passage cells**. The suberin in the casparian strip often stains similarly to the secondary walls in the xylem. Draw and label the cross section.

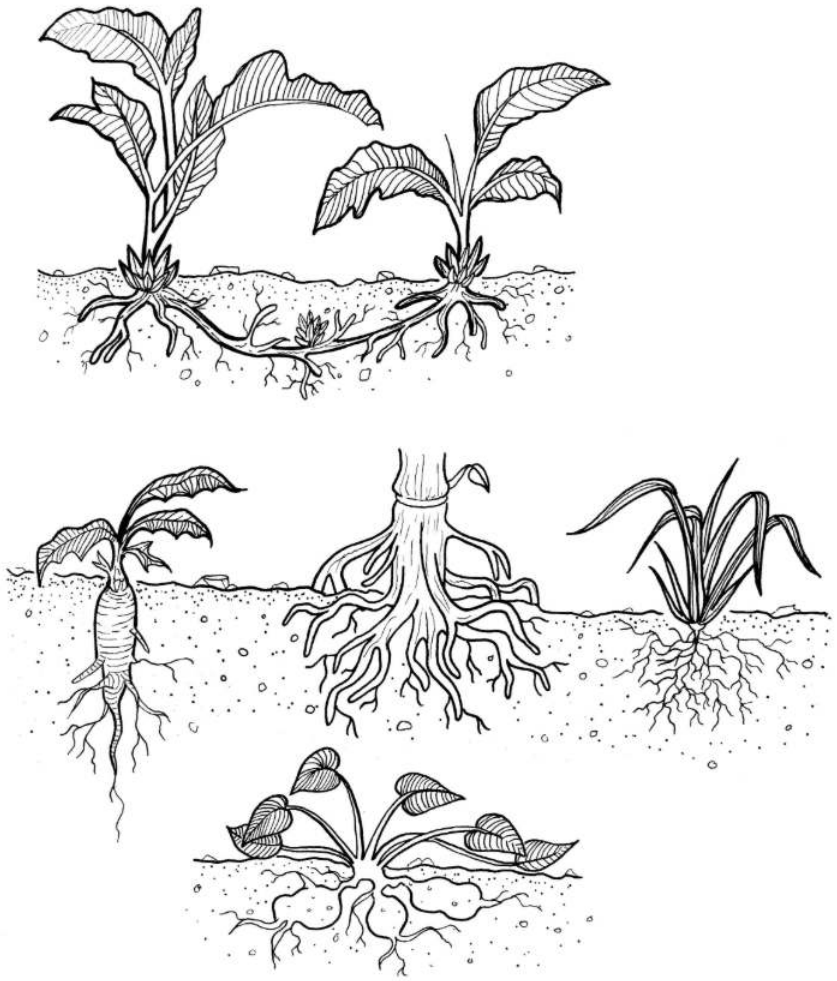


Figure 27.3.4: Types of root systems

In the figure above, label any adventitious roots, prop roots, and storage roots. Label each system as either netted or taproot (except the topmost root system, which is an underground stem).

C6. Observe a prepared slide of a Coleus shoot tip long section. Locate the meristems, tissues, and specialized cells. Draw what you see and label any important features.

Flowchart of Shoot Development

Below is a flowchart of eudicot shoot development in primary growth. There are two lines that will later lead to the secondary meristems. With this information and the filled in boxes, you should be able to determine which meristems and tissues go in the empty boxes.

Fill in the diagram below with the following terms: **cortex, epidermis, fascicular cambium, ground meristem, pith rays, procambium** and **primary xylem.**. Choose a different color to represent meristems and tissues, then color the boxes accordingly.

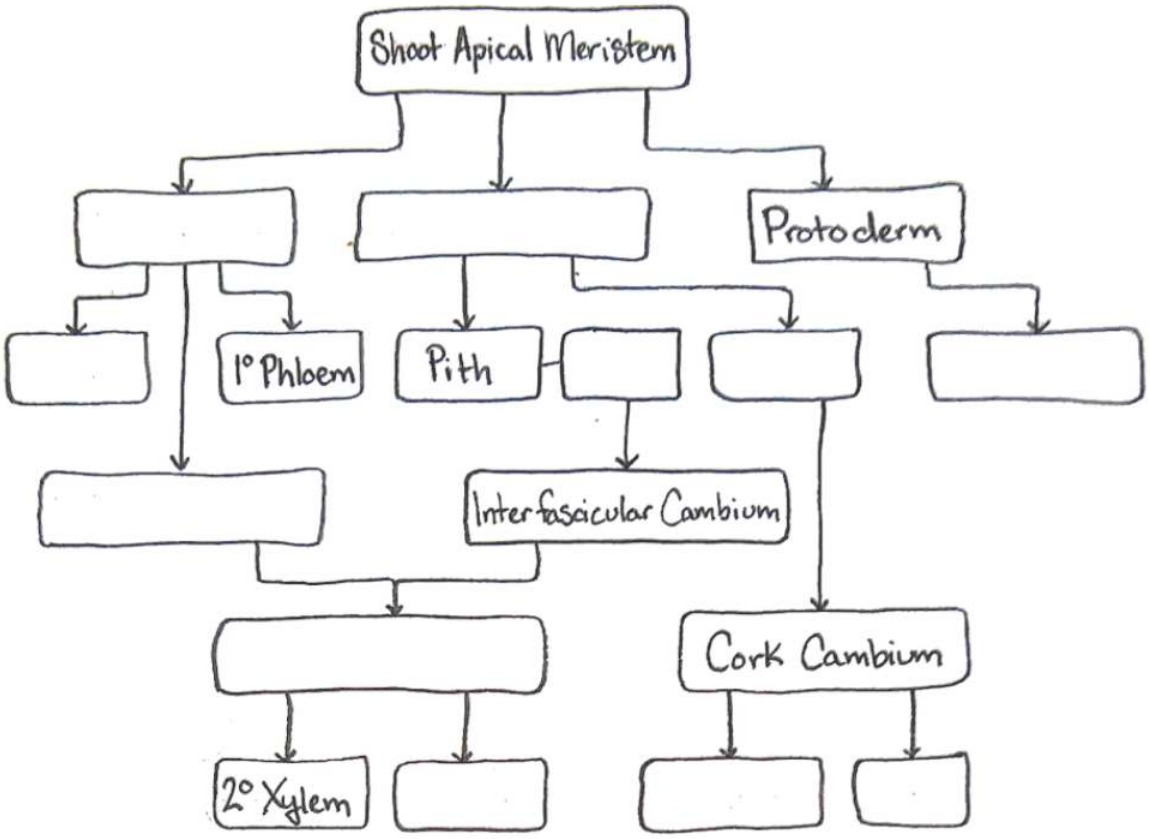


Figure 27.3.5: Flowchart of eudicot shoot development in primary growth

C7. Using a slide of a monocot stem, draw and label the **xylem, phloem,** a **vessel element, sieve tube element,** and **companion cell.**

C8. Observe a cross section of a young *Helianthus* (or other eudicot) stem. Draw and label the **epidermis, cortex, primary phloem, primary xylem**, **pith,** and **pith rays**.

What cell types (-enchymas) can you identify in the *Tilia* cross section? Which tissue(s) do you find each cell type in?

C9. Draw a cross section of a mesophytic leaf, labeling each structure or tissue with its name and function. Consider simplifying the image to use as an easy reference.

In the leaf you are viewing, are there more stomata on the upper or lower epidermis? Can you think of any reasons why this might be?

How does the structure of the spongy mesophyll contribute to its function?

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CHAPTER OVERVIEW

28: Plant Growth

Learning Objectives

Content Objectives

- Observe and classify different types of secondary plant growth.
- Identify structures associated with the lateral growth of plants.

Skill Objectives

- Draw and label parts of a vascular bundle.
- Label the structures of a woody stem.

28.1: Introduction
28.2: Exercise
28.3: Lab Report

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28.1: Introduction

Over the course of each different plant's evolutionary history, environmental pressures can lead to modifications of plant tissues and organs in predictable ways that we can then classify. For example, plants that frequently encounter drought will experience selection for the ability to access water when there is no water in the environment. The plants that evolve water-storage will have better survival in this environment and are more likely to successfully reproduce. However, animals in this dry environment might then seek out their water-filled tissues. Plants that either hide or protect these water reserves are more likely to survive and experience less herbivory. Over time, as the environment continues to select for these defenses, they improve. And thus, over long periods of evolutionary time, one lineage of plants evolves into cacti, while another related lineage evolves in a different environment to be carnations.

There are many selective pressures on plants. limate has a strong selective effect on the anatomy and morphology of plants, particularly water availability and access to sunlight. Herbivory is another strong selective pressure, as plants cannot run away from their predators. Instead, they must evolve other deterrents while balancing the energy costs required for these adaptations with energy devoted to reproduction. A third strong selective pressure is nutrient availability, which is often determined by the soil environment. For example, serpentine soils have low levels of nitrogen and calcium. Plants in serpentine habitats often evolve the ability to trap and digest insects to absorb the missing nutrients. These "carnivorous" plants are not heterotrophic, because they do not use the trapped insects as an energy source.

Plant growth regulators (phytohormones) are small organic molecules that occur naturally in plants. Phytohormones can regulate plant physiological growth processes such as flowering, germination, stem elongation, seed dormancy, fruit ripening, and gene expression.

There are several phytohormones such as gibberellic acid that can increase stem growth parameters. Ethylene can influence fruit ripening.

Gokhan Hacisalihoglu

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The two questions you answered above are essential to understanding the evolution of **secondary growth**. In secondary growth, primary tissues and residual meristematic tissues produce secondary meristems, which then produce secondary tissues. Whereas primary tissues allow for vertical growth, secondary tissues allow for lateral growth: they allow stems and roots to become wider. How might this impact the ability of a plant to grow taller?

In addition to growing wider, secondary growth exchanges the living epidermis for a thick layer of dead, waterproofed cells called cork. The cork and a few other layers of tissue comprise something called the periderm, or perhaps more familiarly called **bark**. Consider the tradeoffs between having a living exterior with guard cells vs. a thick layer of waterproofed dead cells. How might this impact the ability of the plant to interact with the outer environment? How might this impact the ability of the outer environment to interact with the interior of the plant?

Variations on this type of growth appear in a few places, but the evolution of gymnosperms (conifers and their relatives) is when the more typical secondary growth appears in evolutionary history. As you will see in later labs, the gymnosperms evolved during a period in Earth's history when inland seas were drying out and plants were migrating further from the water. This group of plants is specialized for growing tall (think Coast redwoods) or living in harsh, low-water environments. As a general rule, monocots do not undergo secondary growth, so this lab will only address eudicots.

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28.2: Exercise

Secondary Tissues in the Root

In roots, the formation of both secondary meristems involves the pericycle. The pericycle and some **residual procambium** join together to form the **vascular cambium**, a secondary meristem that produces vascular tissue. The other secondary meristem, the **cork cambium**, is initially formed solely from the pericycle.

Each of these secondary meristems divides in two directions to form a different secondary tissue to the inside and outside of the meristematic layer, respective to the center of the plant.

The vascular cambium produces **secondary xylem** to the inside of the root and **secondary phloem** to the outside.

The cork cambium produces **phelloderm**, a storage tissue, to the inside of the root and **cork**, a protective layer of dead, suberized cells, toward the outside. These three layers -- phelloderm, cork cambium, and cork -- are referred to as a **periderm**. As the first periderm layer is formed, it separates the epidermis, cortex, and endodermis from the conductive tissues of the root. The epidermis and cortex cells die and are shed as secondary growth proceeds.

> The pine root below has one layer of periderm fully formed. Can you label the tissues and meristems described above? What are the large holes in the root?

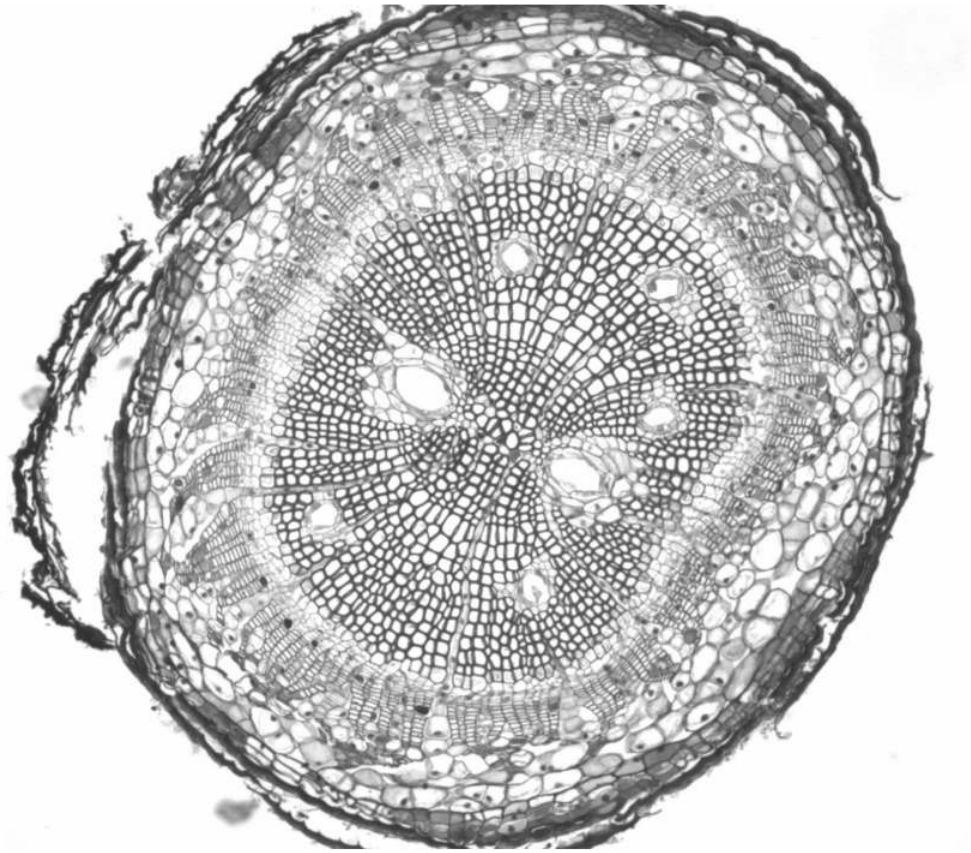


Figure 28.2.1: Pine Root Cross Section

Secondary Tissues in the Shoot

Secondary Vascular Tissue

Though the secondary tissues are all the same, the sequence of secondary meristem development in shoots is a bit different than in roots. Shoots have no pericycle, so the secondary meristems must be formed from different tissues. In shoots, the vascular

cambium is formed from residual procambium within the vascular bundles (**fascicular cambium**) joined by tissue in the pith rays (**interfascicular cambium**).

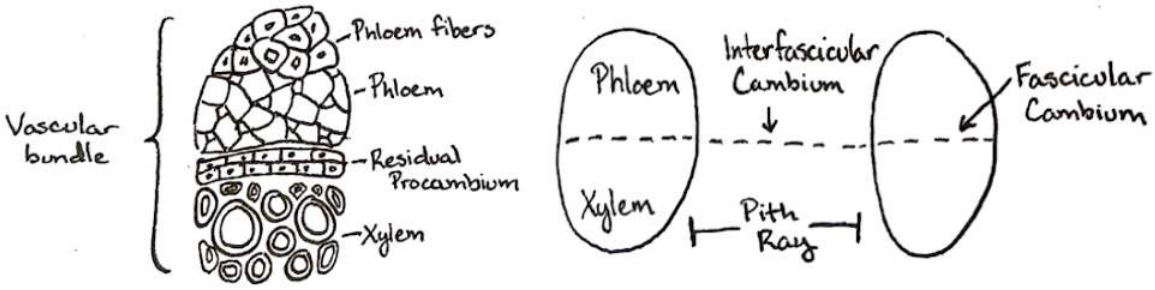


Figure 28.2.1: The vascular bundle of a eudicot stem

This layer of cells will divide to produce secondary xylem toward the inside of the stem and secondary phloem toward the outside. The conducting cells in the secondary xylem are dead at maturity. As the stem matures, these dead cells become separated from the living cells and the plant would be unable to direct the transport of materials into and out of the center of the plant. To solve this, plants have pathways of living cells that traverse the xylem and phloem called rays. **Xylem rays** radiate outward from the center of the stem until they reach the vascular cambium. At this point, they become **phloem rays**. Some of these will balloon out into large deltas of cells in the phloem tissue, while others will remain narrow strips of cells.

The secondary phloem becomes densely packed with layers **secondary phloem fibers** that provide structural support for the stem to grow tall.

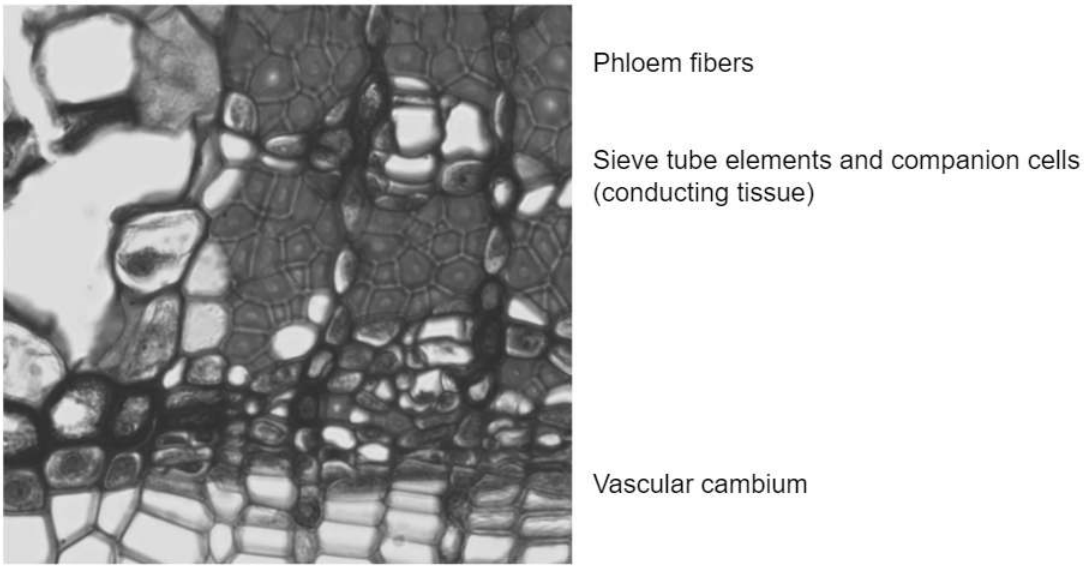


Figure 28.2.2: Close up of vascular cambium

Vessel elements in secondary xylem

In the secondary xylem, different diameter conducting cells form in wet and dry conditions. Larger diameter cells form in the wet season when water is plentiful. As the seasons transition to drier whether or approach freezing conditions, cells are smaller. Because most climates have a wet and dry season each year, you can track years of growth by the formation of these **annual growth rings** in the secondary xylem.

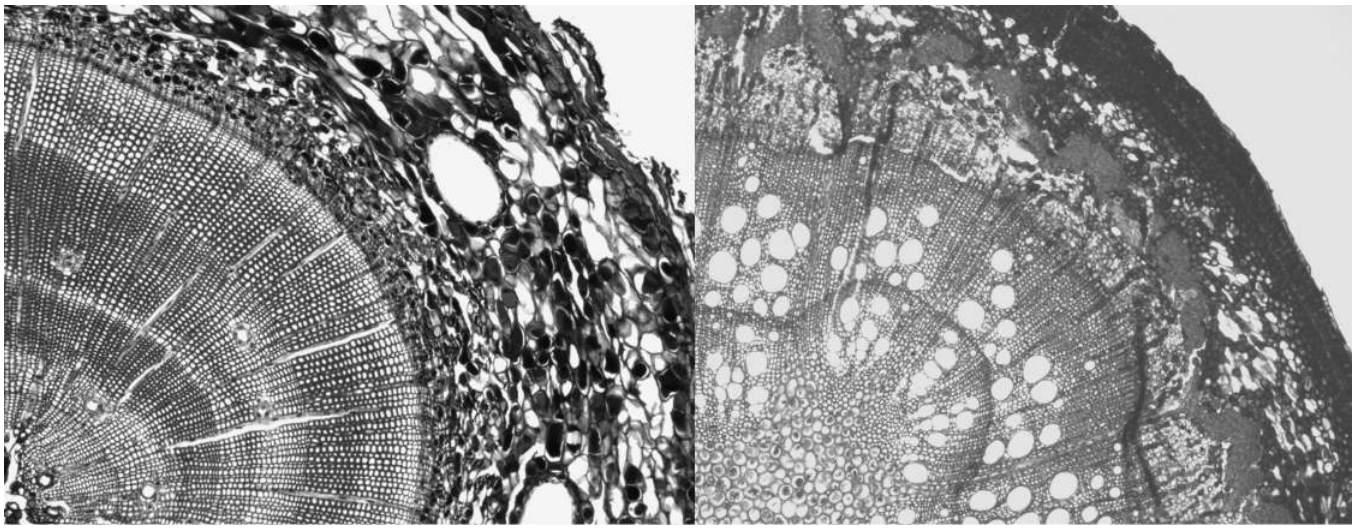


Figure 28.2.3: Tilia stem cross section

On the left is a pine stem, on the right is an oak. In the pine stem, the cells are a more similar diameter because they are all tracheids. On the right, The largest diameter cells are vessel elements. How old are each of these stems?

Resin canals

In answer to the previous question about the roots, those giant holes in the pine stem (as in the roots) are resin canals. These canals transport a sticky substance called resin. Much like the latex produced in some flowering plants, resin acts as a defense compound and a wound sealant.

Resin canals are also present in some groups of angiosperms, particularly tropical trees. Why might these structures occur more commonly in tropical plants?

The cork cambium is formed directly from the cortex. Layers of periderm are continually formed and shed until the cortex runs out. At this point, secondary phloem de-differentiates and forms the new layers of cork cambium.

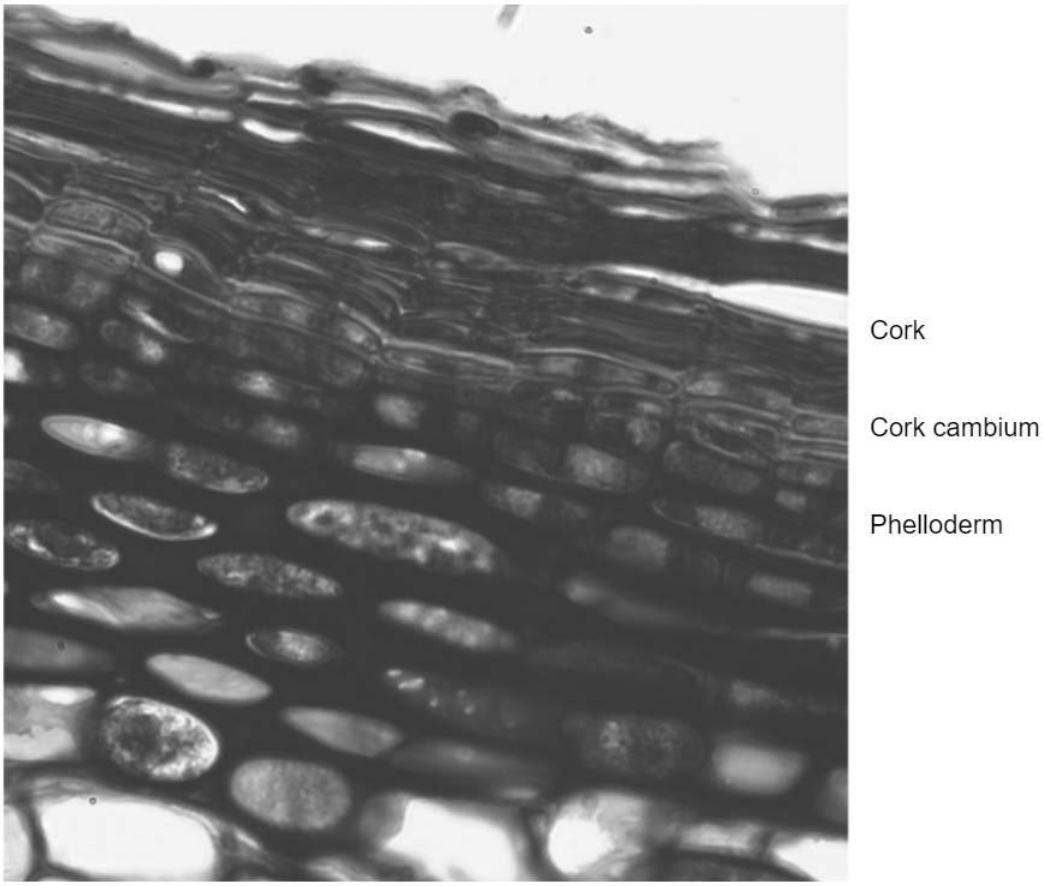


Figure 28.2.4: Periderm

Because the periderm is suberized and dead, it cannot have living guard cells to regulate pores and control gas exchange with the environment. Because the cells on the inside are still alive and respiring, gas exchange is still necessary. This is accomplished through tears in the periderm called **lenticels** (shown below).

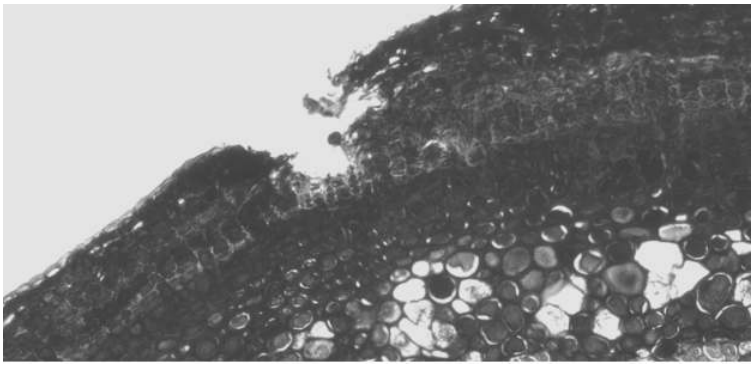


Figure 28.2.5: Lenticel

In a mature woody stem, the layers of periderm are referred to as the **outer bark** and the secondary phloem is the **inner bark**. The secondary xylem is called **wood**. Secondary xylem that is still actively involved in conducting water is called **sapwood**, while inactive xylem is called **heartwood**. The cross section below is too young to have these last two features.

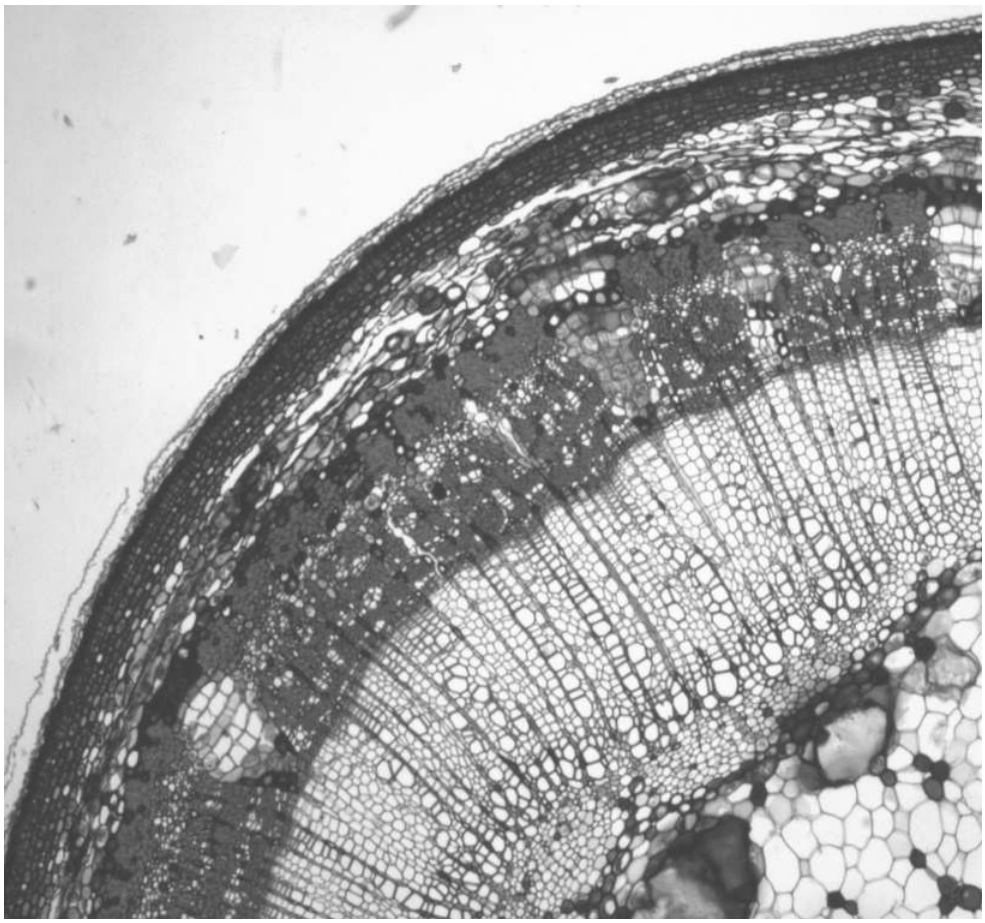


Figure 28.2.6: Cross section of a woody stem

In the stem cross section above, label the following: **pith, secondary xylem, xylem ray, vascular cambium, secondary phloem, phloem ray, cortex, phelloderm, cork cambium, cork,** and **epidermis**. Next, indicate where the oldest and youngest xylem cells are located.

Note: the newest cells will be located closest to the meristem that produced them!

Where is the vascular cambium?

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28.3: Lab Report

Secondary Tissues in the Root

The pine root below has one layer of periderm fully formed. Can you label the tissues and meristems described above? What are the large holes in the root?

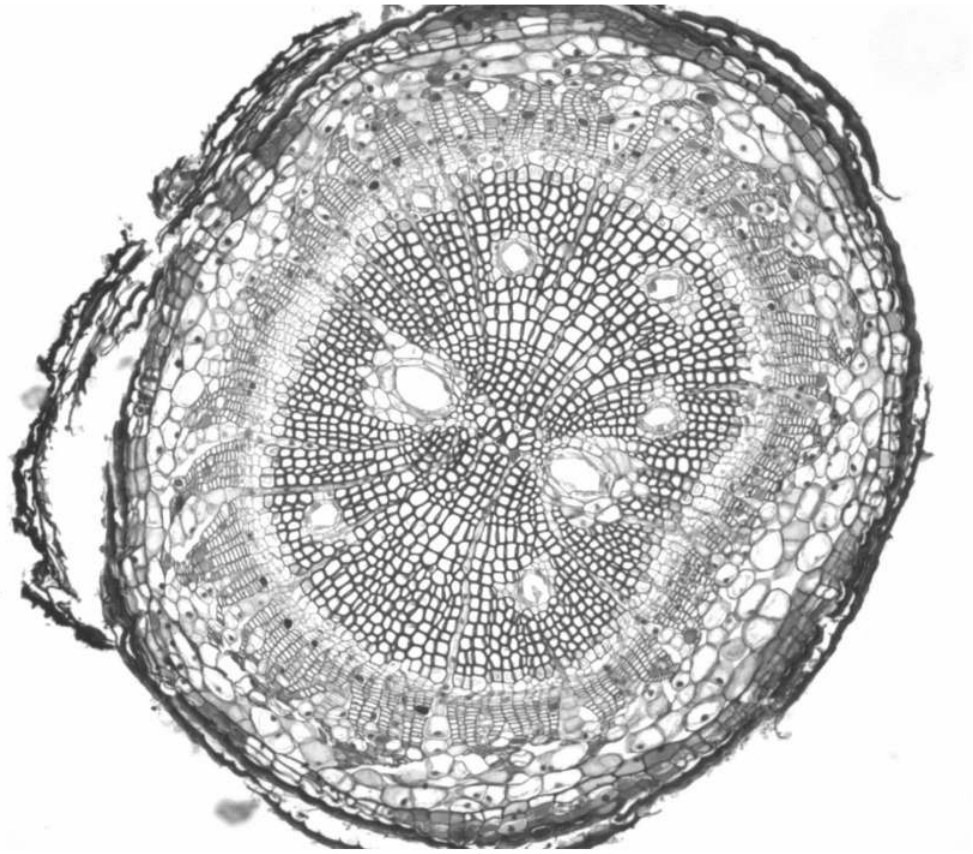


Figure 28.3.1: Pine Root Cross Section

Secondary Tissues in the Shoot

Secondary Vascular Tissue

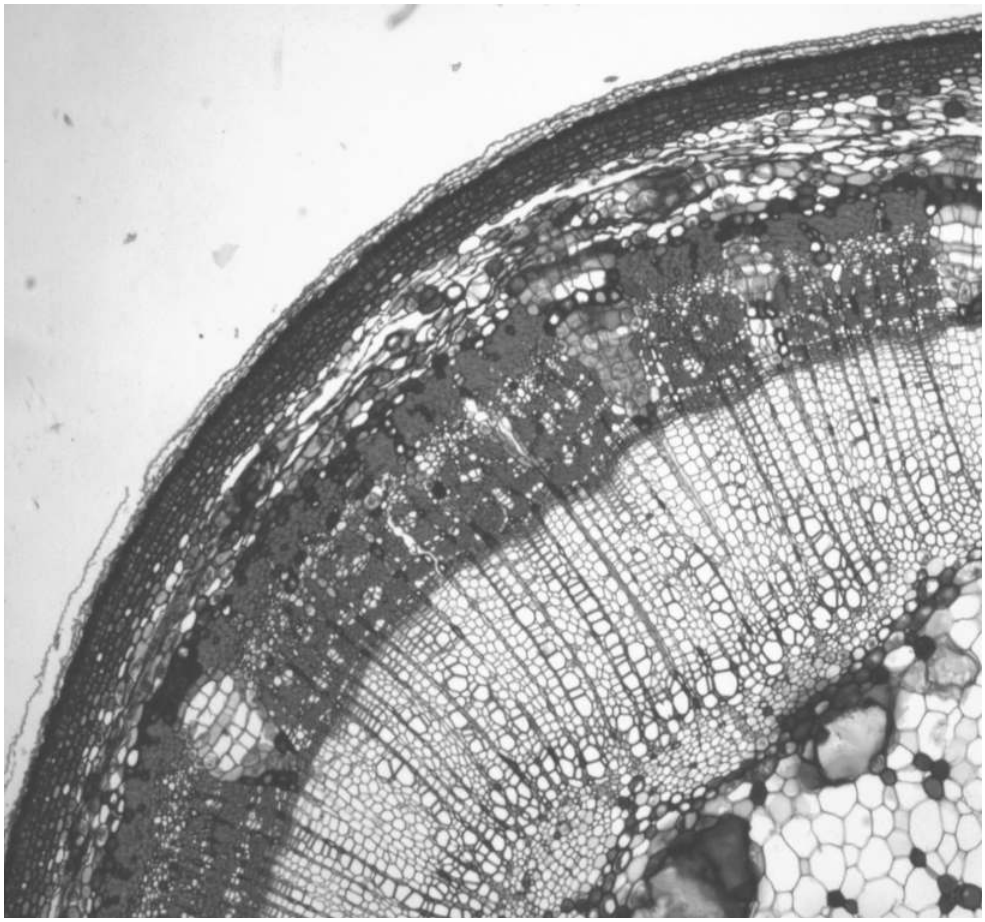


Figure 28.3.2: Cross section of a woody stem

In the stem cross section above, label the following: **pith, secondary xylem, xylem ray, vascular cambium, secondary phloem, phloem ray, cortex, phelloderm, cork cambium, cork,** and **epidermis**. Next, indicate where the oldest and youngest xylem cells are located.

Note: the newest cells will be located closest to the meristem that produced them!

Where is the vascular cambium?

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CHAPTER OVERVIEW

29: Animal Tissues

Learning Objectives

Content Objectives

- Describe epithelial tissues
- Discuss the different types of connective tissues in animals
- Describe three types of muscle tissues
- Describe nervous tissue

Skill Objectives

- Compare and contrast the four tissue types, epithelial, connective, muscular, and nervous.
- Draw and label select tissue examples provided in lab.

29.1: Introduction
29.2: Exercise
29.3: Lab Report

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29.1: Introduction

The tissues of multicellular, complex animals are four primary types: epithelial, connective, muscle, and nervous. Recall that tissues are groups of similar cells (cells carrying out related functions). These tissues combine to form organs—like the skin or kidney—that have specific, specialized functions within the body. Organs are organized into organ systems to perform functions; examples include the circulatory system, which consists of the heart and blood vessels, and the digestive system, consisting of several organs, including the stomach, intestines, liver, and pancreas. Organ systems come together to create an entire organism.

Epithelial Tissues

Epithelial tissues cover the outside of organs and structures in the body and line the lumens of organs in a single layer or multiple layers of cells. The types of epithelia are classified by the shapes of cells present and the number of layers of cells. Epithelia composed of a single layer of cells is called simple epithelia; epithelial tissue composed of multiple layers is called stratified epithelia. Table 33.2 summarizes the different types of epithelial tissues.

Different Types of Epithelial Tissues

Epithelial tissue types

Cell shape	Description	Location
squamous	flat, irregular round shape	simple: lung alveoli, capillaries; stratified: skin, mouth, vagina
cuboidal	cube shaped, central nucleus	glands, renal tubules
columnar	tall, narrow, nucleus toward base; tall, narrow, nucleus along cell	simple: digestive tract; pseudostratified: respiratory tract
transitional	round, simple but appear stratified	urinary bladder

Table 33.2

Squamous Epithelia

Squamous epithelial cells are generally round, flat, and have a small, centrally located nucleus. The cell outline is slightly irregular, and cells fit together to form a covering or lining. When the cells are arranged in a single layer (simple epithelia), they facilitate diffusion in tissues, such as the areas of gas exchange in the lungs and the exchange of nutrients and waste at blood capillaries.

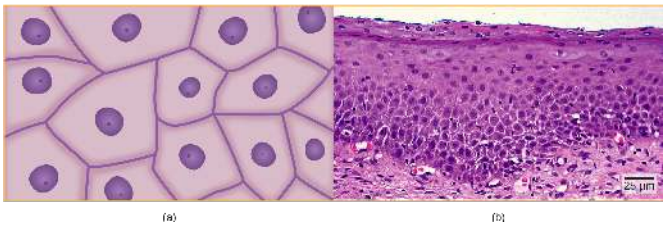


Figure 33.7 Squamous epithelia cells (a) have a slightly irregular shape, and a small, centrally located nucleus. These cells can be stratified into layers, as in (b) this human cervix specimen. (credit b: modification of work by Ed Uthman; scale-bar data from Matt Russell)

Figure 33.7a illustrates a layer of squamous cells with their membranes joined together to form an epithelium. Image Figure 33.7b illustrates squamous epithelial cells arranged in stratified layers, where protection is needed on the body from outside abrasion and damage. This is called a stratified squamous epithelium and occurs in the skin and in tissues lining the mouth and vagina.

Cuboidal Epithelia

Cuboidal epithelial cells, shown in Figure 33.8, are cube-shaped with a single, central nucleus. They are most commonly found in a single layer representing a simple epithelia in glandular tissues throughout the body where they prepare and secrete glandular material. They are also found in the walls of tubules and in the ducts of the kidney and liver.



Figure 33.8 Simple cuboidal epithelial cells line tubules in the mammalian kidney, where they are involved in filtering the blood.

Columnar Epithelia

Columnar epithelial cells are taller than they are wide: they resemble a stack of columns in an epithelial layer, and are most commonly found in a single-layer arrangement. The nuclei of columnar epithelial cells in the digestive tract appear to be lined up at the base of the cells, as illustrated in Figure 33.9. These cells absorb material from the lumen of the digestive tract and prepare it for entry into the body through the circulatory and lymphatic systems.

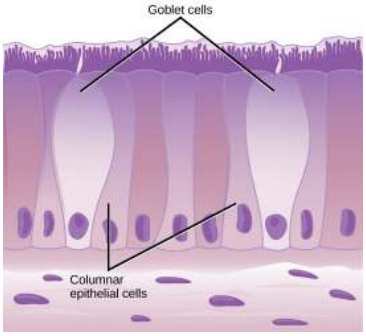


Figure 33.9 Simple columnar epithelial cells absorb material from the digestive tract. Goblet cells secrete mucus into the digestive tract lumen.

Columnar epithelial cells lining the respiratory tract appear to be stratified. However, each cell is attached to the base membrane of the tissue and, therefore, they are simple tissues. The nuclei are arranged at different levels in the layer of cells, making it appear as though there is more than one layer, as seen in Figure 33.10. This is called pseudostratified, columnar epithelia. This cellular covering has cilia at the apical, or free, surface of the cells. The cilia enhance the movement of mucus and trapped particles out of the respiratory tract, helping to protect the system from invasive microorganisms and harmful material that has been breathed into the body. Goblet cells are interspersed in some tissues (such as the lining of the trachea). The goblet cells contain mucus that traps irritants, which in the case of the trachea keep these irritants from getting into the lungs.

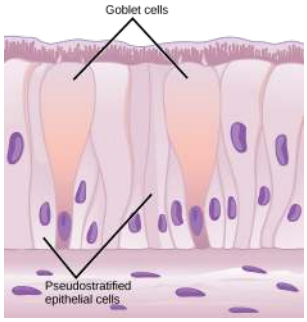


Figure 33.10 Pseudostratified columnar epithelia line the respiratory tract. They exist in one layer, but the arrangement of nuclei at different levels makes it appear that there is more than one layer. Goblet cells interspersed between the columnar epithelial cells secrete mucus into the respiratory tract.

Transitional Epithelia

Transitional or uroepithelial cells appear only in the urinary system, primarily in the bladder and ureter. These cells are arranged in a stratified layer, but they have the capability of appearing to pile up on top of each other in a relaxed, empty bladder, as illustrated in Figure 33.11. As the urinary bladder fills, the epithelial layer unfolds and expands to hold the volume of urine introduced into it. As the bladder fills, it expands and the lining becomes thinner. In other words, the tissue transitions from thick to thin.

Visual Connection

Visual Connection

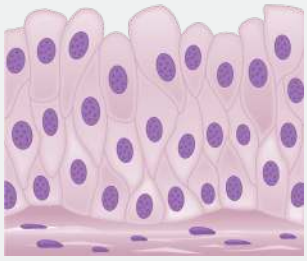


Figure 33.11 Transitional epithelia of the urinary bladder undergo changes in thickness depending on how full the bladder is.

Which of the following statements about types of epithelial cells is false?

a. Simple columnar epithelial cells line the tissue of the lung.
b. Simple cuboidal epithelial cells are involved in the filtering of blood in the kidney.
c. Pseudostratisfied columnar epithilia occur in a single layer, but the arrangement of nuclei makes it appear that more than one layer is present.
d. Transitional epithelia change in thickness depending on how full the bladder is.

Connective Tissues

Connective tissues are made up of a matrix consisting of living cells and a nonliving substance, called the ground substance. The ground substance is made of an organic substance (usually a protein) and an inorganic substance (usually a mineral or water). The principal cell of connective tissues is the fibroblast. This cell makes the fibers found in nearly all of the connective tissues. Fibroblasts are motile, able to carry out mitosis, and can synthesize whichever connective tissue is needed. Macrophages, lymphocytes, and, occasionally, leukocytes can be found in some of the tissues. Some tissues have specialized cells that are not found in the others. The matrix in connective tissues gives the tissue its density. When a connective tissue has a high concentration of cells or fibers, it has proportionally a less dense matrix.

The organic portion or protein fibers found in connective tissues are either collagen, elastic, or reticular fibers. Collagen fibers provide strength to the tissue, preventing it from being torn or separated from the surrounding tissues. Elastic fibers are made of the protein elastin; this fiber can stretch to one and one half of its length and return to its original size and shape. Elastic fibers provide flexibility to the tissues. Reticular fibers are the third type of protein fiber found in connective tissues. This fiber consists of thin strands of collagen that form a network of fibers to support the tissue and other organs to which it is connected. The various types of connective tissues, the types of cells and fibers they are made of, and sample locations of the tissues is summarized in Table 33.3.

Connective Tissues

Tissue	Cells	Fibers	Location
loose/areolar	fibroblasts, macrophages, some lymphocytes, some neutrophils	few: collagen, elastic, reticular	around blood vessels; anchors epithelia
dense, fibrous connective tissue	fibroblasts, macrophages	mostly collagen	irregular: skin; regular: tendons, ligaments
cartilage	chondrocytes, chondroblasts	hyaline: few: collagen fibrocartilage: large amount of collagen	shark skeleton, fetal bones, human ears, intervertebral discs
bone	osteoblasts, osteocytes, osteoclasts	some: collagen, elastic	vertebrate skeletons
adipose	adipocytes	few	adipose (fat)
blood	red blood cells, white blood cells	none	blood

Table 33.3

Loose/Areolar Connective Tissue

Loose connective tissue, also called areolar connective tissue, has a sampling of all of the components of a connective tissue. As illustrated in Figure 33.12, loose connective tissue has some fibroblasts; macrophages are present as well. Collagen fibers are relatively wide and stain a light pink, while elastic fibers are thin and stain dark blue to black. The space between the formed elements of the tissue is filled with the matrix. The material in the connective tissue gives it a loose consistency similar to a cotton ball that has been pulled apart. Loose connective tissue is found around every blood vessel and helps to keep the vessel in place. The tissue is also found around and between most body organs. In summary, areolar tissue is tough, yet flexible, and comprises membranes.

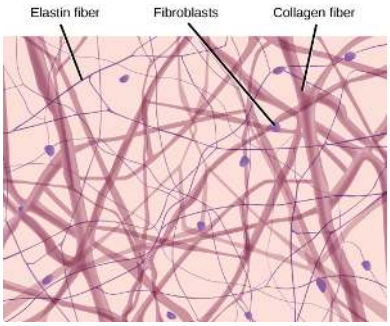


Figure 33.12 Loose connective tissue is composed of loosely woven collagen and elastic fibers. The fibers and other components of the connective tissue matrix are secreted by fibroblasts.

Fibrous Connective Tissue

Fibrous connective tissues contain large amounts of collagen fibers and few cells or matrix material. The fibers can be arranged irregularly or regularly with the strands lined up in parallel. Irregularly arranged fibrous connective tissues are found in areas of the body where stress occurs from all directions, such as the dermis of the skin. Regular fibrous connective tissue, shown in Figure 33.13, is found in tendons (which connect muscles to bones) and ligaments (which connect bones to bones).

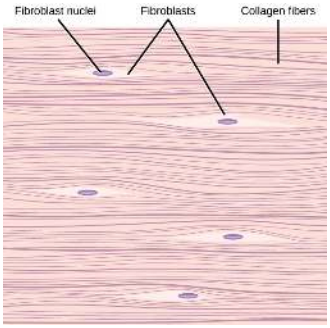


Figure 33.13 Fibrous connective tissue from the tendon has strands of collagen fibers lined up in parallel.

Cartilage

Cartilage is a connective tissue with a large amount of the matrix and variable amounts of fibers. The cells, called chondrocytes, make the matrix and fibers of the tissue. Chondrocytes are found in spaces within the tissue called lacunae.

A cartilage with few collagen and elastic fibers is hyaline cartilage, illustrated in Figure 33.14. The lacunae are randomly scattered throughout the tissue and the matrix takes on a milky or scrubbed appearance with routine histological stains. Sharks have cartilaginous skeletons, as does nearly the entire human skeleton during a specific pre-birth developmental stage. A remnant of this cartilage persists in the outer portion of the human nose. Hyaline cartilage is also found at the ends of long bones, reducing friction and cushioning the articulations of these bones.

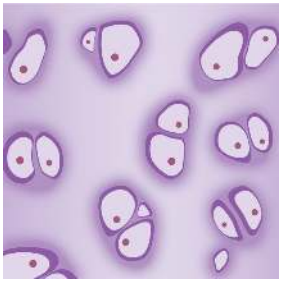


Figure 33.14 Hyaline cartilage consists of a matrix with cells called chondrocytes embedded in it. The chondrocytes exist in cavities in the matrix called lacunae.

Elastic cartilage has a large amount of elastic fibers, giving it tremendous flexibility. The ears of most vertebrate animals contain this cartilage as do portions of the larynx, or voice box. Fibrocartilage contains a large amount of collagen fibers, giving the tissue tremendous strength. Fibrocartilage comprises the intervertebral discs in vertebrate animals. Hyaline cartilage found in movable joints such as the knee and shoulder becomes damaged as a result of age or trauma. Damaged hyaline cartilage is replaced by fibrocartilage and results in the joints becoming “stiff.”

Bone

Bone, or osseous tissue, is a connective tissue that has a large amount of two different types of matrix material. The organic matrix is similar to the matrix material found in other connective tissues, including some amount of collagen and elastic fibers. This gives strength and flexibility to the tissue. The inorganic matrix consists of mineral salts—mostly calcium salts—that give the tissue hardness. Without adequate organic material in the matrix, the tissue breaks; without adequate inorganic material in the matrix, the tissue bends.

There are three types of cells in bone: osteoblasts, osteocytes, and osteoclasts. Osteoblasts are active in making bone for growth and remodeling. Osteoblasts deposit bone material into the matrix and, after the matrix surrounds them, they continue to live, but in a reduced metabolic state as osteocytes. Osteocytes are found in lacunae of the bone. Osteoclasts are active in breaking down bone for bone remodeling, and they provide access to calcium stored in tissues. Osteoclasts are usually found on the surface of the tissue.

Bone can be divided into two types: compact and spongy. Compact bone is found in the shaft (or diaphysis) of a long bone and the surface of the flat bones, while spongy bone is found in the end (or epiphysis) of a long bone. Compact bone is organized into subunits called osteons, as illustrated in Figure 33.15. A blood vessel and a nerve are found in the center of the structure within the

Haversian canal, with radiating circles of lacunae around it known as lamellae. The wavy lines seen between the lacunae are microchannels called canaliculi; they connect the lacunae to aid diffusion between the cells. Spongy bone is made of tiny plates called trabeculae; these plates serve as struts to give the spongy bone strength. Over time, these plates can break causing the bone to become less resilient. Bone tissue forms the internal skeleton of vertebrate animals, providing structure to the animal and points of attachment for tendons.

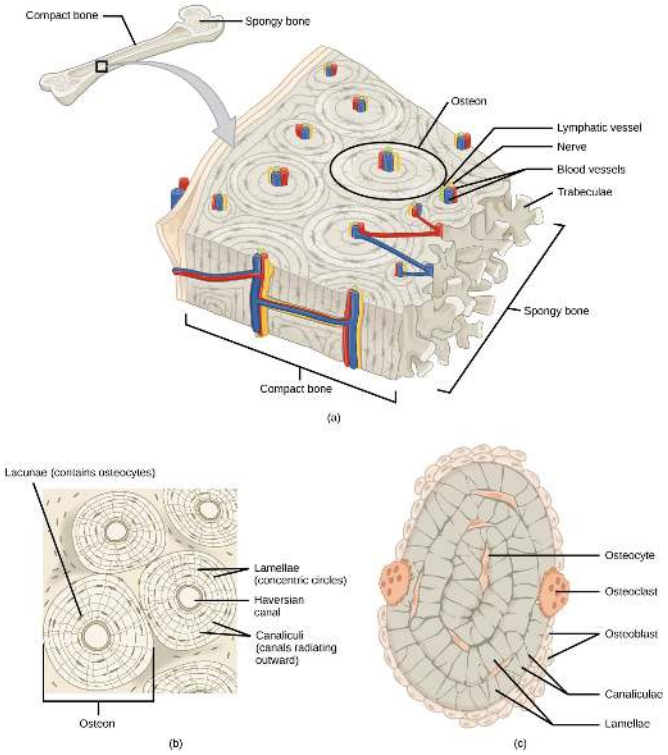


Figure 33.15 (a) Compact bone is a dense matrix on the outer surface of bone. Spongy bone, inside the compact bone, is porous with web-like trabeculae. (b) Compact bone is organized into rings called osteons. Blood vessels, nerves, and lymphatic vessels are found in the central Haversian canal. Rings of lamellae surround the Haversian canal. Between the lamellae are cavities called lacunae. Canaliculi are microchannels connecting the lacunae together. (c) Osteoblasts surround the exterior of the bone. Osteoclasts bore tunnels into the bone and osteocytes are found in the lacunae.

Adipose Tissue

Adipose tissue, or fat tissue, is considered a connective tissue even though it does not have fibroblasts or a real matrix and only has a few fibers. Adipose tissue is made up of cells called adipocytes that collect and store fat in the form of triglycerides, for energy metabolism. Adipose tissues additionally serve as insulation to help maintain body temperatures, allowing animals to be endothermic, and they function as cushioning against damage to body organs. Under a microscope, adipose tissue cells appear empty due to the extraction of fat during the processing of the material for viewing, as seen in Figure 33.16. The thin lines in the image are the cell membranes, and the nuclei are the small, black dots at the edges of the cells.

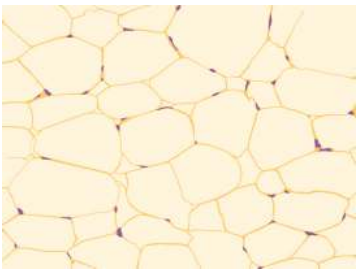


Figure 33.16 Adipose is a connective tissue is made up of cells called adipocytes. Adipocytes have small nuclei localized at the cell edge.

Blood

Blood is considered a connective tissue because it has a matrix, as shown in Figure 33.17. The living cell types are red blood cells (RBC), also called erythrocytes, and white blood cells (WBC), also called leukocytes. The fluid portion of whole blood, its matrix, is commonly called plasma.

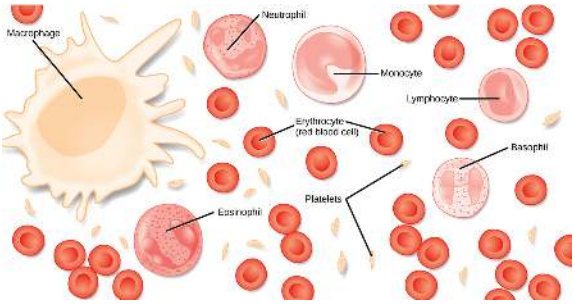


Figure 33.17 Blood is a connective tissue that has a fluid matrix, called plasma, and no fibers. Erythrocytes (red blood cells), the predominant cell type, are involved in the transport of oxygen and carbon dioxide. Also present are various leukocytes (white blood cells) involved in immune response.

The cell found in greatest abundance in blood is the erythrocyte. Erythrocytes are counted in millions in a blood sample: the average number of red blood cells in primates is 4.7 to 5.5 million cells per microliter. Erythrocytes are consistently the same size in a species, but vary in size between species. For example, the average diameter of a primate red blood cell is 7.5 µl, a dog is close at 7.0 µl, but a cat's RBC diameter is 5.9 µl. Sheep erythrocytes are even smaller at 4.6 µl. Mammalian erythrocytes lose their nuclei and mitochondria when they are released from the bone marrow where they are made. Fish, amphibian, and avian red blood cells maintain their nuclei and mitochondria throughout the cell's life. The principal job of an erythrocyte is to carry and deliver oxygen to the tissues.

Leukocytes are the predominant white blood cells found in the peripheral blood. Leukocytes are counted in the thousands in the blood with measurements expressed as ranges: primate counts range from 4,800 to 10,800 cells per µl, dogs from 5,600 to 19,200 cells per µl, cats from 8,000 to 25,000 cells per µl, cattle from 4,000 to 12,000 cells per µl, and pigs from 11,000 to 22,000 cells per µl.

Lymphocytes function primarily in the immune response to foreign antigens or material. Different types of lymphocytes make antibodies tailored to the foreign antigens and control the production of those antibodies. Neutrophils are phagocytic cells and they participate in one of the early lines of defense against microbial invaders, aiding in the removal of bacteria that has entered the body. Another leukocyte that is found in the peripheral blood is the monocyte. Monocytes give rise to phagocytic macrophages that clean up dead and damaged cells in the body, whether they are foreign or from the host animal. Two additional leukocytes in the blood are eosinophils and basophils—both help to facilitate the inflammatory response.

The slightly granular material among the cells is a cytoplasmic fragment of a cell in the bone marrow. This is called a platelet or thrombocyte. Platelets participate in the stages leading up to coagulation of the blood to stop bleeding through damaged blood vessels. Blood has a number of functions, but primarily it transports material through the body to bring nutrients to cells and remove waste material from them.

Muscle Tissues

There are three types of muscle in animal bodies: smooth, skeletal, and cardiac. They differ by the presence or absence of striations or bands, the number and location of nuclei, whether they are voluntarily or involuntarily controlled, and their location within the body. Table 33.4 summarizes these differences.

Types of Muscles

Type of Muscle	Striations	Nuclei	Control	Location
smooth	no	single, in center	involuntary	visceral organs
skeletal	yes	many, at periphery	voluntary	skeletal muscles
cardiac	yes	single, in center	involuntary	heart

Table 33.4

Smooth Muscle

Smooth muscle does not have striations in its cells. It has a single, centrally located nucleus, as shown in Figure 33.18. Constriction of smooth muscle occurs under involuntary, autonomic nervous control and in response to local conditions in the tissues. Smooth muscle tissue is also called non-striated as it lacks the banded appearance of skeletal and cardiac muscle. The walls of blood vessels, the tubes of the digestive system, and the tubes of the reproductive systems are composed of mostly smooth muscle.

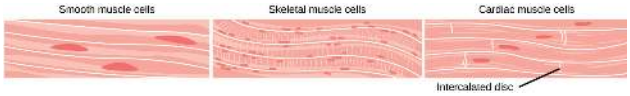


Figure 33.18 Smooth muscle cells do not have striations, while skeletal muscle cells do. Cardiac muscle cells have striations, but, unlike the multinucleate skeletal cells, they have only one nucleus. Cardiac muscle tissue also has intercalated discs, specialized regions running along the plasma membrane that join adjacent cardiac muscle cells and assist in passing an electrical impulse from cell to cell.

Skeletal Muscle

Skeletal muscle has striations across its cells caused by the arrangement of the contractile proteins actin and myosin. These muscle cells are relatively long and have multiple nuclei along the edge of the cell. Skeletal muscle is under voluntary, somatic nervous system control and is found in the muscles that move bones. Figure 33.18 illustrates the histology of skeletal muscle.

Cardiac Muscle

Cardiac muscle, shown in Figure 33.18, is found only in the heart. Like skeletal muscle, it has cross striations in its cells, but cardiac muscle has a single, centrally located nucleus. Cardiac muscle is not under voluntary control but can be influenced by the autonomic nervous system to speed up or slow down. An added feature to cardiac muscle cells is a line than extends along the end of the cell as it abuts the next cardiac cell in the row. This line is called an intercalated disc: it assists in passing electrical impulse efficiently from one cell to the next and maintains the strong connection between neighboring cardiac cells.

Nervous Tissues

Nervous tissues are made of cells specialized to receive and transmit electrical impulses from specific areas of the body and to send them to specific locations in the body. The main cell of the nervous system is the neuron, illustrated in Figure 33.19. The large structure with a central nucleus is the cell body of the neuron. Projections from the cell body are either dendrites specialized in receiving input or a single axon specialized in transmitting impulses. Some glial cells are also shown. Astrocytes regulate the chemical environment of the nerve cell, and oligodendrocytes insulate the axon so the electrical nerve impulse is transferred more efficiently. Other glial cells that are not shown support the nutritional and waste requirements of the neuron. Some of the glial cells are phagocytic and remove debris or damaged cells from the tissue. A nerve consists of neurons and glial cells.

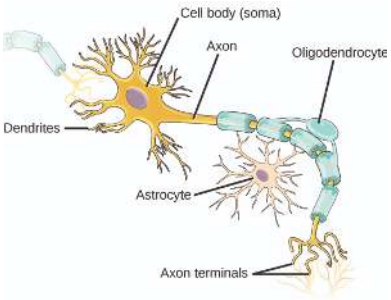


Figure 33.19 The neuron has projections called dendrites that receive signals and projections called axons that send signals. Also shown are two types of glial cells: astrocytes regulate the chemical environment of the nerve cell, and oligodendrocytes insulate the axon so the electrical nerve impulse is transferred more efficiently.

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29.2: Exercise

Exercise A.

For each microscope slide available in lab draw the tissue and complete the following:

1. Specific name of tissue (ex: blood)
2. General category of tissue (ex: connective)
3. Location of tissue (ex: found in blood vessels)
4. Function of tissue (ex: carry oxygen, carbon dioxide, nutrients)

For all microscope drawings include the total magnification under which you drew the tissue and label major structures or parts you can see in your drawing (ex: erythrocyte, leukocyte).

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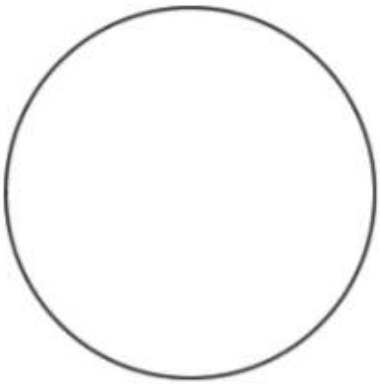
29.3: Lab Report

Exercise A.

For each microscope slide available in lab draw the tissue and complete the following:

1. Specific name of tissue (ex: blood)
2. General category of tissue (ex: connective)
3. Location of tissue (ex: found in blood vessels)
4. Function of tissue (ex: carry oxygen, carbon dioxide, nutrients)

For all microscope drawings include the total magnification under which you drew the tissue and label major structures or parts you can see in your drawing (ex: erythrocyte, leukocyte).



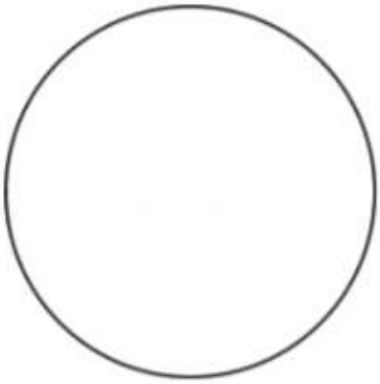
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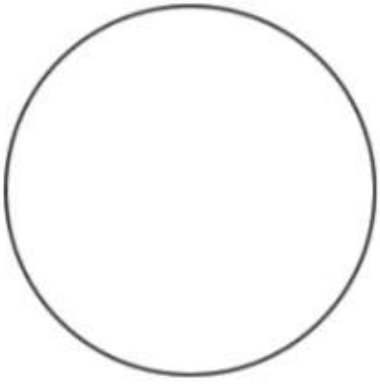
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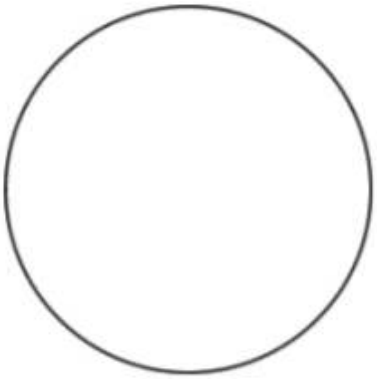
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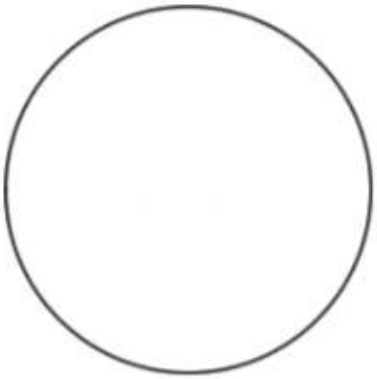
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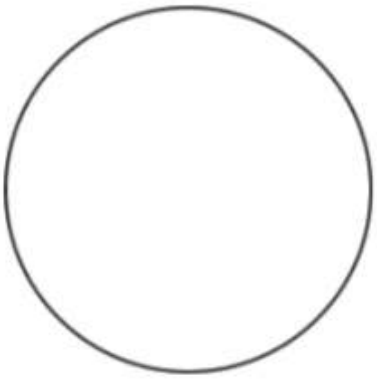
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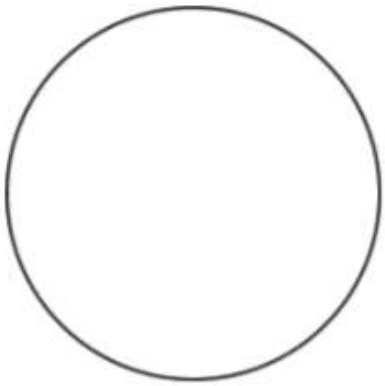
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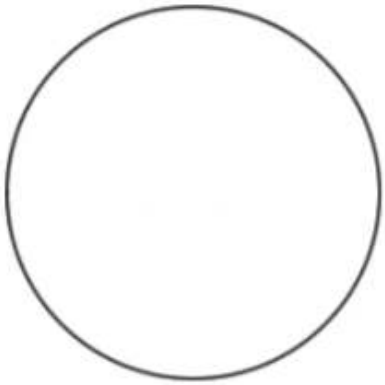
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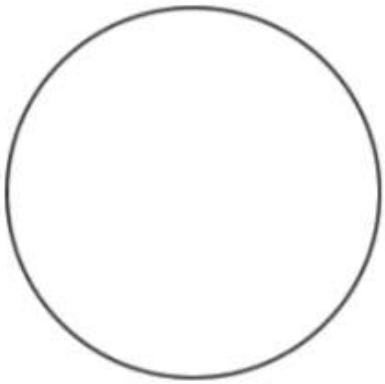
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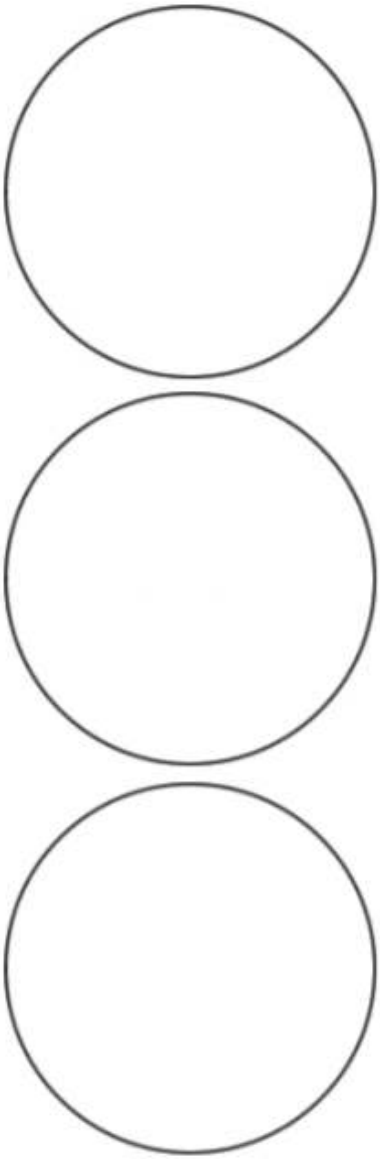
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CHAPTER OVERVIEW

30: Comparative Animal Anatomy

Learning Objectives

Content Objectives

- Define homeostasis.
- Explain animal body systems and adaptations to the environment

Skill Objectives

- Dissect and draw invertebrate and vertebrate animals
- Compare and contrast anatomy and physiological systems
- Identify and examine structures and organs in different animals

30.1: Introduction
30.2: Exercise
30.3: Lab Report

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30.1: Introduction

The structures of animals consist of primary tissues that make up more complex organs and organ systems. Homeostasis allows an animal to maintain a balance between its internal and external environments.

Homeostasis describes the dynamic balance of the body's internal environment and the effort to maintain a constant, stable inside. There are many body components that contribute to homeostasis. Animals use homeostatic mechanisms to regulate things like pH, water and ion content, body temperature, blood pressure, and blood glucose. Not all animal bodies regulate all of these examples.

Animals vary in form and function. From a sponge to a worm to a goat, an organism has a distinct body plan that limits its size and shape. Animals' bodies have evolved to interact with their environments, whether in the deep sea, a rainforest canopy, or the desert. Therefore, a large amount of information about the structure of an organism's body (anatomy) and the function of its cells, tissues and organs (physiology) can be learned by studying that organism's environment.

In lab we will be dissecting several animals in order to compare their anatomy and physiology. We will dissect a squid (mollusc), a frog (amphibian), a pigeon (bird), and a rat (mammal). The structure of an organism's body (anatomy) largely determines and limits the functionality (physiology) of that body. As you dissect animals in our lab, focus on the structures you are encountering and how they determine function. Consider what the role of that structure is in relation to other organs and how structures are put together into body systems.

We will see components and discuss functionality of the following systems:

1. Integumentary System
2. Musculoskeletal System
3. Digestive System
4. Circulatory System
5. Respiratory System
6. Nervous and Sensory Systems
7. Reproductive and Endocrine Systems
8. Osmoregulatory System

1. Integumentary System:

The integumentary system includes all coverings of the animal body. As you dissect animals in lab notice the different kinds of skin and integument and the structures that are derivatives of this system (hair and hair follicles, whiskers, bumps, horns, etc.).

2. Musculoskeletal System:

Muscle cells are specialized for contraction. Muscles allow for motions such as walking, and they also facilitate bodily processes such as respiration and digestion. The body contains three types of muscle tissue: skeletal muscle, cardiac muscle, and smooth muscle. Recall the differences in these three muscle types that you saw under the microscope.

Skeletal muscle tissue forms skeletal muscles, which attach to bones or skin and control locomotion and any movement that can be consciously controlled. Because it can be controlled by thought, skeletal muscle is also called voluntary muscle. Skeletal muscles are long and cylindrical in appearance; when viewed under a microscope, skeletal muscle tissue has a striped or striated appearance. The striations are caused by the regular arrangement of contractile proteins (actin and myosin). Actin is a globular contractile protein that interacts with myosin for muscle contraction. Skeletal muscle also has multiple nuclei present in a single cell.

Smooth muscle tissue occurs in the walls of hollow organs such as the intestines, stomach, and urinary bladder, and around passages such as the respiratory tract and blood vessels. Smooth muscle has no striations, is not under voluntary control, has only one nucleus per cell, is tapered at both ends, and is called involuntary muscle.

Cardiac muscle tissue is only found in the heart, and cardiac contractions pump blood throughout the body and maintain blood pressure. Like skeletal muscle, cardiac muscle is striated, but unlike skeletal muscle, cardiac muscle cannot be consciously controlled and is called involuntary muscle. It has one nucleus per cell, is branched, and is distinguished by the presence of intercalated disks.

A skeletal system is necessary to support the body, protect internal organs, and allow for the movement of an organism. There are three different skeleton designs that fulfill these functions: hydrostatic skeleton, exoskeleton, and endoskeleton.

Hydrostatic Skeleton

A hydrostatic skeleton is a skeleton formed by a fluid-filled compartment within the body, called the coelom. The organs of the coelom are supported by the aqueous fluid, which also resists external compression. This compartment is under hydrostatic pressure because of the fluid and supports the other organs of the organism. This type of skeletal system is found in soft-bodied animals such as sea anemones, earthworms, Cnidaria, and other invertebrates.

Exoskeleton

An exoskeleton is an external skeleton that consists of a hard encasement on the surface of an organism. For example, the shells of crabs and insects are exoskeletons. This skeleton type provides defence against predators, supports the body, and allows for movement through the contraction of attached muscles. As with vertebrates, muscles must cross a joint inside the exoskeleton. Shortening of the muscle changes the relationship of the two segments of the exoskeleton. Arthropods such as crabs and lobsters have exoskeletons that consist of 30–50 percent chitin, a polysaccharide derivative of glucose that is a strong but flexible material. Chitin is secreted by the epidermal cells. The exoskeleton is further strengthened by the addition of calcium carbonate in organisms such as the lobster. Because the exoskeleton is acellular, arthropods must periodically shed their exoskeletons because the exoskeleton does not grow as the organism grows.

Endoskeleton

An endoskeleton is a skeleton that consists of hard, mineralized structures located within the soft tissue of organisms. An example of a primitive endoskeletal structure is the spicules of sponges. The bones of vertebrates are composed of tissues, whereas sponges have no true tissues. Endoskeletons provide support for the body, protect internal organs, and allow for movement through contraction of muscles attached to the skeleton.

The human skeleton is an endoskeleton that consists of 206 bones in the adult. It has five main functions: providing support to the body, storing minerals and lipids, producing blood cells, protecting internal organs, and allowing for movement. The skeletal system in vertebrates is divided into the axial skeleton (which consists of the skull, vertebral column, and rib cage), and the appendicular skeleton (which consists of the shoulders, limb bones, the pectoral girdle, and the pelvic girdle).

3. Digestive System:

Animals obtain their nutrition from the consumption of other organisms. Depending on their diet, animals can be classified into the following categories: plant eaters (herbivores), meat eaters (carnivores), and those that eat both plants and animals (omnivores). The nutrients and macromolecules present in food are not immediately accessible to the cells. There are a number of processes that modify food within the animal body in order to make the nutrients and organic molecules accessible for cellular function. As animals evolved in complexity of form and function, their digestive systems have also evolved to accommodate their various dietary needs.

Herbivores, Omnivores, and Carnivores

Herbivores are animals whose primary food source is plant-based. Examples of herbivores include vertebrates like deer, koalas, and some bird species, as well as invertebrates such as crickets and caterpillars. These animals have evolved digestive systems capable of handling large amounts of plant material. Herbivores can be further classified into frugivores (fruit-eaters), granivores (seed eaters), nectivores (nectar feeders), and folivores (leaf eaters).

Carnivores are animals that eat other animals. The word carnivore is derived from Latin and literally means “meat eater.” Wild cats such as lions and tigers are examples of vertebrate carnivores, as are snakes and sharks, while invertebrate carnivores include sea stars, spiders, and ladybugs. Obligate carnivores are those that rely entirely on animal flesh to obtain their nutrients; examples of obligate carnivores are members of the cat family, such as lions and cheetahs. Facultative carnivores are those that also eat non-animal food in addition to animal food. Note that there is no clear line that differentiates facultative carnivores from omnivores; dogs would be considered facultative carnivores.

Omnivores are animals that eat both plant- and animal-derived food. In Latin, omnivore means to eat everything. Humans, bears, and chickens are example of vertebrate omnivores; invertebrate omnivores include cockroaches and crayfish.

Invertebrate Digestive Systems

Animals have evolved different types of digestive systems to aid in the digestion of the different foods they consume. The simplest example is that of a gastrovascular cavity and is found in organisms with only one opening for digestion. Platyhelminthes (flatworms), Ctenophora (comb jellies), and Cnidaria (coral, jelly fish, and sea anemones) use this type of digestion. Gastrovascular cavities are typically a blind tube or cavity with only one opening, the “mouth”, which also serves as an “anus”. Ingested material enters the mouth and passes through a hollow, tubular cavity. Cells within the cavity secrete digestive enzymes that break down the food. The food particles are engulfed by the cells lining the gastrovascular cavity.

The alimentary canal is a more advanced system: it consists of one tube with a mouth at one end and an anus at the other. Earthworms are an example of an animal with an alimentary canal. Once the food is ingested through the mouth, it passes through the esophagus and is stored in an organ called the crop; then it passes into the gizzard where it is churned and digested. From the gizzard, the food passes through the intestine, the nutrients are absorbed, and the waste is eliminated as feces, called castings, through the anus.

Vertebrate Digestive Systems

Vertebrates have evolved more complex digestive systems to adapt to their dietary needs. Some animals have a single stomach, while others have multi-chambered stomachs. Birds have developed a digestive system adapted to eating unmasticated food.

Monogastric: Single-chambered Stomach

As the word monogastric suggests, this type of digestive system consists of one (“mono”) stomach chamber (“gastric”). Humans and many animals have a monogastric digestive system. The process of digestion begins with the mouth and the intake of food. The teeth play an important role in masticating (chewing) or physically breaking down food into smaller particles. The enzymes present in saliva also begin to chemically break down food. The esophagus is a long tube that connects the mouth to the stomach. Using peristalsis, or wave-like smooth muscle contractions, the muscles of the esophagus push the food towards the stomach. In order to speed up the actions of enzymes in the stomach, the stomach is an extremely acidic environment, with a pH between 1.5 and 2.5. The gastric juices, which include enzymes in the stomach, act on the food particles and continue the process of digestion. Further breakdown of food takes place in the small intestine where enzymes produced by the liver, the small intestine, and the pancreas continue the process of digestion. The nutrients are absorbed into the blood stream across the epithelial cells lining the walls of the small intestines. The waste material travels on to the large intestine where water is absorbed and the drier waste material is compacted into feces; it is stored until it is excreted through the rectum.

Avian

Birds face special challenges when it comes to obtaining nutrition from food. They do not have teeth and so their digestive system must be able to process un-masticated food. Birds have evolved a variety of beak types that reflect the vast variety in their diet, ranging from seeds and insects to fruits and nuts. Because most birds fly, their metabolic rates are high in order to efficiently process food and keep their body weight low. The stomach of birds has two chambers: the proventriculus, where gastric juices are produced to digest the food before it enters the stomach, and the gizzard, where the food is stored, soaked, and mechanically ground. The undigested material forms food pellets that are sometimes regurgitated. Most of the chemical digestion and absorption happens in the intestine and the waste is excreted through the cloaca.

Parts of the Digestive System

The vertebrate digestive system is designed to facilitate the transformation of food matter into the nutrient components that sustain organisms.

Oral Cavity

The oral cavity, or mouth, is the point of entry of food into the digestive system. The food consumed is broken into smaller particles by mastication, the chewing action of the teeth. All mammals have teeth and can chew their food.

The extensive chemical process of digestion begins in the mouth. As food is being chewed, saliva, produced by the salivary glands, mixes with the food. Saliva is a watery substance produced in the mouths of many animals. There are three major glands that secrete saliva—the parotid, the submandibular, and the sublingual. Saliva contains mucus that moistens food and buffers the pH of the food. Saliva also contains immunoglobulins and lysozymes, which have antibacterial action to reduce tooth decay by inhibiting growth of some bacteria. Saliva also contains an enzyme called salivary amylase that begins the process of converting starches in the food into a disaccharide called maltose. Another enzyme called lipase is produced by the cells in the tongue. Lipases are a class of enzymes that can break down triglycerides. The lingual lipase begins the breakdown of fat components in the food. The chewing and wetting action provided by the teeth and saliva prepare the food into a mass called the bolus for swallowing. The tongue helps in swallowing—moving the bolus from the mouth into the pharynx. The pharynx opens to two passageways: the trachea, which leads to the lungs, and the esophagus, which leads to the stomach. The trachea has an opening called the glottis, which is covered by a cartilaginous flap called the epiglottis. When swallowing, the epiglottis closes the glottis and food passes into the esophagus and not the trachea. This arrangement allows food to be kept out of the trachea.

Esophagus

The esophagus is a tubular organ that connects the mouth to the stomach. The chewed and softened food passes through the esophagus after being swallowed. The smooth muscles of the esophagus undergo a series of wave like movements called peristalsis that push the food toward the stomach. The peristalsis wave is unidirectional—it moves food from the mouth to the stomach, and reverse movement is not possible. The peristaltic movement of the esophagus is an involuntary reflex; it takes place in response to the act of swallowing.

A ring-like muscle called a sphincter forms valves in the digestive system. The gastro-esophageal sphincter is located at the stomach end of the esophagus. In response to swallowing and the pressure exerted by the bolus of food, this sphincter opens, and the bolus enters the stomach. When there is no swallowing action, this sphincter is shut and prevents the contents of the stomach from traveling up the esophagus. Many animals have a true sphincter; however, in humans, there is no true sphincter, but the esophagus remains closed when there is no swallowing action. Acid reflux or “heartburn” occurs when the acidic digestive juices escape into the esophagus.

Stomach

A large part of digestion occurs in the stomach. The stomach is a saclike organ that secretes gastric digestive juices. The pH in the stomach is between 1.5 and 2.5. This highly acidic environment is required for the chemical breakdown of food and the extraction of nutrients. When empty, the stomach is a rather small organ; however, it can expand to up to 20 times its resting size when filled with food. This characteristic is particularly useful for animals that need to eat when food is available.

The stomach is also the major site for protein digestion in animals other than ruminants. Protein digestion is mediated by an enzyme called pepsin in the stomach chamber. Pepsin is secreted by the chief cells in the stomach in an inactive form called pepsinogen. Pepsin breaks peptide bonds and cleaves proteins into smaller polypeptides; it also helps activate more pepsinogen, starting a positive feedback mechanism that generates more pepsin. Another cell type—parietal cells—secrete hydrogen and chloride ions, which combine in the lumen to form hydrochloric acid, the primary acidic component of the stomach juices. Hydrochloric acid helps to convert the inactive pepsinogen to pepsin. The highly acidic environment also kills many microorganisms in the food and, combined with the action of the enzyme pepsin, results in the hydrolysis of protein in the food. Chemical digestion is facilitated by the churning action of the stomach. Contraction and relaxation of smooth muscles mixes the stomach contents about every 20 minutes. The partially digested food and gastric juice mixture is called chyme. Chyme passes from the stomach to the small intestine. Further protein digestion takes place in the small intestine. Gastric emptying occurs within two to six hours after a meal. Only a small amount of chyme is released into the small intestine at a time. The movement of chyme from the stomach into the small intestine is regulated by the pyloric sphincter.

When digesting protein and some fats, the stomach lining must be protected from getting digested by pepsin. There are two points to consider when describing how the stomach lining is protected. First, as previously mentioned, the enzyme pepsin is synthesized in the inactive form. This protects the chief cells, because pepsinogen does not have the same enzyme functionality of pepsin. Second, the stomach has a thick mucus lining that protects the underlying tissue from the action of the digestive juices. When this mucus lining is ruptured, ulcers can form in the stomach. Ulcers are open wounds in or on an organ caused by bacteria (*Helicobacter pylori*) when the mucus lining is ruptured and fails to reform.

Small Intestine

Chyme moves from the stomach to the small intestine. The small intestine is the organ where the digestion of protein, fats, and carbohydrates is completed. The small intestine is a long tube-like organ with a highly folded surface containing finger-like projections called the villi. The apical surface of each villus has many microscopic projections called microvilli. These structures are lined with epithelial cells on the luminal side and allow for the nutrients to be absorbed from the digested food and absorbed into the blood stream on the other side. The villi and microvilli, with their many folds, increase the surface area of the intestine and increase absorption efficiency of the nutrients. Absorbed nutrients in the blood are carried into the hepatic portal vein, which leads to the liver. There, the liver regulates the distribution of nutrients to the rest of the body and removes toxic substances, including drugs, alcohol, and some pathogens.

The human small intestine is over 6m long and is divided into three parts: the duodenum, the jejunum, and the ileum. The "C-shaped," fixed part of the small intestine is called the duodenum. The duodenum is separated from the stomach by the pyloric sphincter which opens to allow chyme to move from the stomach to the duodenum. In the duodenum, chyme is mixed with pancreatic juices in an alkaline solution rich in bicarbonate that neutralizes the acidity of chyme and acts as a buffer. Pancreatic juices also contain several digestive enzymes. Digestive juices from the pancreas, liver, and gallbladder, as well as from gland cells of the intestinal wall itself, enter the duodenum. Bile is produced in the liver and stored and concentrated in the gallbladder. Bile contains bile salts which emulsify lipids while the pancreas produces enzymes that catabolize starches, disaccharides, proteins, and fats. These digestive juices break down the food particles in the chyme into glucose, triglycerides, and amino acids. Some chemical digestion of food takes place in the duodenum. Absorption of fatty acids also takes place in the duodenum.

The second part of the small intestine is called the jejunum. Here, hydrolysis of nutrients is continued while most of the carbohydrates and amino acids are absorbed through the intestinal lining. The bulk of chemical digestion and nutrient absorption occurs in the jejunum.

The ileum is the last part of the small intestine and here the bile salts and vitamins are absorbed into blood stream. The undigested food is sent to the colon from the ileum via peristaltic movements of the muscle. The ileum ends and the large intestine begins at the ileocecal valve. The vermiform, "worm-like," appendix is located at the ileocecal valve. The appendix of humans secretes no enzymes and has an insignificant role in immunity.

Large Intestine

The large intestine reabsorbs the water from the undigested food material and processes the waste material. The human large intestine is much smaller in length compared to the small intestine but larger in diameter. It has three parts: the cecum, the colon, and the rectum. The cecum joins the ileum to the colon and is the receiving pouch for the waste matter. The colon is home to many bacteria or "intestinal flora" that aid in the digestive processes. The colon can be divided into four regions, the ascending colon, the transverse colon, the descending colon and the sigmoid colon. The main functions of the colon are to extract the water and mineral salts from undigested food, and to store waste material. Carnivorous mammals have a shorter large intestine compared to herbivorous mammals due to their diet.

Rectum and Anus

The rectum is the terminal end of the large intestine. The primary role of the rectum is to store the feces until defecation. The feces are propelled using peristaltic movements during elimination. The anus is an opening at the far-end of the digestive tract and is the exit point for the waste material. Two sphincters between the rectum and anus control elimination: the inner sphincter is involuntary and the outer sphincter is voluntary.

Accessory Organs

The organs discussed above are the organs of the digestive tract through which food passes. Accessory organs are organs that add secretions (enzymes) that catabolize food into nutrients. Accessory organs include salivary glands, the liver, the pancreas, and the gallbladder. The liver, pancreas, and gallbladder are regulated by hormones in response to the food consumed.

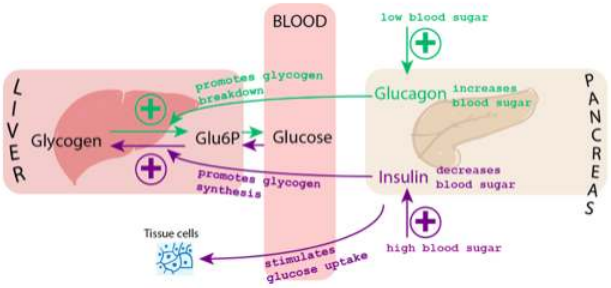
The liver is the largest internal organ in humans and it plays a very important role in digestion of fats and detoxifying blood. The liver produces bile, a digestive juice that is required for the breakdown of fatty components of the food in the duodenum. The liver also processes the vitamins and fats and synthesizes many plasma proteins.

The pancreas is another important gland that secretes digestive juices. The chyme produced from the stomach is highly acidic in nature; the pancreatic juices contain high levels of bicarbonate, an alkali that neutralizes the acidic chyme. Additionally, the pancreatic juices contain a large variety of enzymes that are required for the digestion of protein and carbohydrates.

The gallbladder is a small organ that aids the liver by storing bile and concentrating bile salts. When chyme containing fatty acids enters the duodenum, the bile is secreted from the gallbladder into the duodenum.

Liver

The liver is an organ within the digestive system and is responsible for maintaining sugar levels in the blood as part of homeostasis. After a large meal, the liver converts extra glucose into **glycogen**, a polysaccharide that stores glucose. A hormone called **insulin** is produced by the pancreas stimulates glycogen production. When levels of glucose in the blood drop, the liver breaks down glycogen back into glucose for the blood to circulate throughout the body. A hormone called **glucagon** produced by the pancreas stimulates this process. All cells of the body require glucose for cellular respiration to make energy.



The liver receives blood from the small intestines through the **hepatic portal vein**. After a large meal, the hepatic vein would transport glucose rich blood from the small intestines to the liver. Blood leaves the liver and returns to the heart through the **hepatic vein**.

4. Circulatory System:

The cardiovascular system has three major jobs in most animals

1. Transport
   - Gases (Oxygen, carbon dioxide)
   - Nutrients to cells
   - Metabolic wastes
   - Water
   - Hormones
2. Homeostasis
3. Protection
   - Blood clotting factors
   - White blood cells to fight against infection

Some invertebrates (like cnidarians) rely on a gastrovascular cavity for these functions, or diffuse gases and metabolic wastes directly through their skin. Larger and more complex animals have open or closed circulatory systems. In closed circulatory systems, the blood or equivalent transport medium is entirely within vessels in the body. Humans, other vertebrates, and squid have closed circulatory systems. In open circulatory systems, like those found in insects and many other invertebrates, blood circulates through vessels and open sinuses and can mix with interstitial fluid (this mix is called hemolymph).

Circulatory systems typically require a pump, also known as the heart, to contract and push the fluid around the body. Many animals have more than one heart for this purpose.

5. Respiratory System:

Exchange of oxygen and carbon dioxide requires a wet, thin surface for diffusion to occur. Respiratory surfaces may be internal (lungs) or external (gills) and depend on the animals environment. Small animals in wet environments can have thin skin for direct cutaneous respiration.

6. Nervous and Sensory Systems:

Nervous systems are unique to animals and are critical for detecting and interpreting information, making decisions, and regulating body functions and movements. Nervous systems are constructed from neurons and glia. Neurons are the main functional cells while glia play a variety of support roles.

Nervous systems develop as interconnected networks of cells. The largest nervous organs are the brains of vertebrates, while animals as simple as jellyfish have a 'nerve net'. A critical concept related to nervous systems is consciousness, or 'self-awareness'. That idea will be discussed from one perspective in the next lab, distinguishing sensation from perception.

The exercises here will review cellular function and structure, and explore several basic neural networks within the larger body. We will use a combination of models and microscopic analysis, gross anatomical dissection, and physiological exercises to study nervous systems. An overview of the components of nervous systems.

Overview of the Components of Nervous Systems:

Cellular Components	
Cell Structures	**Cell Functions**
Neurons: • Cells with membrane extensions, or *neurites* (axons {1 per neuron} & dendrites), interconnected at synapses. Identified by the extent of neurite branching: ◦ Multipolar = 2 or more dendrites ◦ Bipolar = 1 axon + 1 dendrite ◦ Unipolar = 1 neurite (functions as dendrite and axon, seen in specialized sensory neurons)	• Excitable cells based on maintenance of ion—concentration gradients (for Na+ and K+) and membrane ion channel proteins. • Membrane polarity (or membrane potential, or voltage) for a cell **at rest** is negative inside relative to out, based on an excess flow of K+ out of the cell. • An '**action potential**' is the activation of a neuron, and the membrane polarity reverses, due to the inward flow of Na+.
• **Synapses** – physical junctions between neurons, as well as between neurons and other cells, that allow for communication.	• **Chemical synapses** (most common type) use an action potential to signal the exocytosis of neurotransmitter chemicals (over 100 different neurotransmitters known in humans). Each neurotransmitter requires a **receptor protein** (multiple subtypes known for each different neurotransmitter). • **Electrical synapses** are direct membrane junctions between cells that allow continuations of action potentials.
Glia (or glial cells, or neuroglia): • Oligodendrocytes & Schwann cells	• Cells that wrap axons in myelin sheaths (glial membrane extensions) that increase action potential velocity.
• Astrocytes, Satellite cells & Ependymal cells	• Cells that form borders between nervous tissues and other body tissues.
• Microglia	• Immune cells that clear cellular debris from nervous tissues.

The essential component is neurons, the major functional cells in nervous tissue. In many sensory organs, additional cells and tissues will contribute to the process of signal transduction.

Signal transduction is the process of a receptor detecting specific forms of matter or energy, and activating chemical and electrical changes in neurons. The neurons can then communicate with other neurons in the nervous system via synapses and networks to coordinate responses.

Receptor is a term used for the part of a sensory organ that detects the signal. 'Receptor' can refer to specific protein molecules which first interact with the matter or energy, the cell(s) that contains those proteins, or an assembly of cells in the larger organ.

Sensory Organs:

The major sensory organs can be grouped based on various characteristics, i.e. what type of matter or energy they detect and subsequently 'transduce' to produce our perceptions (e.g. vision, taste). Eventually, there are electrical and chemical signals within our brains. Specific organs include:

Sensory Organ (Major Receptor)	Matter/Energy Detected By Receptor	General Anatomy and Physiology	Perceived Sensation
Eye (Retina)	Visible light (Electromagnetic Radiation)	Multilayered nervous sheet within the eye with muscles and lenses for focusing	Vision
Ear (Cochlea)	Physical force (Sound)	Flexible 'hair' cells that release signal molecules based on waves in fluid started by the motion of the **eardrum**	Hearing
Ear (Semicircular Canals, Saccule, Utricle)	Physical Force	Flexible 'hair' cells that release signal molecules based on waves in fluid started by the motion of the **head**	Movement
Nose (Olfactory Epithelium)	Chemicals ('Odorants')	A layer of neurons at the top of the nasal cavity	Smell ('Olfaction')
Tongue (Taste Buds)	Chemicals	Clusters of epithelial cells that release signals to neurons if specific chemicals are present (e.g. sodium ions)	Taste ('Gustation')
Skin (Mechanoreceptors)	Physical Force	Various neurons that respond to physical movements	Touch
Skin (Thermoreceptors)	Heat Transfer	Specific neurons that respond to **increases** in temperature	Hot
Skin (Thermoreceptors)	Heat Transfer	Specific neurons that respond to **decreases** in temperature	Cold
Muscles & Tendons	Physical Force	Neurons responding to stretch and contraction of muscles & tendons	Body Position (Proprioception)
Most of the body	Various Signals	Neurons responding to physical force, temperature, and specific chemicals to warn of (potential) damage.	Pain ('Nociception')

In order to investigate and understand sensory processes, we will investigate their anatomical structures (at macro- and microscopic levels) and physiological functions. An important distinction to consider is how humans can functionally separate **sensation** (activation of the different receptors) as compared to **perception** (the conscious awareness of the sensation). This distinction reveals how sensory deficits can result from damage in brain regions, even though the sensory organ is intact. Also, we may have perceptions that are only present in the brain, even though the sensory organs are silent.

7. Reproductive and Endocrine Systems:

Animal reproduction is necessary for the survival of a species. In the animal kingdom, there are innumerable ways that species reproduce. Asexual reproduction produces genetically identical organisms (clones), whereas in sexual reproduction, the genetic material of two individuals combines to produce offspring that are genetically different from their parents.

Generally, the producers of sperm (the small gamete) are called males, producers of the eggs (the large gamete) are called females. Any other characteristics that may often be associated with sex, such as chromosomes, parental care, or sexual behavior, are not universal throughout the animal kingdom. During sexual reproduction the male gamete (sperm) may be placed inside the female's body for internal fertilization, or the sperm and eggs may be released into the environment for external fertilization.

Various internal and external structures allow animals to reproduce, including gonads, tubules to allow gametes to exit the body, and external structures for gamete deposition or transfer.

Many reproductive organs (like gonads) are also endocrine organs, and reproduction is one of the major processes the endocrine system controls. The endocrine system is the system that produces hormones, which are chemicals the body uses to communicate between organs and systems and control major processes like reproduction, growth, metabolism, and the stress response (and many more!). Many organs that are part of other systems produce hormones (like the gonads, pancreas, and brain) and there are some glands specific to the endocrine system (thyroid, pituitary, pineal). Glands are often hard to find in dissections as they tend to be small and soft and difficult to differentiate from other tissue.

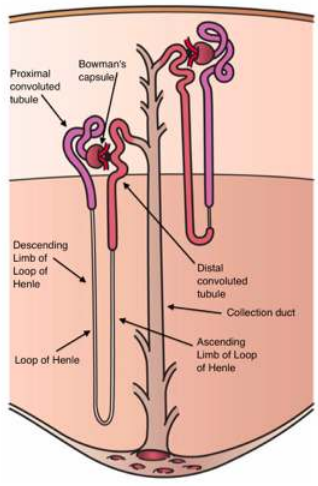
8. Osmoregulatory System:

Kidneys

The kidneys are part of the urinary system. As they produce urine to release nitrogenous wastes from the body the kidneys also maintain homeostasis through pH balance and water-salt balance in **osmoregulation**. These bean shaped organs are located along the dorsal wall of the abdominal cavity.

Observe the kidney models available in the lab. Locate the outer renal cortex tissue and the more internal renal medulla. The renal pelvis is the area that collects the urine. Find the renal artery and the renal vein.

The functioning unit of the kidney is called the **nephron.** Part of the nephron is located in the cortex and part in the medulla. Use the picture below and the models in the lab to identify the following components of the nephron.



- Glomerulus
- Bowman's capsule
- Proximal tubule
- Distal tubule
- Loop of Henle (descending limb and ascending limb)
- Collecting duct

- Peritubular capillaries

Urine production in the kidney involves four main steps:

1. **Filtration**: molecules move out of the glomerulus into Bowman's capsule. Large molecules like proteins and blood cells are too big to be filtered and remain in the blood.
2. **Reabsorption**: glucose and amino acids move from the proximal tubule back into the blood stream through peritubular capillaries.
3. **Secretion**: Substances like histamines, H+, and ammonia get secreted into the nephron from the peritubular capillaries
4. **Water reabsorption**: both the Loop of Henle and the collecting duct reabsorb water to maintain the blood volume

Kidneys are also important in **osmoregulation**, maintaining an internal salt/water balance. The kidney can produce large amounts of dilute urine or small amounts of concentrated urine depending on the needs of the body. The pituitary glad produces antidiuretic hormone (ADH) which controls the concentration of urine output. ADH specifically acts on the collecting duct making it more or less permeable to water. Kidneys also play a role in pH balance.

LICENSES AND ATTRIBUTIONS

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30.2: Exercise

Dissection is a powerful tool that provides us a profound understanding of our own anatomy and physiology as living, breathing creatures and also helps us to develop a stronger understanding of evolutionary relationships between taxonomic groups. What sort of dissection experiences do you already have? As you begin this sequence of dissections, please keep in mind several things. Dissection should be done thoughtfully and respectfully. It is important to take your time carefully and to think about what you are doing. This care will help you to preserve structures for the next several weeks and will make review easier. Also, merely identifying structures is insufficient to develop fully an appreciation for how these structures work. You must think about the function of these structures. How do they work?! Also, please do these dissections respectfully. These organisms were euthanized so that we might have a wonderful opportunity to learn something greater about the living world. Please keep that in mind as you are working.

Squid Dissection

Procedure:

External Anatomy

1. Place the squid in the dissection pan with the **mantle** (major body part) facing away from you and the **tentacles** and **arms** towards you.
2. Turn the squid so that the **siphon** faces you. It is located between the eyes. By expelling water through the siphon the squid can effectively move through the water.
3. Notice the **chromatophores** on the mantle. They allow the squid to change color and blend into the environment.
4. At the pointed tip of the mantle there are two **fins** that help stabilize and propel the squid
5. Notice the **eyes** on either side-they are well developed and allow the squid to have excellent vision
6. Distinguish between the tentacles and the arms. The tentacles are longer and are used to pass food to the arms.
7. Count the number of arms. How many are there?
8. Notice the **suction cups** on both the tentacles and the arms. How does the distribution of the suction cups differ between these two structures?
9. Pull back the arms and locate the **beak** or mouth in the middle

Draw the external anatomy of your squid. Label the listed structures on your drawing and give functions.

- Tentacles
- Arms
- Suction cups
- Beak
- Eyes
- Mantle
- Fins
- Siphon

Dissection

1. Ensure the siphon is facing up. Grab the mantle with your hand and squeeze until you "pop the collar". There should now be space under the mantle. Using scissors, cut the mantle up the middle from the funnel to the tip at the top of the squid. Be careful to lift up with the scissors while cutting to avoid cutting the internal organs. The mantle should now fall open. Pin the mantle sides to your dissecting tray using dissecting pins.
2. Spread the mantle open and try to identify the following internal structures
    1. Feathery gills
    2. Heart, located at the base of each gill. Squid actually have three hearts!
    3. Liver, probably yellowish in color and long in shape running down the middle
    4. Ink sack, which looks like a small silver fish. If you find it, cut it out at both ends and you can extract some of the ink and try to write with it!

5. The pen, which is all that remains of the shell. To try and find the pen, lift the head of the squid and place it down over the organs. You should notice a pointy area along the midline of the body, the tip of the pen. If you grasp the tip and pull the pen will release from the mantle. It resembles a transparent feather.

Internal Anatomy

Draw the internal anatomy of your squid. Label the listed structures on your drawing and give functions.

- Esophagus
- Stomach
- Caecum
- Ink sac
- Gills
- Hearts
- Pen
- Brain
- Eyes: cut open an eye, taking the lens out.
  - What shape is the lens?
  - How is this lens similar to and different from the lens in the eye of a terrestrial mammal?
- Gonads: is your squid male or female? How can you tell?
- Nidamental gland (if female)

Frog Dissection

Procedure:

External Anatomy

1. Place the frog in the dissection pan legs down.
    1. Identify the eyes, covered by a **nicitating membrane**, the **external nares** (nostrils), and the **tympanum** located behind each eye.
        1. What is the function of the tympanum?
    2. Examine the front and back limbs. How many phalanges are on the hindfeet? The forefeet? Which pair of limbs is the longest? How does this assist the frog in its movement?
2. Mouth
    1. Turn the frog over and open the mouth as wide as you can. You can cut the hinges of the jaw if necessary. Identify the following structures:
        1. Two **vomerine teeth** located in the middle of the roof of the mouth
        2. Maxillary teeth (smaller) located on the sides of the upper jaw
        3. **Tongue**
        4. **Pharynx** (located behind the tongue)
        5. **Esophagus**, the opening leading to the stomach
        6. **Glottis**, slit where air passes through to enter the **trachea**, which leads to the lungs
        7. **Eustacian tubes** (2) openings that lead to the ears. They are located in the angle of the jaw.

Draw the external anatomy of your frog. Label the listed structures on your drawing and give functions.

- Eyes
- Nares
- Tympanum
- Mouth with opening to esophagus and trachea
- Skin - Describe the skin. What do you notice about it? Why is the color and texture the way that it is?

Dissection

1. Body Dissection
    1. Place the frog belly side up in the dissecting tray. You can pin down the limbs if necessary.
    2. Lift up the skin with forceps midway between the hind legs of the frog. Use scissors to cut the skin along the midline of the frog starting between the hind legs and ending at the neck. Be careful not too cut too deeply.
    3. Cut the skin horizontally above the hind legs and below the front legs creating skin flaps.
    4. Pick up a skin flap with forceps and use a scalpel to separate the skin from the muscle below.
    5. Pin the skin flaps to the dissection tray.
    6. Repeat the same procedure to cut through the muscles. Create one long incision along the midline of the frog from between the hind legs to the neck. Be careful not to cut too deeply and damage the internal organs. When you reach the area just below the front legs of the frog, turn your scissors sideways to cut through the chest bones and avoid damaging the heart and lungs. Then make horizontal incisions above the rear legs and between the front legs. Use forceps and a scalpel to separate the muscle from the tissue below. Then pin the muscle to the dissection tra

Internal Anatomy

1. The most prominent organ is the **liver**, dark brown in color, and taking up most of the abdominal cavity
2. Identify the **lungs**, two small pouches on opposite sides of the frog midline. They may be partially hidden by the liver.
3. Lift up the liver and underneath locate the **gallbladder.**
4. Identify the **heart** covered by the protective **pericardium**. Frogs have a three chambered heart with two atria and one ventricle. Try to locate these different areas of the frog heart.
    1. How is it a disadvantage to have a 3 chambered heart?
5. The **stomach** is a j-shaped organ located underneath the left lobe of the liver. It connects to the **esophagus** bringing food from the mouth and the **small intestine** used for nutrient absorption.
6. The small intestine connect to the **large intestine** which carry any undigested material to the **cloaca**. Frogs have one opening to the outside environment and the cloaca receives materials from the intestine, the urinary system and the reproductive system.
7. Find the **pancreas**, a yellow ribbon located between the stomach and the small intestine.
8. Locate the **spleen**, shaped similarly to a pea and located near the stomach.
9. You will be able to see the yellow, finger like, **fat bodies**, which the frog uses to store fat.
10. The **kidneys** of the frog are long and narrow and located along the back body wall.
11. Try to find the **mesonephric ducts**, thin white tubes that carry urine from the kidney to the cloaca.
12. If your frog is female, the abdominal cavity will be filled with black and white eggs. The eggs are stored in the **ovaries.**
13. If you have a male frog, locate the **testes**. The testes are shaped like a bean and located at the top of the kidneys. They are yellow/tan in color.
    1. Do you have a male or female frog?

Draw the internal anatomy of your frog. Label the listed structures on your drawing and give functions.

- Liver
- Lungs
- Gallbladder
- Heart
    - Once you have completed your dissection, take the heart out and cut it open. How many chambers does it have?
- Esophagus
- Stomach
- Small intestine
- Large intestine
- Pancreas
- Spleen
- Fat bodies
- Kidneys

- Ovaries or testes

Pigeon Dissection

Procedure:

External Anatomy

1. Place the pigeon in the dissection pan with ventral side up.
2. Note the major anatomy of the bird
    1. Head with beak and large eyes
    2. Wings
    3. Trunk
    4. Legs
3. Feel the ventral side of the bird and find the **keeled sternum**. This bone is flat in humans, but has a keel on it in birds for muscle attachment
4. On the legs, note the angle of the joints. Where is the **femur** located? Where are the knee and ankle joints? Compare bird legs with joints to that of a mammal.

What major anatomical modifications do birds have for flight?

Draw the external anatomy of your pigeon. Label the listed structures on your drawing and give functions.

- Eyes
- Nares
- Beak
- Wings
- Sternum
- Legs
- Feet with claws
- Cloaca

Dissection

1. With the ventral side of your bird up, first use a scalpel to cut the skin from neck to vent and peel the skin back. Note how thin the skin is, and the large **pectoralis** muscle underneath.
2. Feel down the sternum until you find the end, where the belly is soft.
3. Using a scalpel or scissors cut the skin underneath the sternum from one side to the other, exposing the organs underneath.
4. Cut up each side of the bird, until you come to a shoulder joint.
5. Using a scissors, cut through the bone at each shoulder joint.
6. You should now be able to lift the sternum with muscle attached up towards the head of the bird, and get a good view of all the organs inside.
7. Note that birds do not have a separate thoracic and abdominal cavity, as we will see in mammals.

Internal Anatomy

Draw the internal anatomy of your pigeon. Label the listed structures on your drawing and give functions.

- Esophagus
- Proventriculus
- Gizzard
- Intestine
- Pancreas
- Spleen
- Heart
- Lungs

- How do birds ventilate their lungs?
- Gonads: is your bird male or female? How can you tell?

Rat Dissection

Procedure:

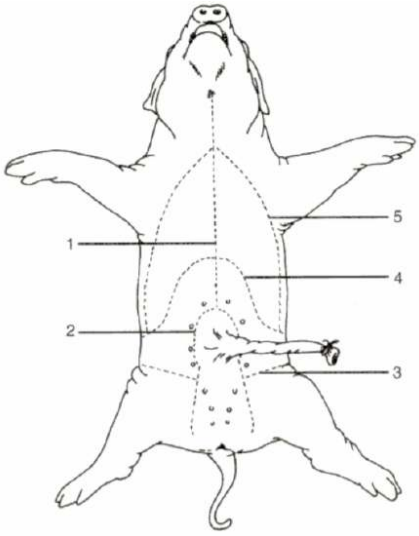
External Anatomy

1. Based on the external anatomy is your rat male or female? How can you tell?
2. Can you locate any hair on your rat, a common characteristic of mammals? Where is there the most hair?

Draw the external anatomy of your rat. Label the listed structures on your drawing and give functions.

- Mouth
- Nose
- Eyes
- Body
- Feet with claws
- Tail

The Incision



Place your rat in the dissecting pan ventral side up. Use string to tie the legs behind the back of the pan. Use scissors to cut through the skin and muscles according to the diagram.

Your rat may be filled with water and preservative, drain over the sink if necessary.

Neck Region:

Using the diagram to the right begin your dissection in the neck region. Try to cut as little as possible. Once you open the body cavity, you will generally be able to separate the different organs by simply pulling them apart with your fingers, forceps, or a probe. The more you cut things up, the harder it will be to figure out what you're looking at. Cut midline on the ventral surface of the neck to expose the underlying muscles. Carefully separate the muscles to observe the underlying structures. Locate and understand the functions of the following structures:

- **Larynx**: an enlarged structure on the trachea. If you cut it open, you can see the vocal cords inside.
- **Thymus Gland**: an endocrine (hormone-secreting) gland that helps regulate the immune system. It's a large, spongy structure covering the ventral surface of the trachea, extending up along either side, and also resides over the heart. It is easy to cut so be extra careful.
- **Thyroid Gland**: another endocrine gland; it's a small bilobed (two parts) structure just posterior to the larynx. The thyroid secretes hormones that help regulate metabolism.
- **Trachea**: the airway; it's reinforced with rings of cartilage so it does not collapse.
- **Esophagus**: carries food from mouth to stomach; soft and muscular so it can move a food bolus by peristalsis. It is located dorsal to the trachea (but appears behind it because the specimen is upside down).

Thoracic Cavity:

Vertebrates have true coeloms (a body cavity). In mammals, the coelom is divided into two main cavities: the thoracic cavity, which contains the lungs, and the abdominal cavity, which contains the digestive system. The thoracic cavity and the abdominal cavity are separated by the diaphragm. Note the many membranes lining the coelom and holding the organs in place. Look for these structures in the thoracic cavity:

- **Lungs**: they have several lobes. Note how spongy the tissue is.

- **Heart**: muscular and easy to find. The heart is surrounded by a pericardial sac. Note the aorta, where high-pressure blood leaves the heart on its way to the systemic circulation. You may also see the right and left carotid arteries, which supply blood to the head. For now, don't spend too much time on the various lobes of the heart and the many blood vessels. Come back to these later.
- **Diaphragm**: a sheet of muscle and connective tissue that helps in breathing and divides the two cavities described here.

Abdominal Cavity (Digestion & Absorption):

Locate and understand the functions of the following structures:

- **Liver**: very large and dark. It has several lobes. You'll need to lift it out of the way to see the organs beneath. The liver produces bile that is stored in the gall bladder. The gall bladder is a small organ attached to the underside of the liver; it's usually greenish due to the bile. It connects to the small intestine by the bile duct.
- **Stomach**: a muscular, sac-like organ that sits posterior to and to the left of the liver. Here is where the gastric juices released by glands continue enzymatic digestion started in the mouth. In particular, proteins are hydrolyzed via pepsin. At each end of the stomach are valves that regulate food entering and leaving the stomach. At the esophagus is the cardiac sphincter valve, and at the duodenum is the pyloric sphincter valve. View the inside of the stomach by slicing it open lengthwise.
- **Small & Large Intestine**: tube-like structures that continue the movement of food (now called chyme). The small intestine is first. The initial part of the small intestine (duodenum) is responsible for the final steps of enzymatic digestion and then eventually absorption of the degraded molecules. The large intestine primarily functions to compact the remaining waste material by absorbing water invested in the digestion and lubrication process. Also absorption of vitamins completes here.
- **Rectum**: the final portion of the large intestine where wastes are stored before elimination through the anus.
- **Mesenteries**: thin, transparent sheets of connective tissue containing blood vessels connecting the intestine and other organs.
- **Pancreas**: white and looks a little bit like cauliflower and located along the underside of the stomach, a pancreatic duct leads to the duodenum – first part of the small intestine. The pancreas also makes insulin, which is necessary for the proper uptake of sugars from the blood. It secretes digestive enzymes and buffers as well that contribute to the digestion of material within the small intestine.
- **Spleen**: The spleen is a flat organ located near the stomach. It performs several functions related to producing and maturing new blood cells and eliminating old ones. Blood passes through open sinuses in the spleen, rather than being confined to narrow blood vessels.

QUESTION

1. What do the hard and soft palate separate?

Cut into the neck region. Make sure you can locate the following structures:

1. Trachea
2. Thymus
3. Thyroid
4. Esophagus

QUESTION

1. Is the trachea in front of or behind the esophagus?

Cut into the thoracic cavity beneath the rib cage. Make sure you can locate the following structures:

1. Heart
2. Lungs
3. Bronchi
4. Diaphragm

QUESTIONS

1. How many chambers does the rat heart have?
2. How does the size of the rat lungs compare to the size of the frog lungs you dissected previously?
3. What role does the diaphragm play in respiration?

4. What cavity contains the lungs?
5. What cavity contains the heart?

Focus next on the abdominal cavity. First look at the digestive system organs. Make sure you can locate the following organs:

1. Stomach
2. Spleen
3. Liver
4. Gallbladder
5. Small intestine
6. Large intestine
7. Pancreas

QUESTIONS

1. What is the function of the liver?
2. What is the function of the gallbladder?
3. What type of digestion occurs in the stomach?
4. Name the three sections of the small intestines in order.
5. Name one process that occurs in the large intestine.
6. Which digestive organs located in the abdominal cavity are considered to be accessory organs?

Also in the abdominal cavity you will find the excretory system organs. Make sure you can locate:

- Kidneys
- Bladder

Finally in the abdominal cavity are the reproductive organs. If you have a female rat look at another group's male rat and vice versa. You should be able to find:

- Testes (male)
- Uterus with horn (female)
- Ovary (female)

The arteries and veins are challenging to identify, especially if the pig is not injected with dye. Arteries carry blood away from the heart. Veins return blood to the heart. Try to identify the following:

1. Aorta
2. Pulmonary artery
3. Coronary arteries
4. Jugular vein
5. Carotid artery
6. Renal artery
7. Renal vein

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30.3: Lab Report

Part 1

Squid Dissection

Draw the external anatomy of your squid. Label the listed structures on your drawing and give functions.

- Tentacles
- Arms
- Suction cups
- Beak
- Eyes
- Mantle
- Fins
- Siphon

Draw the internal anatomy of your squid. Label the listed structures on your drawing and give functions.

- Esophagus
- Stomach
- Caecum
- Ink sac
- Gills
- Hearts
- Pen
- Brain
- Eyes: cut open an eye, taking the lens out.
  - What shape is the lens?
  - How is this lens similar to and different from the lens in the eye of a terrestrial mammal?
- Gonads: is your squid male or female? How can you tell?
- Nidamental gland (if female)

Frog Dissection

Draw the external anatomy of your frog. Label the listed structures on your drawing and give functions.

- Eyes
- Nares
- Tympanum
- Mouth with opening to esophagus and trachea
- Skin - Describe the skin. What do you notice about it? Why is the color and texture the way that it is?

Draw the internal anatomy of your frog. Label the listed structures on your drawing and give functions.

- Liver
- Lungs
- Gallbladder
- Heart
  - Once you have completed your dissection, take the heart out and cut it open. How many chambers does it have?
- Esophagus
- Stomach
- Small intestine
- Large intestine
- Pancreas

- Spleen
- Fat bodies
- Kidneys
- Ovaries or testes

Part 2

Pigeon Dissection

Draw the external anatomy of your pigeon. Label the listed structures on your drawing and give functions.

- Eyes
- Nares
- Beak
- Wings
- Sternum
- Legs
- Feet with claws
- Cloaca

On the legs, note the angle of the joints. Where is the **femur** located? Where are the knee and ankle joints? Compare bird legs with joints to that of a mammal.

What major anatomical modifications do birds have for flight?

Draw the internal anatomy of your pigeon. Label the listed structures on your drawing and give functions.

- Esophagus
- Proventriculus
- Gizzard
- Intestine
- Pancreas
- Spleen
- Heart
- Lungs
    - How do birds ventilate their lungs?
- Gonads: is your bird male or female? How can you tell?

Rat Dissection

Draw the external anatomy of your rat. Label the listed structures on your drawing and give functions.

- Mouth
- Nose
- Eyes
- Body
- Feet with claws
- Tail

Draw the internal anatomy of your rat. Label the listed structures on your drawing and give functions. You may want to draw multiple internal pictures.

Neck region

1. Trachea
2. Thymus
3. Thyroid
4. Esophagus

Thoracic cavity

1. Heart
2. Lungs
3. Bronchi
4. Diaphragm

QUESTIONS

1. How many chambers does the rat heart have?
2. How does the size of the rat lungs compare to the size of the frog lungs you dissected previously?
3. What role does the diaphragm play in respiration?
4. What cavity contains the lungs?
5. What cavity contains the heart?

Abdominal cavity

1. Stomach
2. Spleen
3. Liver
4. Gallbladder
5. Small intestine
6. Large intestine
7. Pancreas
8. Kidneys
9. Bladder
10. Testes (male)
11. Uterus with horn (female)
12. Ovary (female)

QUESTIONS

1. What is the function of the liver?
2. What is the function of the gallbladder?
3. What type of digestion occurs in the stomach?
4. Name the three sections of the small intestines in order.
5. Name one process that occurs in the large intestine.
6. Which digestive organs located in the abdominal cavity are considered to be accessory organs?

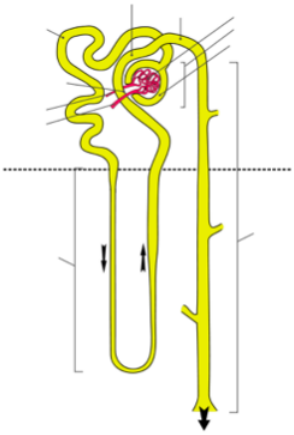
The arteries and veins are challenging to identify, especially if the pig is not injected with dye. Arteries carry blood away from the heart. Veins return blood to the heart. Try to identify the following:

1. Aorta
2. Pulmonary artery
3. Coronary arteries
4. Jugular vein
5. Carotid artery
6. Renal artery
7. Renal vein

Questions

1. Which parts of the nephron are located in the renal cortex?
2. Which components of the nephron are located in the medulla?

Label the parts of the nephron on the diagram below and indicate where the different urine production steps occur.



Focus on Filtration

Blood entering the glomerulus contains cells, proteins, glucose, amino acids, salts, urea, and water. Fill in the table below indicating which molecules will leave the glomerulus and enter the Bowman's capsule. Write yes or no for each and state why based on size (small or big).

Substance	Enter Bowman's Capsule?	Why?
Cells		
Proteins		
Glucose		
Amino acids		
Salts		
Urea		
Water		

Focus on Reabsorption

When the filtrate enters the proximal tubule it contains the following molecules: glucose, water, urea, amino acids, and salts. Water and salts are passively reabsorbed to maintain blood volume and pH as part of homeostasis.

Questions

1. What would happen to the blood volume over time if water were not reabsorbed?
2. How would this lack of water reabsorption affect blood pressure?
3. The table below has several different events that would impact osmoregulation. Fill in the chart with either "increase" or "decrease" to explain how the kidney would help maintain homeostasis.

Event	Change in blood concentration	ADH output	Water Reabsorption	Type of Urine produced
Dehydration due to sunbathing in the afternoon and forgetting your water bottle				Scant, concentrated
Drinking large amounts of water throughout the day				Copious, dilute
Going to the move theater and eating a large bucket of salty popcorn without water to wash it down				Scant, concentrated

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CHAPTER OVERVIEW

31: Experimental Physiology

Learning Objectives

Content Objectives

- Understand aspects of animal physiology.
- Observe the function of the cardiovascular system in a model organism, *Daphnia*.

Skill Objectives

- Expose *Daphnia* to various concentrations of caffeine and observe the results.
- Write a report detailing the results of your experiment.

31.1: Introduction
31.2: Exercise
31.3: Lab Report

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31.1: Introduction

We will be using *Daphnia magna* to explore basic principles of animal physiology. *Daphnia* is a genus of small crustaceans in the phylum Arthropoda. They are a good model organism for the study of animal physiology as we can see some organs and their functions under a microscope.



Figure 31.1.1: Female adult Daphnia magna with eggs. Attribution: "Female adult of the water flea Daphnia magna by Hajime Watanabe(opens in new window)" by dullhunk(opens in new window) is licensed under CC BY 2.0(opens in new window).

Female adult Daphnia magna with eggs. Attribution: "Female adult of the water flea Daphnia magna by Hajime Watanabe(opens in new window)" by dullhunk(opens in new window) is licensed under CC BY 2.0(opens in new window).

Among the organs visible in the *Daphnia* is the heart, located on the dorsal surface of the animals' body. Crustaceans, like other arthropods, have an **open circulatory system**, where the heart pumps blood into open sinuses throughout body. While the baseline heart rate is established by autorhythmic cells in the heart, it can be affected by a variety of environmental factors.

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31.2: Exercise

Getting Started

1. Use a pipette to place one *Daphnia* on a concave microscope slide. Do not put a cover slip over your live specimen.
2. Look at your specimen under the microscope. Keep the light low and turn off when not looking at your specimen (this keeps the live *Daphnia* more comfortable).
3. Find the major body parts under the microscope
    - Head with eyes and antenna
    - Legs
    - Intestine
    - Heart
    - Ovary, brood pouch, embryos (if gravid female)
4. Locate the heart, practice counting heart beats
    - Heart rate = beats per minute
    - Count the number of heart beats in 15 seconds, multiply by 4

The Effects of Caffeine on Heart Rate

Think about what effect caffeine might have on heart rate. You may want to do some research in an online database in order to come up with your hypothesis.

1. Detail your hypothesis in your lab notebook

2. Place one *Daphnia* in the control solution (freshwater), wait 2 minutes for it to acclimate with the light off, then record its heart rate in beats per minute.

3. Place the *Daphnia* in .1 mg/L caffeine solution, wait 2 minutes for it to acclimate with the light off, then record its heart rate in beats per minute.

4. Place the *Daphnia* in 1 mg/L caffeine solution, wait 2 minutes for it to acclimate with the light off, then record its heart rate in beats per minute.

5. Place the *Daphnia* in 10 mg/L caffeine solution, wait 2 minutes for it to acclimate with the light off, then record its heart rate in beats per minute.

6. If time permits, repeat this experiment with a second *Daphnia*.

Design Your Own Experiment

Now that you've seen the effects of caffeine on heart rate, what else do you think might affect heart rate? Can you think of factors that might affect cardiac physiology? Further database research or observations may give you some ideas.

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31.3: Lab Report

1. Background information and notes

2. Hypothesis

3. Predictions

4. Detailed methods and notes

5. Combined results of class data on the effects of caffeine on heart rate.

6. Compare the results to your hypothesis. Was your hypothesis supported? Why or why not?

7. Write a 1-paragraph discussion of your experiment, including comparison to two published sources. How does your data fit in to the current knowledge on effects of caffeine on heart rate? You may look for papers on other organisms or related questions. Put your results in the context of the field.

8. If an original experiment was done, complete parts 1-7 for that experiment.

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CHAPTER OVERVIEW

32: Field Biology and Ecology

Learning Objectives

Content Objectives

- Define ecosystem and ecology.
- Understand how energy flows through trophic levels.
- Define population and community.
- Identify the types of symbiosis.

Skill Objectives

- Design a model food web and identify nutrient flow.
- Explore two different types of symbiosis.
- Model and identify issues associated with eutrophication.

32.1: Introduction
32.2: Exercise
32.3: Lab Report

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32.1: Introduction

Ecology

Ecology is the study of the interactions of living organisms with their environment. One core goal of ecology is to understand the distribution and abundance of living things in the physical environment. Attainment of this goal requires the integration of scientific disciplines inside and outside of biology, such as mathematics, statistics, biochemistry, molecular biology, physiology, evolution, biodiversity, geology, and climatology.

Levels of Ecological Study

When a discipline such as biology is studied, it is often helpful to subdivide it into smaller, related areas. For instance, cell biologists interested in cell signaling need to understand the chemistry of the signal molecules (which are usually proteins) as well as the result of cell signaling. Ecologists interested in the factors that influence the survival of an endangered species might use mathematical models to predict how current conservation efforts affect endangered organisms.

To produce a sound set of management options, a *conservation biologist* needs to collect accurate data, including current population size, factors affecting reproduction (like physiology and behavior), habitat requirements (such as plants and soils), and potential human influences on the endangered population and its habitat (which might be derived through studies in sociology and urban ecology). Within the discipline of ecology, researchers work at four general levels, which sometimes overlap. These levels are organism, population, community, and ecosystem (Figure 44.2).

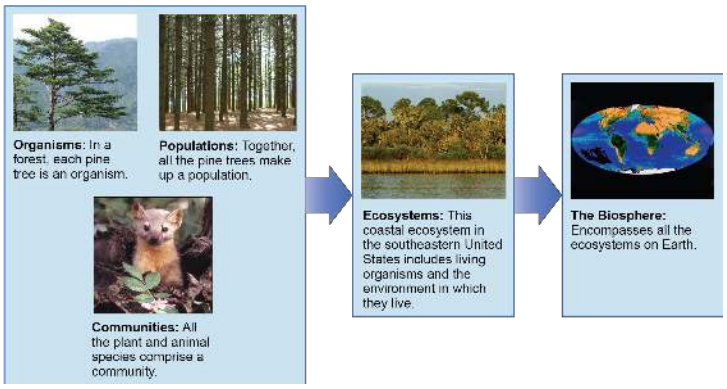
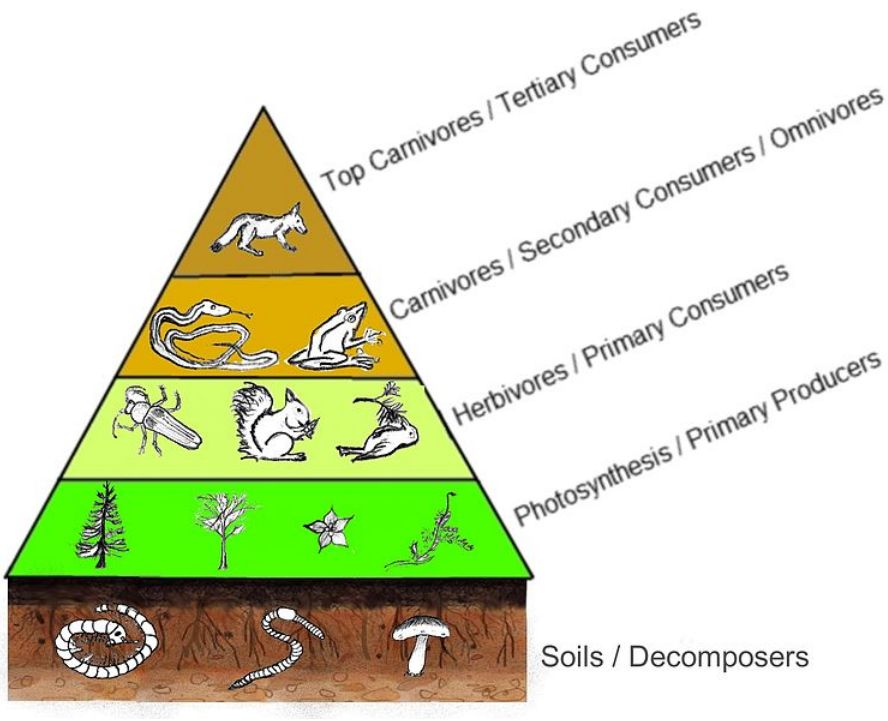


Figure 44.2 Ecologists study within several biological levels of organization. (credit “organisms”: modification of work by yeowatzup”/Flickr; credit “populations”: modification of work by "Crystl"/Flickr; credit “communities”: modification of work by US Fish and Wildlife Service; credit “ecosystems”: modification of work by Tom Carlisle, US Fish and Wildlife Service Headquarters; credit “biosphere”: NASA)

Ecosystem & Eutrophication

An **ecosystem** is defined as an association of life and the physical environment. Ecosystems take into account both living organisms and the nonliving components like water, soil, light, etc. Ecosystems can be either terrestrial (dessert, forest, grassland) or aquatic (coral reef, pond, estuary).

Within ecosystems scientists study **energy transformations**. Energy typically enters the ecosystem from the sun, is transferred to photosynthetic organisms (primary producers) and then to organisms that need to eat others for energy, the consumers or heterotrophs. An ecosystem will typically have several levels of consumers. The transfer of energy from organism to organism can be illustrated through **trophic levels.** A trophic level includes all organisms the same number of transfer steps away from the energy input into an ecosystem. A simple trophic level diagram for a forest ecosystem is illustrated below. Notice the photosynthetic organisms like plants are on the first trophic level, one step away from the energy source. The squirrel is a primary consumer (eating plants directly) but on the second tropic level two steps away from the primary energy source. At each step along the pathway some energy is lost as heat. Therefore at higher tropic levels there are fewer organisms (as noted by the pyramid shape) and most ecosystems support no more than four or five total trophic levels.



Ecosystems are changing due to human behaviors. Humans negatively impact natural ecosystems through activities such as deforestation, hunting, and pollution. Today's lab focuses on the impact of **eutrophication** on aquatic ecosystems. Through eutrophication bodies of water acquire extremely high concentrations of nutrients. The source of these nutrients can be natural or artificial. Humans cause cultural eutrophication through behaviors like run off from agricultural fields, wastewater from sewage treatment plants, and excel detergents running into bodies of water. The excess nutrients fuel photosynthesis causing an increased growth in algae, a photosynthetic primary producer protist, and an algae bloom. During the bloom the algae cover the surface of the water. When the algae die, decomposers in the ecosystem break down the protists using up the oxygen available in the aquatic environment through respiration. As oxygen levels decrease (hypoxia), fish die, and the balance of the ecosystem is destroyed.

In an effort to understand ecosystems and human impact on ecosystems scientists often use models. Modeling of ecosystems serves two main functions. First, the model represents scientist's best understanding of the relationships/functions that define the ecosystem. Second, models allow scientists to investigate questions that would be impossible to pursue in reality.

Ecosystem models fall into two categories, analytical models and simulation models. Analytical models use math to explain simple linear relationships. Simulation models, more widely used, are used to illustrate complex non linear relationships in ecosystems. Since the natural ecosystem has numerous interactions between living and non living components ecosystem models must simplify this real world situation. Models incorporate only the most important components or group similar components in an effort to effectively represent the ecosystem in a straightforward fashion.

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32.2: Exercise

Modeling a Food Web

Draw a food web. If you are on a field trip, include organisms that you see in the same area, interacting together. If in the lab, try to include organisms that you have seen or learned about. Make sure to include a **primary producer**, at least **two levels of consumers**, and a **decomposer**. Label which organisms are **autotrophs** and which are **heterotrophs**. Use arrows to show how energy is transferred and lost throughout this web.

What is the ultimate source of energy for your food web?

What happens to this energy as it moves through the food web? Where does it end up?

Nutrient Cycling in Ecosystems

In drawing your food web, you depicted a combination of the flow of energy and the cycling of nutrients through an ecosystem. Where as energy has an input and an output, nutrients are continually recycled. These elements often cycle between the biotic and abiotic components in an ecosystem.

For example, water is composed of hydrogen and oxygen atoms. Water molecules precipitate from the atmosphere and fall as rain, saturating the soil. A plant absorbs those molecules through its roots, transporting them up to its leaves. Some of these water molecules will be broken apart during the process of photosynthesis, the oxygen exiting the plant as O_2 gas that you will breathe in while the hydrogen might be used to form molecules of glucose that you will eat. The rest of the water exits the plant through its stomata, evaporating back into the atmosphere in a process called **transpiration**, the evaporation of water from plant tissues. The oxygen atoms that you breathed in will be used for cellular respiration and be joined back to hydrogen molecules to once again form water. This water might exit your body as vapor on your breath or as perspiration and return directly to the atmosphere. It might also soak into your clothes as sweat or exit as urine, be processed in a wastewater facility, then sent out to the ocean where it will evaporate back into the atmosphere.

The atoms of hydrogen and oxygen are broken apart and reassembled into other molecules multiple times in this process, but the overall outcome is a cycle, with atoms traveling between the atmosphere, hydrosphere, lithosphere, and biosphere, but rarely ever exiting our Earth system.

> In the space below, diagram the flow of water through the ecosystem around you.
>
> What is the role of plants in the global cycling of water?

Mycorrhizal Networks

A **symbiosis** (sym- meaning shared, bio- meaning life) is when two or more organisms of different species live in close proximity to one another, sharing some aspect of their life cycle. A **mutualism** is a type of symbiosis in which both partners get a net benefit from the interaction. In other types of symbiosis, only one partner benefits and in a **parasitic** symbiosis, it is to the detriment of the other partner.

Most plants form a mutualistic symbiosis with fungi called a **mycorrhizal** relationship (myco- meaning fungus, rhiz- meaning root). In this particular partnership, the fungus enters the plant through the roots and takes sugars from the plant. In exchange, the plant takes water and dissolved nutrients (particularly nitrogen and phosphorus) from the fungus. Though both of these organisms lose something in the exchange, it is something that they tend to have a surplus of, while the thing they get in return is one that they would not have access to alone.

> The fungus obtains its sugars from the living plant tissue. What terms could you apply to this organism?
>
> The plant synthesizes its own sugars during photosynthesis. What terms could you apply to this organism?

Somewhat recently, a researcher named Suzanne Simard began tracking the exchange of nutrients through mycorrhizae. Not just from tree to fungus, but from tree to tree via connections to the same mycorrhizal partner. By providing certain trees with carbon dioxide containing a heavier isotope of carbon (C14), Dr. Simard could track the sugars formed from those heavy carbon atoms as they moved from tree to tree. What she found was that the connections extended beyond just one tree to another, but that almost all of the trees within her plot were connected through a network of different mycorrhizal fungi. Trees of different species and fungi of

different species could exchange nutrients with each other. Further study revealed that the exchange was not limited to nutrients and water, but also included transfer of plant defense compounds that worked like raising an alarm, cuing the other plants to start producing defensive chemicals.

> How might this information change the way we study ecosystems?
>
> Sketch a few of the above ground plants that you can see (or imagine) and connect them below ground via a mycorrhizal network. Use arrows to indicate the flow of sugar through this network. Add in some disturbance that would set off a plant's immune system (attack of the caterpillars, fungal infection, etc…) and depict the movement of the signal throughout the plant community.

Some plants have evolved to be parasitic by taking advantage of the mycorrhizal network. These plants no longer photosynthesize. Instead, they form relationships with mycorrhizal fungi that are attached to other plants, siphoning sugars from those plants through the fungal connection. For example, the parasitic plant *Allotropa* parasitizes the Matsutake mushroom, which is mycorrhizal with tanoaks and a few other trees.

> Add a parasitic plant into your ecosystem above and use arrows to depict the movement of sugars.
>
> Are these parasitic plants autotrophic or heterotrophic? How would you classify them, using ecology terminology? Explain your reasoning.

Each species in an ecosystem is considered a **population**. This represents a group of actually or potentially interbreeding organisms and is an important area of study for both ecologists and geneticists. When multiple species in an ecosystem are considered together, this is called a **community**. For example, a forest could be composed of a single population of redwood trees, but it is more likely to be a community, including other tree species like Sitka spruce and Douglas-fir, and animals like the Northern spotted owl or Coho salmon. All living organisms are considered to be a part of the community in a given ecosystem.

> What is the difference between a community and an ecosystem?

Community Interactions

Pollination

One important community interaction we study in botany (in addition to mycorrhizae) is **pollination**. This is the transfer of pollen from one plant to another and can be mediated by animals, water, wind, or another mechanism. Since we are talking about community interactions, we will focus on animal pollination. Many flowering plants have evolved over time to attract a specific animal pollinator. Because the plant often provides food in the form of nectar or pollen, the pollinator often relies on this relationship as well and coevolves with the plant. One interesting example of this coevolution is the pollination of figs by parasitic wasps.

The Story of the Fig and the Wasp

Figs are made of many small flowers in an inside-out inflorescence called a syconium. How, then, are these flowers pollinated to develop into fruits? Figs are pollinated by tiny chalcid wasps known as fig wasps. In the spring, female fig wasps laden with pollen and fertilized eggs enter into the fig syconium through a small opening at the top. As she enters, her wings are ripped off, making her unable to leave again. She has reached her destination.

As she walks along the tops of the flowers, which are in their female stage, she lays her eggs inside the ovaries by inserting a long tube called an ovipositor down through the style.

Pollen falls off of her body onto the flowers and travels down the styles as well to fertilize the ovaries. Each flower she deposits an egg into will develop into a wasp instead of a fruit. However, some of the styles are longer than others, and in these she will not be able to lay her eggs. These flowers will develop into the fig fruit, while the others will serve as nurseries for the fig's strange pollinators.

The male wasps hatch first. Blind and wingless, they roam around the enclosed inflorescence, impregnating their still-sleeping sisters. As carbon dioxide builds up within the chamber, they begin to suffocate and burrow out through the sides of the figs, allowing oxygen to flow back in.

After their brothers have died, the females hatch, climbing over the flowers that are now in their male stage, collecting pollen and stuffing it into pouches on their bodies. The young female wasps, laden with pollen and fertilized eggs, leave the fig through the tunnels their brothers made and fly off to find a developing fig to lay their eggs and pollinate the enclosed florets. The timeline of a fig wasp's life is intricately interwoven into the phenology of the fig tree.

View the diagram on the following page and identify the stages in the connected life cycles of these organisms. If available in lab or on your field trip, cut open a fig to view the florets developing inside the syconium.

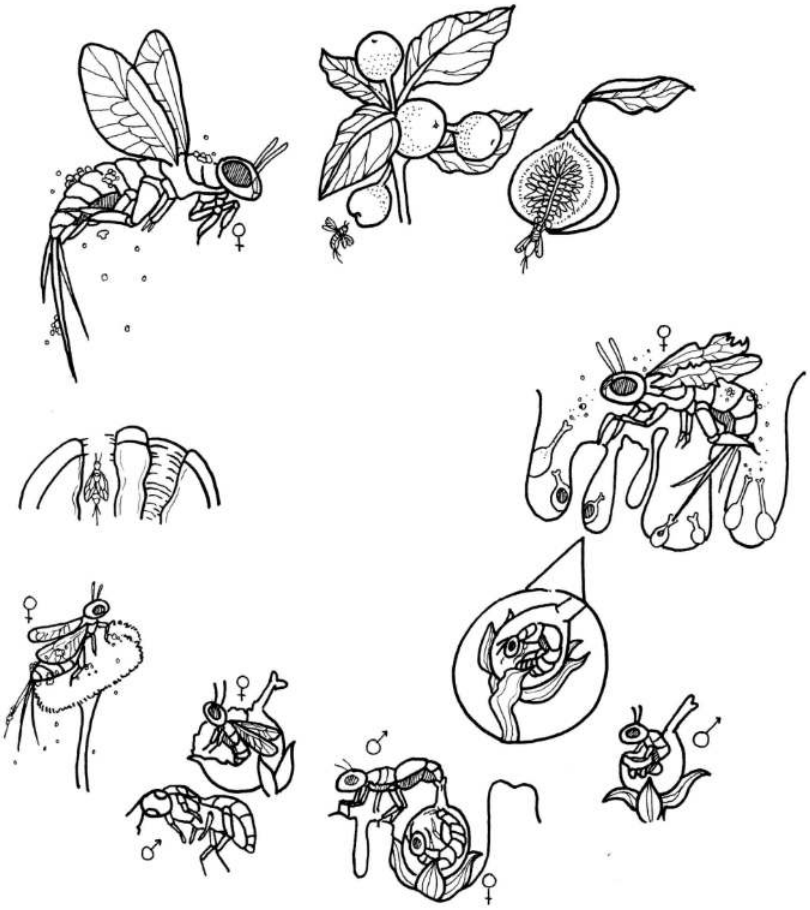


Figure 32.2.1: Fig Wasp Pollination Diagram

Is this relationship a parasitism or mutualism? Explain your reasoning.

Contributors and Attributions

- Maria Morrow (College of the Redwoods)

Ecosystem & Eutrophication - Silver Springs

This lab will utilize the Silver Spring model, an analytical model, developed by H. T. Odum in 1957. Silver Springs is a real aquatic ecosystem located in central Florida. Odum developed his model to illustrate energy flow through the different trophic levels. The main organisms in Sliver Sping organized by trophic level are:

- *First trophic level*: Eelgrass and algae are the main photosynthetic organisms.
- *Second trophic level*: Invertebrates, turtles, and fish are the herbivores.
- *Third tropic level*: Both fish and invertebrates are carnivores and prey upon the herbivores.
- *Fourth tropic level*: The top carnivores consist of gar and bass, which eat the other fish species at lower trophic levels.
- *Decomposers*: Bacteria and crayfish are the main decomposers.

The Silver Springs model is on Blackboard as an Excel file. The general model structure is pictured on the next page. Odum's model contains five state variables representing energy inputs into the four tropic levels and the decomposers. Flux rates describe the addition/removal of energy to/from the various parts of the system and the rate of transfer of energy between the components of the system. In the Excel file you can change the initial values **(X)** of any or all of the state variables and coefficients **(P)**, and control the length of time simulated. Following a simulation "run" you will be easily able to view graphs of the state variables or community respiration versus time. To change the initial values, simply type over the current value and press return. Use the model to investigate the scenarios below:

We will use the model in two different scenarios in today's lab, to understand eutrophication and to determine the impact of bread on the ecosystem.

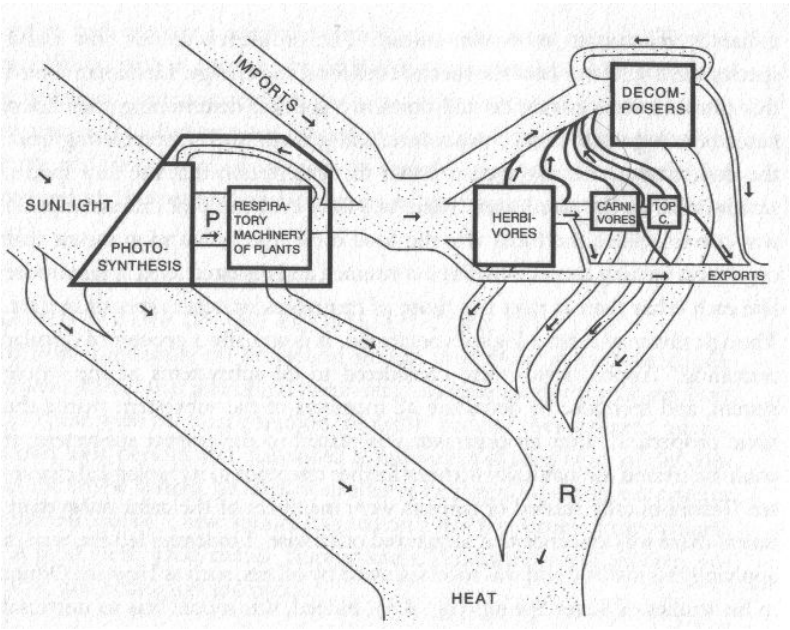


Figure 32.2.2: Odum's Silver Spring Model

Activity I

To understand more about eutrophication, you will conduct a laboratory experiment (Part 1) and use the Silver Springs computer model (Part 2)

Part 1

One potential way to decrease cultural eutrophication is by having higher trophic level organisms consume the algae. We will investigate this possibility using *Daphnia*, a zooplankton that feeds on green algae, and an alga species called *Chorella*. Although the information we gain from this activity is useful, it is an over simplification as to what might really occur in the aquatic environment.

We will use a **colorimeter** to measure the algae population. A colorimeter measures absorbance, the amount of light that is absorbed by a solution rather than the amount of light that can pass through. As the algae population increases (number of algae cells), the absorbance will also increase. It is a direct relationship.

Procedure

1. Obtain two cuvettes and a transfer pipette.
2. Fill one cuvette with distilled water. This cuvette is the blank
3. Place the blank into the colorimeter and zero it. This provides a baseline measure for the experiment. Save the blank cuvette for later use.
4. Using the disposable pipette, fill the second cuvette with the *Chorella* algae. Use the pipette to try and disperse the algae evenly throughout the cuvette.

5. Add 10 *daphnia* to the cuvette and immediately measure the absorbance using the colorimeter. Record your reading in the table below.
6. Remove the cuvette from the colorimeter and allow it to sit, undisturbed for 30 minutes.
7. After 30 minutes, place the blank cuvette (with distilled water) back into the colorimeter. Use the blank to re-zero the machine.
8. Remove the blank cuvette and put the algae/*Daphnia* cuvette into the colorimeter. Measure the absorbance and record your reading in the table below.

Table 1: Colorimeter data of *Daphnia* feeding on *Chorella*

	Absorbance value
Before feeding (time zero)	
After feeding (time 30 minutes)	

Questions

1. How did the absorbance change from time 0 to time 30 minutes? Did the absorbance increase, decrease, or stay the same?
2. What does the absorbance value change tell you about the concentration of the *Chorella*? Did it increase, decrease, or stay the same?
3. What does the absorbance value chance tell you about the behavior of the *Daphnia*? Did they consume *Chorella?* How do you know?
4. In a real aquatic ecosystem, do you think zooplankton could decrease the impact of cultural eutrophication and algae blooms? Explain why or why not.

Part 2

Now that you have observed trophic interactions and cultural eutrophication in a lab experiment, apply that knowledge to the computer model(opens in new window). Use the model to analyze the sensitivity of the Silver Springs ecosystem to cultural eutrophication. Remember that alga is a photosynthetic producer on the first trophic level. The *Daphnia* would be one of the herbivores in the environment on the second trophic level. Change the levels in the model to simulate a eutrophication situation. View the graphs to see how the different trophic level populations change through the simulation.

Questions

Based on your findings with the model, answer the following questions:

1. What is the maximum number of producers the ecosystem can support before higher trophic levels begin to decline?
2. What happens to community respiration in the simulated algae bloom? Does it increase, decrease, or stay the same?
3. What happens to the decomposer population in the simulated algae bloom? Does it increase, decrease, or stay the same? Why?
4. Which group of carnivores (which trophic level) is more greatly impacted by the algae bloom? Why do you think this is the case?

Activity II

Eutrophication is not the only way that human activities affect aquatic ecosystems. Tourism in the Silver Spring area has grown lately as a result of more of vacationers visiting Central Florida. When visiting Silver Spring, tourists enjoy feeding the ducks. But, the ducks are gaining weight and becoming dependent on the tourists as a food source disrupting the normal food chain in the environment. Some environmental organizations have voiced displeasure regarding the dependence of ducks on bread handouts from visitors to the lake. Is it feasible to stop feeding the ducks or have they become too reliant on the tourists and the bread handouts?

Task

Use the model to analyze the sensitivity of long-term duck populations to an increase or decrease in bread input. There is a separate variable for bread and the ducks would be on the second trophic level as herbivores. View the graphs to see how the different

trophic level populations change through the simulation.

QUESTIONS

Based on your findings with the model, answer the following questions:

1. Is an elimination of bread feasible? Why or why not?
2. How would the system respond to increasing levels of tourism, assuming bread levels also increased? What trophic levels are most impacted by increased levels of bread?
3. What is the role of bread in the system, and how does its presence or absence impact the ecology of Silver Springs?
4. What would be your suggestion to the managing board of Silver Springs? Should they completely eliminate the tourists feeding the ducks? Why or why not?

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Ecology Game:

Islands have always provided scientists unique locations to learn about the natural world. Many of Charles Darwin's key observations that led to his understanding of natural selection came from species living on the many islands that make up the Galapagos archipelago. For example, Darwin observed different species of finches were found on different islands and that the shape of the finches' beaks corresponded to the type of food they consumed. Further research on the finches of the Galapagos by Dr. Peter Grant and Dr. Rosemary Grant found that even within an island, changes in environmental conditions (i.e. drought, disease) could influence the adaptations observed in the finches.

Because islands are isolated from the mainland and each other by water and often long distances, they can be **colonized** by new species that have intentionally or unintentionally dispersed to an island. In biology, we call this the **founder effect**, which is when a new population is established. A founding population is often small and has a different allele frequency than the original population. It is important to remember that every species that finds itself in a new habitat may not be ideally suited for the environmental conditions. Is the habitat, food, climate appropriate for the new species needs? Is there competition for any of these resources with species already living on the island? Large islands that are close to the mainland tend to support more species than small islands that are far from the mainland. As we discuss in class, we know that no two species can occupy the same **ecological niche**—basically, two species cannot co-exist if they are competing for the same resource. While niches can overlap significantly between two species, they cannot be exactly alike. Any habitat has a limited number of species that can be supported by the available resources. We know that the most successful species are those with adaptations best suited to the environment in which they find themselves and those that can best utilize the available resources. These individuals are able to survive and reproduce on the island and a new population is established there. Occasionally, if there is a mismatch in the individual's

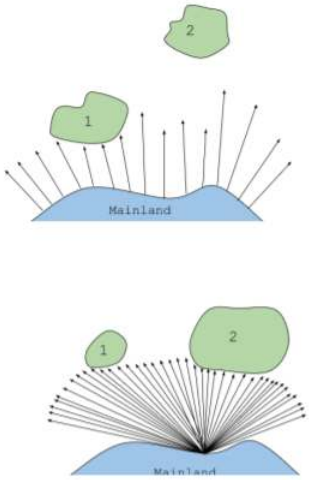


Figure 1 A & B – Both the distance of an island from the mainland (1A) and the size of an island (1B) can influence the number of species it can support.

traits and the environment in which they are located or the environmental conditions change unfavorably the individual may no longer succeed in that environment. This can lead to local extinction which is also known as **extirpation**. An extirpated species can recolonize if environmental conditions become favorable.

Today, you will be playing the role of an individual of one of the six different species on an ancient continent to the East in a game we call the Ecology Game. The objective of the game is to cross the ocean, colonizing islands as you go. However, every species in the game has its own ecological needs and each island along the way has specific environmental conditions and the ability to support different numbers of species. If you are a successful colonizer, a population of your species will exist on the island even after you have moved on. Please note that in order to colonize and establish a new population in real life, you would need a sexually mature male and female or a pregnant female. However, in this game, we are simplifying this concept by using a model of a single individual. Every species will not be able to colonize every island successfully and you have to compete with the other species who are also trying to make it to the new continent. The islands are of varying sizes and successful colonization may cover a small area or a large area depending on the size of the island. The winner is determined by a combination of the order that you reach the new continent and the island area (not how many islands, but how much island space) you have successfully colonized. Remember to think strategically! The Ecology Game is not always about speed, but thinking about your species, its ecological niche, and what we know about colonization and adaptation. Just because you are the first species to reach the new continent that does not mean you win the game. Think of today like an ecological game of chess!

Set up (per group—groups should have no more than 6 students):

- 1 board map
- 2 six-sided dice
- 1 cup of 6 species sticks
- 4 bags of colored game pieces (if groups are larger than 4 and additional game pieces are required use coins or see Manal for additional colored pieces)
- 6 species cards
- 24 island cards
- Environmental factor cards

To Begin:

1. Each person in the group should draw a species stick out of the cup. You will play the role of an individual of this species for the entire game.
2. Read the associated species card. This will tell you about your ecological niche and any movement limitations you will have in the game.
3. Each player can place their colored game piece at any location on the mainland to start the game.
4. Roll the dice. The player with the highest roll will go first and play will move counterclockwise around the group.

To Play:

1. Player 1 will roll both die. The sum of the numbers shown on the roll indicates how many squares the player can move.

> Note
>
> You cannot move diagonally but you can move vertically and horizontally and you can move forwards or backwards. Some species have limitations on the maximum number of moves they can make. If your roll exceeds the maximum number of moves for your species, you must stop at your maximum number. Every species can stop in the ocean.

2. With each roll, player 1 will also draw an environmental factor card which may influence your movement along the way. This card may affect all players, a subset of players, or just the player drawing the card.

3. If you land on any part of an island, choose the island card and determine if your species can successfully colonize the island. If so, fill that player's name/species name in the appropriate slot on the card (remember, some islands can only hold a few species) and place a colored game piece on the island to indicate that it has been colonized by you.

> **Note**
> On the next turn, a species on an island can leave from any part of the island.

4. Play continues in a counterclockwise fashion.

5. The game is complete for an individual species when they have reached the new continent. At this point, they no longer roll the dice and are no longer affected by environmental factor cards.

6. Play continues until all species have reached the new continent.

7. Calculate the winners using the formulas below.

Who Won?

- Each player's score is determined by adding up the total area your species covered (including bonuses) and dividing by your finish place modifier.
  - 1st place = 1
  - 2nd place = 1.5
  - 3rd place = 2
  - 4th place = 2.5
- For example: If my species covers 144 units of land and I had a bonus of 25 units of land for a total of 169 and I came in second place. My score = 122.7

$$\frac{(144\ units\ covered + 25\ bonus\ units)}{1.5\ (finish\ place\ modifier)} = 122.7\ (final\ score) \tag{32.2.1}$$

Questions:

1. What did the game clear up for you that you might not have understood fully about ecology and evolution?

2. Movement from the mainland to an island or from one island to another island represents what force that can cause a population to evolve?

3. If once your species has colonized an island and remains there and also becomes isolated from other populations over time, what possible outcome might occur given the passage of enough time?

4. When an island has multiple habitat types what does this say about the island's ability to support populations of different species?

5. How suitable are small islands to support multiple species? Explain your answer.

6. Once colonizing an island, a species may thrive or fail there. Describe how a species can either thrive or fail using terms and ideas from lecture. To help you answer the question, draw how a population's size will change over time if the species is K-selected.

Figure 1 is adapted from Wikimedia Commons material and this lab is licensed under a Creative Commons Attribution License License (3.0).

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32.3: Lab Report

Modeling a Food Web

1. Draw a food web.

2. What is the ultimate source of energy for your food web?

3. What happens to this energy as it moves through the food web? Where does it end up?

Nutrient Cycling in Ecosystems

1. In the space below, diagram the flow of water through the ecosystem around you.

What is the role of plants in the global cycling of water?

Mycorrhizal Networks

1. The fungus obtains its sugars from the living plant tissue. What terms could you apply to this organism?
2. The plant synthesizes its own sugars during photosynthesis. What terms could you apply to this organism?

Somewhat recently, a researcher named Suzanne Simard began tracking the exchange of nutrients through mycorrhizae. Not just from tree to fungus, but from tree to tree via connections to the same mycorrhizal partner. By providing certain trees with carbon dioxide containing a heavier isotope of carbon (C14), Dr. Simard could track the sugars formed from those heavy carbon atoms as they moved from tree to tree. What she found was that the connections extended beyond just one tree to another, but that almost all of the trees within her plot were connected through a network of different mycorrhizal fungi. Trees of different species and fungi of different species could exchange nutrients with each other. Further study revealed that the exchange was not limited to nutrients

and water, but also included transfer of plant defense compounds that worked like raising an alarm, cuing the other plants to start producing defensive chemicals.

3. How might this information change the way we study ecosystems?

4. Sketch a few of the above ground plants that you can see (or imagine) and connect them below ground via a mycorrhizal network. Use arrows to indicate the flow of sugar through this network. Add in some disturbance that would set off a plant's immune system (attack of the caterpillars, fungal infection, etc…) and depict the movement of the signal throughout the plant community.
5. Add a parasitic plant into your ecosystem above and use arrows to depict the movement of sugars.
6. Are these parasitic plants autotrophic or heterotrophic? How would you classify them, using ecology terminology? Explain your reasoning.
7. What is the difference between a community and an ecosystem?

Community Interactions

8. Is the relationship between the fig and the wasp a parasitism or mutualism? Explain your reasoning.

Contributors and Attributions

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Ecosystem & Eutrophication - Silver Springs

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Part 2

Questions

Based on your findings with the model, answer the following questions:

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Activity II

QUESTIONS

Based on your findings with the model, answer the following questions:

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environmental conditions. Is the habitat, food, climate appropriate for the new species needs? Is there competition for any of these resources with species already living on the island? Large islands that are close to the mainland tend to support more species than small islands that are far from the mainland. As we discuss in class, we know that no two species can occupy the same **ecological niche**—basically, two species cannot co-exist if they are competing for the same resource. While niches can overlap significantly between two species, they cannot be exactly alike. Any habitat has a limited number of species that can be supported by the available resources. We know that the most successful species are those with adaptations best suited to the environment in which they find themselves and those that can best utilize the available resources. These individuals are able to survive and reproduce on the island and a new population is established there. Occasionally, if there is a mismatch in the individual's traits and the environment in which they are located or the environmental conditions change unfavorably the individual may no longer succeed in that environment. This can lead to local extinction which is also known as **extirpation**. An extirpated species can recolonize if environmental conditions become favorable.

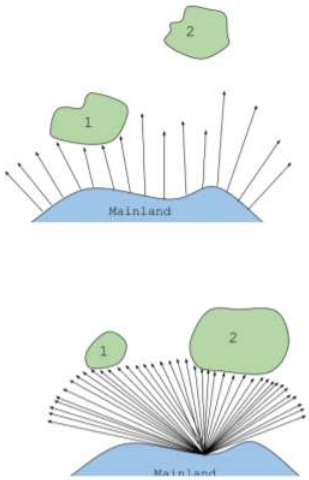


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3. If you land on any part of an island, choose the island card and determine if your species can successfully colonize the island. If so, fill that player's name/species name in the appropriate slot on the card (remember, some islands can only hold a few species) and place a colored game piece on the island to indicate that it has been colonized by you.

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CHAPTER OVERVIEW

33: Animal Behavior

Learning Objectives

Content Objectives

- Understand how to study and quantify animal behavior
- Compare ways scientists study animal behavior in the lab and field

Skill Objectives

- Design an experiment to evaluate animal behavior
- Quantify animal behavior and use to test hypotheses

33.1: Introduction
33.2: Exercise
33.3: Lab Report

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33.1: Introduction

Behavior is the change in activity of an organism in response to a stimulus. Behavioral biology is the study of the biological and evolutionary bases for such changes. The idea that behaviors evolved as a result of the pressures of natural selection is not new. Animal behavior has been studied for decades, by biologists in the science of ethology, by psychologists in the science of comparative psychology, and by scientists of many disciplines in the study of neurobiology. Although there is overlap between these disciplines, scientists in these behavioral fields take different approaches. Comparative psychology is an extension of work done in human and behavioral psychology. Ethology is an extension of genetics, evolution, anatomy, physiology, and other biological disciplines. Still, one cannot study behavioral biology without touching on both comparative psychology and ethology.

One goal of behavioral biology is to dissect out the innate behaviors, which have a strong genetic component and are largely independent of environmental influences, from the learned behaviors, which result from environmental conditioning. Innate behavior, or instinct, is important because there is no risk of an incorrect behavior being learned. They are “hard wired” into the system. On the other hand, learned behaviors, although riskier, are flexible, dynamic, and can be altered according to changes in the environment.

Animal behavior can be studied in the lab or field environment. The lab provides an advantage as it is a controlled environment, but many animals do not behave the same in a captive environment and thus behavioral studies must take place in the field. Both lab and field studies are important in our understanding of animal behavior, and which modality of study you choose must take into account the natural history and stress of the animal.

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- **45.7: Behavioral Biology - Proximate and Ultimate Causes of Behavior** by OpenStax is licensed CC BY 4.0.

33.2: Exercise

Lab Experiment: Bean Beetles

Adapted from C. Beck and L. Blumer by Staci Forgey, TCC Biology Faculty

Bean beetles, *Callosobruchus maculatus*, are herbivorous pest insects that are found in Africa and Asia. Females lay their eggs on the surface of beans. Eggs are layed singly, and hatch into larvae (maggots) several days later. The larva then burrows into the bean and will form a pupa 21–30 days after the egg was deposited. They mature 24–36 hours after emergence from the pupa and do not need to feed.

Adults typically live for 1–2 weeks. Mating and oviposition occur during this time period. Females will choose the best substrate (bean) to lay their offspring on, since the larvae cannot move. By choosing a substrate for oviposition, the female chooses the food resource available to her offspring (Brown and Downhower 1988). This is a critical choice for the female, as it influences the growth, survival and future reproductive success of her offspring (Mitchell, 1975, Wasserman and Futuyma, 1981). Females can lay eggs on a wide range of bean species, but very few bean species will result in normal development and the successful emergence of adults. Some species of beans are toxic to larvae (Janzen 1977).

Materials

In class, you will be provided with live cultures of bean beetles containing adults that have been raised on mung beans (*Vigna radiata*).

Female beetles are easily identified in the live cultures because they have two dark stripes on the posterior of the abdomen, whereas the posterior abdomen of males is uniformly light in color.

You will also have access to petri dishes and several types of beans.

Experimental Design

Since the oviposition choices of females influences the survival and future success of their offspring, females may be very sensitive to the species and condition of the beans on which they are depositing eggs.

Each group should design a set of experiments to address whether female bean beetles discriminate between suitable species of beans. We will re-visit the experiment next week and collect our data set. We will choose one experiment to perform as a class.

As a group, list several ways we might examine female bean choice for oviposition. We will pick one experiment from the class to perform. Write your ideas below.

Stop here. We will go over ideas for our experiment as a class and choose one to perform

Outline the agreed on experiment using the criteria below.

1. Formulate a hypothesis for this week's experiment. Be specific!
2. We will re-visit the experiment in a week to collect data on the number of eggs laid. Formulate a hypothesis for this experiment.
3. Identify the independent variable(s).
4. Identify the dependent variable(s).
5. What variables will you keep standard?
6. What is your control?
7. Design data collection tables for both of your experiments on a separate sheet of paper.

For this data, it is most useful to perform a Chi Square analysis. Chi Square statistical test evaluate whether this is a significant difference between groups of data. The null hypothesis (H_0) is the hypothesis that states that there is no difference between the groups of data. The alternative hypothesis, (H_a) is the hypothesis you outlined above.

1. Restate your H_0 hypothesis.
2. H_a hypothesis.
3. If the null hypothesis is correct, what would we expect to see?

4. If our alternative hypothesis is correct, what would we expect to see?

To do this statistical test, we need to calculate observed and expected values for the bean species and for our control. Our expected values are based on our null hypothesis (that there is no difference).

For example, if we had 40 eggs and four different treatment groups, we would expect there to be 10 eggs on each group.

Treatment	Observed number of eggs	Expected number of eggs
Mung beans	5	10
Pinto beans	25	10
Chick peas	10	10
Control	0	10

Fill in the data table below with our observed (what we actually found) and expected values.

Treatment	Observed number of eggs	Expected number of eggs

To calculate the chi-square value, or χ^2, we simply add the square differences, divided by the expected, of all the observed and expected. In mathematical terms:

$\chi^2 = \Sigma \frac{(O-E)^2}{E}$

So for our example from the previous sample table, the first $\frac{(O-E)^2}{E}$ would be $\frac{(5-10)^2}{10} = 2.5$

We would then add to this value all of the other $\frac{(O-E)^2}{E}$ in the table and then add to get the χ^2 value.

Treatment	Observed number of eggs	Expected number of eggs	Observed-Expected	(Observed-Expected)^2	(Observed-Expected)^2/Expected

Compute the χ^2 value for your data by adding all of the (Observed-Expected)2/Expected values.

In order to find something to compare this number with, we need to calculate the degrees of freedom, or the number of different comparisons that can be made within the table. The degrees of freedom are the number of columns (M) minus one times the number of rows (N) minus one.

Degrees of freedom = (M − 1)(N − 1)

A	B
C	D

How many ways can this two by two table be broken into individual comparisons? Hint: Use the formula above.

Calculate the degrees of freedom in your experiment.

Now we can compare against the Chi distribution for the likelihood that our data is generated by chance. Remember, we are looking at the column labeled 0.05 for our p value. This means that at this level, we are 95% sure our results are real. The Chi distribution table gives us a critical value to compare our test value to.

- If test > critical value @ p level, **reject** null hypothesis
- If test < critical value @ alpha level, **fail to reject** null hypothesis

Compare to the Chi-distribution table(opens in new window). Did your results come about by chance?

DF/P	0.995	.990	0.975	.950	.900	.750	.500	.250	.100	.050	.025	.010	.005
1	0.00004	.00016	0.001	0.004	0.016	0.102	0.455	1.323	2.706	3.841	5.024	6.635	7.879
2	0.010	0.020	0.0506	0.103	0.211	0.575	1.386	2.773	4.605	5.991	7.378	9.210	10.597
3	0.072	0.115	0.216	0.351	0.584	1.213	2.366	4.108	6.251	7.815	9.348	11.345	12.838
4	0.207	0.297	0.484	0.711	1.064	1.923	3.357	5.385	7.779	9.488	11.143	13.277	14.860
5	0.412	0.554	0.831	1.145	1.610	2.675	4.351	6.626	9.236	11.070	12.833	15.086	16.750
6	0.676	0.872	1.237	1.635	2.204	3.455	5.348	7.841	10.645	12.592	14.449	16.812	18.548
7	0.989	1.239	1.690	2.167	2.833	4.255	6.346	9.037	12.017	14.067	16.013	18.475	20.278
8	1.344	1.647	2.180	2.733	3.490	5.071	7.344	10.219	13.362	15.507	17.535	20.090	21.955
9	1.735	2.088	2.700	3.325	4.168	5.899	8.343	11.389	14.684	16.919	19.023	21.666	23.589
10	2.156	2.558	3.247	3.940	4.865	6.737	9.342	12.549	15.987	18.307	20.483	23.209	25.188
11	2.603	3.053	3.816	4.575	5.578	7.584	10.341	13.701	17.275	19.675	21.920	24.725	26.757
12	3.074	3.571	4.404	5.226	6.304	8.438	11.340	14.845	18.549	21.026	23.337	26.217	28.300
13	3.565	4.107	5.009	5.892	7.042	9.299	12.340	15.984	19.812	22.362	24.736	27.688	29.819
14	4.075	4.660	5.629	6.571	7.790	10.165	13.339	14.114	21.064	23.685	26.119	29.141	31.319
15	4.601	5.229	6.262	7.261	8.547	11.037	14.339	18.245	22.307	24.996	27.488	30.578	32.801
16	5.142	5.812	6.908	7.962	9.312	11.912	15.339	19.369	23.542	26.296	28.845	32.000	34.267
17	5.697	6.408	7.564	8.672	10.085	12.792	16.338	20.489	24.769	27.587	30.191	33.409	35.718
18	6.265	7.015	8.231	9.390	10.865	13.675	17.338	21.605	25.989	28.869	31.526	34.805	37.156
19	6.844	7.633	8.907	10.117	11.657	14.562	18.338	22.18	27.204	30.144	32.852	36.191	38.582
20	7.434	8.260	9.591	10.851	12.443	15.452	19.337	23.848	28.412	31.410	34.170	37.566	39.997
21	8.034	8.897	10.283	11.591	13.240	16.344	20.337	24.935	29.615	32.671	35.479	38.932	41.401
22	8.643	9.542	10.982	12.338	14.041	17.240	21.337	26.039	30.813	33.924	36.781	40.289	42.796
23	9.260	10.196	11.689	13.091	14.848	18.137	22.337	27.141	32.007	35.172	38.076	41.638	44.181
24	9.886	10.856	12.401	13.848	15.659	19.037	23.337	28.241	33.196	36.415	39.364	42.980	45.559
25	10.520	11.524	13.120	14.611	16.473	19.939	24.337	29.339	34.382	37.652	40.646	44.314	46.928
26	11.160	12.198	13.844	15.379	17.292	20.843	25.336	30.435	35.563	38.885	41.923	45.642	48.290
27	11.808	12.879	14.573	16.151	18.114	21.749	26.336	31.528	36.741	40.113	43.195	46.963	49.645
28	12.461	13.565	15.308	16.928	18.939	22.657	27.336	32.620	37.916	41.337	44.461	48.278	50.993
29	13.121	14.256	16.047	17.708	19.768	23.567	28.336	33.711	39.087	42.557	45.722	49.588	52.336
30	13.787	14.953	16.791	18.493	20.599	24.478	29.336	34.800	40.256	43.773	46.979	50.892	53.672

We will now use this data and information to do a lab write up.

Field Study: Campus Bird Ethology

A number of bird species live at our campus pond, providing a natural experimental setting.

Procedure:

Identify a species of bird at the pond. Write down its common and scientific name.

Watch an individual or group of individuals of the same species for ten minutes and list every behavior you see (examples: rest, peck object, dip head in water, social interaction).

A full list of all the activities or behaviors observed in an animal is called an **ethogram**, and can be an excellent tool for study of that species.

Once you have your list, you will do two kinds of measurements for behavioral observations of your species.

1. Time budget: watch an individual of your species for 5 minutes, keep track of each behavioral change and how long the animal is doing that behavior.
    1. Calculate a percentage of time for each activity you saw. For example, if your bird spent 2 minutes and 10 seconds of the 5 minutes resting it would have spent 43% of its time resting. Convert the time to seconds, then divide time of activity by total time (in this example 130/300 = .4333, x100 for percentage = 43%).
2. Total occurrences: watch an individual of your species for 10 minutes. Using the ethogram/list you made, put a tick mark next to the activity at every occurrence. If you individual was resting, then scratched its head, then went back to resting that would be two tick marks next to "rest" and one next to "scratched head". If you encounter a new behavior during this time add it to your list.

Which type of measurement do you think is better for measuring behaviors in a species? Why? What kinds of information might you want to study using time budget or total occurrence data?

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33.3: Lab Report

Lab Experiment: Bean Beetles

Background information

Hypothesis and predictions

Materials and methods (include experimental design)

1. Identify the independent variable(s).
2. Identify the dependent variable(s).
3. What variables will you keep standard?
4. What is your control?
5. Design data collection tables for both of your experiments on a separate sheet of paper.

For this data, it is most useful to perform a Chi Square analysis. Chi Square statistical test evaluate whether this is a significant difference between groups of data. The null hypothesis (H_0) is the hypothesis that states that there is no difference between the groups of data. The alternative hypothesis, (H_a) is the hypothesis you outlined above.

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To calculate the chi-square value, or χ^2, we simply add the square differences, divided by the expected, of all the observed and expected. In mathematical terms:

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So for our example from the previous sample table, the first $\frac{(O-E)^2}{E}$ would be $\frac{(5-10)^2}{10} = 2.5$

We would then add to this value all of the other $\frac{(O-E)^2}{E}$ in the table and then add to get the χ^2 value.

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In order to find something to compare this number with, we need to calculate the degrees of freedom, or the number of different comparisons that can be made within the table. The degrees of freedom are the number of columns (M) minus one times the number of rows (N) minus one.

Degrees of freedom = (M − 1)(N − 1)

A	B
C	D

How many ways can this two by two table be broken into individual comparisons? Hint: Use the formula above.

Calculate the degrees of freedom in your experiment.

Now we can compare against the Chi distribution for the likelihood that our data is generated by chance. Remember, we are looking at the column labeled 0.05 for our p value. This means that at this level, we are 95% sure our results are real. The Chi distribution table gives us a critical value to compare our test value to.

- If test > critical value @ p level, **reject** null hypothesis
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Compare to the Chi-distribution table(opens in new window). Did your results come about by chance?

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7	0.989	1.239	1.690	2.167	2.833	4.255	6.346	9.037	12.017	14.067	16.013	18.475	20.278
8	1.344	1.647	2.180	2.733	3.490	5.071	7.344	10.219	13.362	15.507	17.535	20.090	21.955
9	1.735	2.088	2.700	3.325	4.168	5.899	8.343	11.389	14.684	16.919	19.023	21.666	23.589
10	2.156	2.558	3.247	3.940	4.865	6.737	9.342	12.549	15.987	18.307	20.483	23.209	25.188
11	2.603	3.053	3.816	4.575	5.578	7.584	10.341	13.701	17.275	19.675	21.920	24.725	26.757
12	3.074	3.571	4.404	5.226	6.304	8.438	11.340	14.845	18.549	21.026	23.337	26.217	28.300
13	3.565	4.107	5.009	5.892	7.042	9.299	12.340	15.984	19.812	22.362	24.736	27.688	29.819
14	4.075	4.660	5.629	6.571	7.790	10.165	13.339	14.114	21.064	23.685	26.119	29.141	31.319
15	4.601	5.229	6.262	7.261	8.547	11.037	14.339	18.245	22.307	24.996	27.488	30.578	32.801
16	5.142	5.812	6.908	7.962	9.312	11.912	15.339	19.369	23.542	26.296	28.845	32.000	34.267
17	5.697	6.408	7.564	8.672	10.085	12.792	16.338	20.489	24.769	27.587	30.191	33.409	35.718
18	6.265	7.015	8.231	9.390	10.865	13.675	17.338	21.605	25.989	28.869	31.526	34.805	37.156
19	6.844	7.633	8.907	10.117	11.657	14.562	18.338	22.18	27.204	30.144	32.852	36.191	38.582
20	7.434	8.260	9.591	10.851	12.443	15.452	19.337	23.848	28.412	31.410	34.170	37.566	39.997
21	8.034	8.897	10.283	11.591	13.240	16.344	20.337	24.935	29.615	32.671	35.479	38.932	41.401
22	8.643	9.542	10.982	12.338	14.041	17.240	21.337	26.039	30.813	33.924	36.781	40.289	42.796
23	9.260	10.196	11.689	13.091	14.848	18.137	22.337	27.141	32.007	35.172	38.076	41.638	44.181
24	9.886	10.856	12.401	13.848	15.659	19.037	23.337	28.241	33.196	36.415	39.364	42.980	45.559
25	10.520	11.524	13.120	14.611	16.473	19.939	24.337	29.339	34.382	37.652	40.646	44.314	46.928
26	11.160	12.198	13.844	15.379	17.292	20.843	25.336	30.435	35.563	38.885	41.923	45.642	48.290
27	11.808	12.879	14.573	16.151	18.114	21.749	26.336	31.528	36.741	40.113	43.195	46.963	49.645
28	12.461	13.565	15.308	16.928	18.939	22.657	27.336	32.620	37.916	41.337	44.461	48.278	50.993
29	13.121	14.256	16.047	17.708	19.768	23.567	28.336	33.711	39.087	42.557	45.722	49.588	52.336
30	13.787	14.953	16.791	18.493	20.599	24.478	29.336	34.800	40.256	43.773	46.979	50.892	53.672

Conclusion:

- Write a paragraph restating your hypothesis and whether you accept or reject your hypothesis

- In this paragraph, **explain** why you accepted or rejected your hypothesis **using data from the experiment**. Include a brief summary of the data—averages, highest, lowest, etc., to help the reader understand your results and why you have come to particular conclusions.
- Discuss your thoughts about the possible reasons for your results (for example, if you chose salt water as a variable, give a possible reason why salt water, in particular, may have generated your results).
- Discuss possible errors that could have occurred in the collection of the data (experimental errors) and describe how these errors may have impacted the data.

Field Study: Campus Bird Ethology

Identify a species of bird at the pond. Write down its common and scientific name.

Watch an individual or group of individuals of the same species for ten minutes and list every behavior you see (examples: rest, peck object, dip head in water, social interaction).

Once you have your list, you will do two kinds of measurements for behavioral observations of your species.

1. Time budget: watch an individual of your species for 5 minutes, keep track of each behavioral change and how long the animal is doing that behavior.
    1. Calculate a percentage of time for each activity you saw. For example, if your bird spent 2 minutes and 10 seconds of the 5 minutes resting it would have spent 43% of its time resting. Convert the time to seconds, then divide time of activity by total time (in this example 130/300 = .4333, x100 for percentage = 43%).
2. Total occurrences: watch an individual of your species for 10 minutes. Using the ethogram/list you made, put a tick mark next to the activity at every occurrence. If you individual was resting, then scratched its head, then went back to resting that would be two tick marks next to "rest" and one next to "scratched head". If you encounter a new behavior during this time add it to your list.

Which type of measurement do you think is better for measuring behaviors in a species? Why? What kinds of information might you want to study using time budget or total occurrence data?

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CHAPTER OVERVIEW

34: Museum Science

Learning Objectives

Content Objectives

- Understand the importance and uses of natural history museum collections
- Explore ecology and evolution in the context of museum collections

Skill Objectives

- Find, explore, and clean data from natural history museum repositories and databases
- Use industry standard mapping software to understand patterns of species distributions

34.1: Introduction
34.2: Exercise
34.3: Lab Report

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34.1: Introduction

What is a Natural History Collection?

Historic definition: “Anything connected with NATURE.”

Modern definition: Definitions from biologists often focus on the scientific study of individual organisms in their environment.

"Natural history is the study of animals and plants – of organisms. ... I like to think, then, of natural history as the study of life at the level of the individual – of what plants and animals do, how they react to each other and their environment, how they are organized into larger groupings like populations and communities“- Marston Bates, 1954

"The close observation of organisms—their origins, their evolution, their behavior, and their relationships with other species“- D.S. Wilcove and T. Eisner, 2000

Marston Bates, The nature of natural history, Scribners, 1954.

D. S Wilcove and T. Eisner, "The impending extinction of natural history," Chronicle of Higher Education 15 (2000): B24

Paleolithic cave paintings- identified “species”



Figure 34.1.1: Chauvet horses(opens in new window) ~31,000BP is licensed under Public Domain(opens in new window)

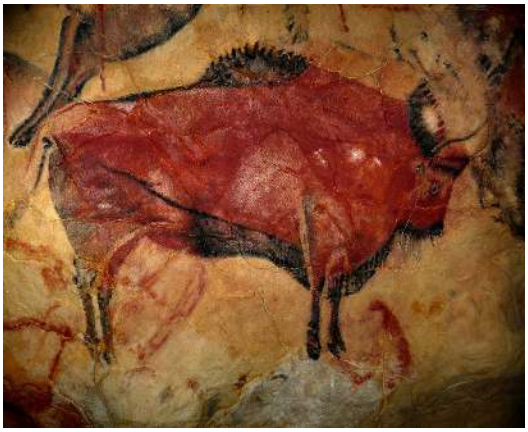


Figure 34.1.2: Altamira Bison(opens in new window) by Rameessos is licensed under Public Domain(opens in new window)

Early Natural History

Naturalists: Gaining much knowledge, but no durable preservation techniques

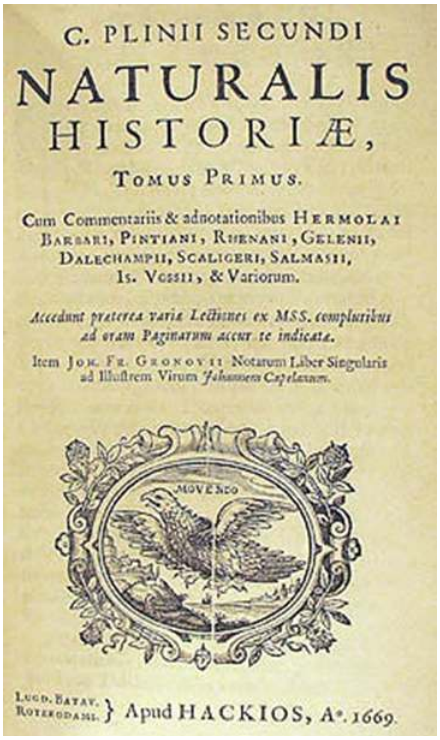
Bacteria and microbes were unknown

Drawings and written descriptions only

- Aristotle (History of Animals)

- Aelian (De Natura Animalium)
- Pliny the Elder (Natural History)

Figure 34.1.3: Naturalis Historia, 1669 edition, title page. The title at the top reads: "Volume I of the Natural History of Gaius Plinius Secundus".



Why Collect Specimens?

Article: *Why Collections Matter*(opens in new window) from The Society for the Preservation of Natural History Collections [SPNHC] - an international organization devoted to the preservation, conservation and management of natural history collections.

1. A physical record to identify new species and subspecies. New, unidentified species can be compared to "type specimens" to determine relatedness.

- Holotype- a single type specimen upon which the description and name of a new species is based.
- Paratype- a specimen of an organism that helps define what the scientific name of a species actually represents, but it is not the holotype.
- Topotype- a specimen of a species collected at the locality at which the original type was obtained.



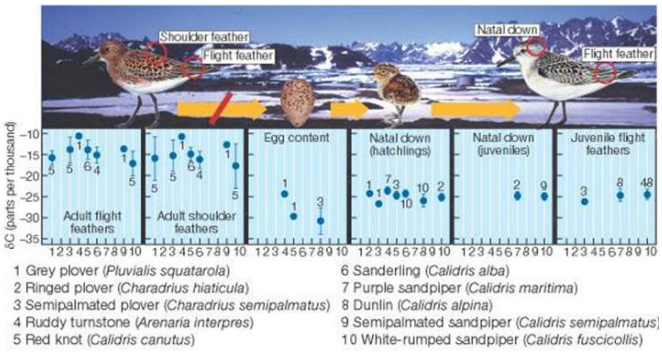
Fig. 1. *Spectrolebias brousseauí*, male (above), holotype, MNKP 10162, 28.4 mm SL and female (below), paratype, MZUSP 109219, 25.3 mm SL.

Figure 34.1.4: [Ichthyology • 2013] Spectrolebias brousseaui • A New Annual Fish (Cyprinodontiformes: Rivulidae: Cynolebiatinae) from the upper río Mamoré basin, Bolivia

2. Monitor physical &/or physiological changes in a population. (See the case studies below for examples.)
3. Law enforcement- identify endangered species and wildlife trafficking.
4. Tissue samples for contaminant/disease analysis. (See the case studies below for examples.)
5. Isotope analysis for determining relatedness or territory distributions, or in the case of Arctic waders, determining how they utilize nutrients to make eggs.

Figure 34.1.5: Arctic waders are “income breeders,” not “capital breeders” from Klaassen, M., Lindström, Å., Meltofte, H. *et al.* Arctic waders are not capital breeders. *Nature* **413**, 794 (2001). [doi.org]

6. Detailed scientific drawings for field identification guides
7. “Teaching collections” which allow students to see and hold various species from other parts of the world.



Types of collections

Microbiological- World Federation of Culture Collections(opens in new window)

Fungi- USDA Database(opens in new window)

Plants- Plant Collection Network- NA(opens in new window)

Invertebrates- Field Museum, Chicago

Herpetological- Smithsonian National Museum of Natural History(opens in new window)

Fish- Northern Carolina Museum of Natural Sciences(opens in new window)

Avian- Western Foundation of Vertebrate Zoology

Mammals- American Museum of Natural History(opens in new window)

The History of Collecting

Collecting natural history and geological specimens was a “fad” in the 18th and 19th centuries. Conservation of species was not considered, and collectors gathered as much as they could to sell to the public. Those in the military would often collect specimens from overseas while on deployment.

"Market collectors took about 10 million Common Murre eggs between 1850 and 1900 from the Farallon Islands off the northern Californian coast, for sale to markets and restaurants in San Francisco. Good egg collectors took egg clutches early in a field season so that birds could re-lay; in contrast, destructive egg collectors would take all of the eggs of all of the birds in a locality – for example, a nesting colony – during the entire breeding season. This type of collecting, combined with the detrimental effects of the millinery trade on birds, caused outrage among ornithologists and interested lay-people around the world, and led to the enaction of laws in the early 20th century limiting bird and egg collecting to those specimens needed only for scientific purposes.”- from WFVZ *The History of Bird and Egg Collecting*(opens in new window)

Laws on Collecting

In the UK, it is illegal to sell eggs, regardless of age.

In the US, State and Federal permits are required to “take” or “salvage” specimens, and to “house” collections- Scientific Collecting permits- California Dept of Fish and Wildlife(opens in new window)

Must follow Endangered Species Act(opens in new window) and CITES (the Convention on International Trade in Endangered Species of Wild Fauna and Flora)(opens in new window) laws.

The primary sources for animal specimens currently are wildlife rehabilitation organizations; wildlife research agencies; birds killed by cats, window strikes, and car impacts. It is rare to actively go out into the wild to collect animal specimens.

It's All About The Data!

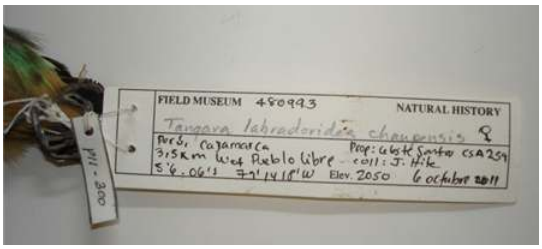


Figure 34.1.6: Labels on specimen- Original tag and museum catalog tag (From Field Museum of Natural History.)

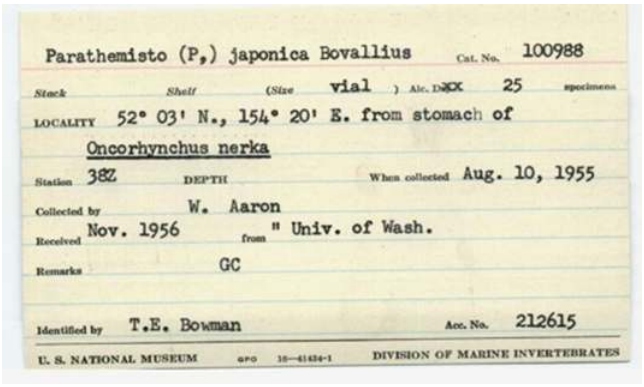


Figure 34.1.7: Data card- More in-depth information kept on file at the museum where the specimen is housed (from The WFVZ)

Methods of preservation

Study skins- skin of animal preserved, often with bones intact

Mammals, birds, reptiles, (and fish)

Flat skin- For large animals that take up a large amount of space

Nests

Egg- egg blowing

Spirit specimens

Skeletons

Fossils

Plant press

Seed banks

Case Study- Influenza

1918 influenza outbreak- 20 million to 40 million people were killed worldwide, including 675,000 people in the United States (peak of 10,000 per week) (Crosby 1989). Influenza virus from preserved bird specimens in the Smithsonian was compared with that in tissue samples from humans infected in 1918 (Taubenberger et al. 1997, Fanning et al. 2002).

These studies showed that the virus responsible for the 1918 pandemic was more similar to strains infecting swine and humans than to avian influenza, suggesting that the pandemic was not caused by the virus jumping from birds to humans, as previously suspected.

Other recent studies have used historical samples to reconstruct the evolutionary history of the virus, providing guidance for future vaccine development (e.g., Ferguson et al. 2003)

[academic.oup.com]

Case Study- Hantavirus

In 1993, the hantavirus outbreak in SW United States killed 70 percent of afflicted individuals. The virus had been found in deer mice in the Southwest before the human outbreak, but little was known about its abundance in natural populations or the causes for its sudden jump into human populations (Yates et al. 2002).

Some citizens expressed concern that the new human infections might be linked to military weapons testing at nearby Fort Wingate (Horgan 1993).

Genetic analysis conclusively showed that hantavirus had been present in rodent populations before the 1993 outbreak, and the close relationships between different strains of the virus and different rodent hosts suggested an ancient association between virus and host (Yates et al. 2002).

Wet El Nino years allow rodent populations to expand, and increase the probability of infecting humans.

[academic.oup.com]

Case Study- Lead Poisoning

California Condors (*Gymnogyps californianus*) were brought to the brink of extinction, in part, because of lead poisoning, and lead poisoning remains a significant threat today. Condors in California remain chronically exposed to harmful levels of lead. 30% of the annual blood samples collected from condors indicate lead exposure (blood lead ≥ 200 ng/mL) that causes significant subclinical health effects. Each year, ∼20% of free-flying birds have blood lead levels (≥450 ng/mL) that indicate the need for clinical intervention to avert morbidity and mortality.

Lead isotopic analysis shows that lead-based ammunition is the principle source of lead poisoning in condors.

The condor's apparent recovery is solely because of intensive ongoing management, with the only hope of achieving true recovery dependent on the elimination or substantial reduction of lead poisoning rates.

[www.pnas.org]

Case study- Mercury

More than 1.6 million Americans are at risk of mercury poisoning. Mercury deposition in fish is the leading cause of governmental advisories against fish consumption in the United States—over 2200 such advisories were issued in 2000 (EPA 2002, Stoner 2002.) Researchers can estimate historical levels of contamination in museum specimens, and construct a baseline against which current levels can be compared.

Analysis of preserved bird specimens from the Swedish Museum of Natural History has shown that concentrations of accumulated mercury increased during the 1940s and 1950s, probably as a result of human industry (Berg et al. 1966)

Museum collections can also be used to allay unwarranted fears. Some studies have demonstrated that the accumulation of toxic compounds, such as mercury, is not occurring in all oceanic fish (Barber et al. 1972, Miller et al. 1972).

Notable Collections

Gallery of Paleontology and Comparative Anatomy(opens in new window)

Western Foundation of Vertebrate Zoology(opens in new window)

British Museum of Natural History(opens in new window)

Local Natural History Collections

U.C. Berkeley- Museum of Vertebrate Zoology(opens in new window)

California Academy of Sciences(opens in new window)

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34.2: Exercise

This term, you will complete a group research project using digitized natural history museum collections. We will be working within the framework of one of the research projects designed by the Biological Collections in Ecology and Evolution Network (BCEENET). Instructions and full project materials will be presented in lab and available on Canvas.

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34.3: Lab Report

Your lab research project will culminate in a poster symposium at the end of the term. Full details will be provided by your instructor.

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35: Appendix A - Lab Safety

The following guidelines were designed by the Biology Department Faculty for use in all biology courses offered at Evergreen Valley College. It is mandatory that students follow these regulations. Students are expected to read these regulations, sign the Safety Form on page 5, and return the bottom half to the instructor. This procedure must be completed before a student can begin any laboratory activity. The student should keep the regulations for future reference.

General*

1. Read all laboratory instructions before coming to class.
2. Dress appropriately for lab: closed-toe shoes, long hair tied back, no dangling jewelry or loose clothing. Avoid wearing contact lenses.
3. Know the location and proper use of the following in your laboratory: first aid kit, fire extinguisher, eye wash, emergency telephone, fire blanket, closest fire alarm, chemical shower, emergency exits, emergency gas shut-off valves, evacuation site for your classroom. Keep the safety equipment and exits free of obstruction.
4. Behave in a professional manner.
5. Only registered students are allowed in the laboratory.
6. Students are not permitted access to prep rooms, laboratory cupboards, or instructor desks without prior approval from the instructor.
7. Students who have any medical conditions (e.g. diabetes, immunological defect, pregnancy, epilepsy) that may necessitate special precautions in the laboratory must inform the professor.
8. To encourage classroom unity and promote safety, please speak English in class.
9. No food or drink (including water) allowed in the laboratory.
10. Keep hands away from face, eyes and clothing while using hazardous solutions or materials. Do not handle contact lenses or apply cosmetics during class.
11. Maintain a clean, open work area free of everything except material related to the exercise.
12. Use all equipment and materials with care and as authorized by instructor.
13. Report all spills or accidents to the instructor. Clean up spills immediately.
14. Wipe down work surfaces at the beginning and end of each lab.
15. Wash your hands at the beginning and end of class.
16. At the end of lab make sure all items are returned to their proper location. Push in chairs. Make sure sinks and your work space are clean and free of debris.

*Covid precautions

1. Perform a Self-Check for the following symptoms listed below prior to coming to lab. If any symptoms are present, do not come to lab.
2. Fever or chills, cough, shortness of breath or difficulty breathing, fatigue, muscle or body aches, new loss of taste or smell, sore throat, congestion or runny nose, nausea or vomiting, diarrhea
3. Wear a mask at all times, regardless of vaccination status.
4. Maintain social distancing, when possible.

Emergency Preparedness

1. During an earthquake stay in the room and immediately get under the desk. After the earthquake follow your instructor’s directions.
2. During a fire or other emergencies requiring evacuation, meet at the designated evacuation location. Students are required to meet and remain at the evacuation site until dismissed by the instructor.
3. During a shelter-in-place emergency, students should remain in the classroom and follow instructions.

Handling Equipment and Materials

1. Properly label all glassware and slides.
2. Keep all equipment and materials away from the edge of the lab benches.

3. Use protective aids (e.g. test-tube clamps, hot pads) when handling hot glassware.
4. Light gas burners only as instructed. Be sure no volatile or flammable materials are being used nearby. Remember to have long hair tied back, no dangling jewelry or loose clothing.
5. When heating a test tube do not look into the mouth of the tube or point it into the direction of any person.
6. Heat sources should only be on when in use. Do not leave heat sources unattended. When in doubt, students should assume a heat source is on or hot.
7. Operate electrical equipment in a dry area with dry hands.
8. When removing an electrical plug from its socket, pull the plug, not the cord.
9. Inform your instructor immediately if equipment or materials are broken or not working properly.
10. Never return reagents back into their original containers. Keep containers capped when not in use.

Handling Hazardous Material

1. Wear appropriate eye protection as directed by the instructor.
2. Wear disposable gloves when handling hazardous materials including chemicals, organisms, or bodily fluids.
3. Wear protective clothing when required by the instructor.
4. Follow correct disposal procedures as detailed in “Cleanup and Disposal.”
5. To prevent contamination by needle stick injuries, always use new needles and lancets.
6. Use the fume hood whenever noxious, corrosive or toxic fumes are produced or released.

Bacteria and Fungi

1. Never open Petri dishes containing bacterial or fungal growth unless directed to do so by the instructor.
2. Dispose of all discarded bacterial and fungal cultures as directed by the instructor.

Cleanup and Disposal

1. Follow correct disposal procedures – dispose of used materials promptly and in the appropriate disposal containers.
    1. Dispose of broken glass in labeled glass disposal containers.
    2. Dispose of needles and lancets in puncture-proof sharp containers.
    3. Dispose of used chemicals in labeled and capped waste containers as directed by your instructor.
    4. For disposal of items contaminated with organisms or bodily fluids,
        1. Decontaminate slides, spectrophotometer tubes, and other glassware in 10% bleach.
        2. Dispose of soft items (i.e. gauze or swabs) in labeled biohazard containers.
        3. Dispose of all animal tissues in labeled biohazard containers.
2. Place micropipette tips, sample vials, and contaminated glassware in a labeled waste container or designated area.
3. See instructor for disposal of cadaver tissues.

Safety Pledge

I have read the Biology Laboratory Safety Guidelines and understand the rules and procedures of proper safety in the biology laboratory course listed below. I recognize my responsibility and pledge to observe all safety rules in the biology laboratory at all times.

I acknowledge that the instructor has covered all safety guidelines and equipment that we may need to use this semester.

Name of Student (please print)_____

Signature of Student _____

Date _____

Course _____ Section _____ Professor _____

Student copy

Safety Pledge

I have read the Biology Laboratory Safety Guidelines and understand the rules and procedures of proper safety in the biology laboratory course listed below. I recognize my responsibility and pledge to observe all safety rules in the biology laboratory at all times.

I acknowledge that the instructor has covered all safety guidelines and equipment that we may need to use this semester.

Name of Student (please print)_____

Signature of Student _____

Date _____

Course _____ Section _____ Professor _____

Instructor copy

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CHAPTER OVERVIEW

36: Appendix B - Brief Introduction to Statistics

36.1: Reading - Descriptive Statistics and Graphing

36.2: Reading - Statistical Analysis

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36.1: Reading - Descriptive Statistics and Graphing

A. How do you summarize data?

Data is summarized in two main ways: summary calculations and summary visualizations

Calculations: What types of measures are used?

To be able to interpret patterns in the data, raw data must first be manipulated and summarized into two categories of measurements: Measures of central tendency and measures of variability. These two categories of measurements encapsulate the first step of scientific inquiry, descriptive statistics.

Measures of central tendency (center) – Provides information of how data cluster around some single middle value. While there are several ways to measure central tendency, the two measures most often used in Ecology are the mean and the median:

- **Mean** (average) – Sum of all individual values (Σx_i) divided by total number of values in sample/population (n). This is the most commonly used measure of central tendency under symmetrical (normal) distribution. It is sensitive to outliers. The sample mean is usually denoted $\bar{x}$.
- **Median** – The middle value when the data set is ordered in sequential rank. This is commonly used when data is skewed and is resistant to outliers. Notice that if there is an even number of values, the median is calculated by adding the middle two values, and dividing by 2 :

  for an odd number of values, ranked smallest to largest: 1, 3, 3, **6**, 7, 8, 9. The median is **6**.

  for an even number of values, ranked smallest to largest: 1, 2, 3, **4, 5**, 6, 7, 9. The median is $(4 + 5) \div 2 \ = 4.5$.

- **Mode** – The most frequently occurring value in the sample. This is less often used in Ecology.

Measures of variability (spread) – Describes how spread out or dispersed the data are. There are two main measures of spread used in biological inquiry:

- **Range** – Quantifies the distance between the largest and smallest data values.
- **Standard deviation** – Quantifies the variation or dispersion from the average of a dataset. A low standard deviation indicates that the data tends to be very close to the mean; a high standard deviation indicates that the data points are spread out over a large range of values. This calculation is sensitive to outliers. The standard deviation of a sample is denoted with a lower case ***s*** and is calculated as follows:

$$s = \sqrt{\frac{(x_i - \bar{x})^2}{n-1}} \tag{36.1.1}$$

  Notice that the numerator in the formula for the standard deviation includes the term $(x_i - \bar{x})$ which measures how far each individual value (x_i) is from the sample mean $\bar{x}$. If the data are highly dispersed/variable, some of these values will be larger, and hence the standard deviation will be large.

- **Standard error (SE)** – Quantifies how different the sample mean (the average of the data values you have) is likely to be from the mean for the whole population mean (i.e., what you are trying to estimate). It tells you how much the sample mean would vary if you were to repeat a study using new samples from within a single population. The standard error is calculated by dividing the standard deviation by the square root of the sample size:

$$SE = \frac{s}{\sqrt{n}} \tag{36.1.2}$$

Visualizing the data: How are tables and graphs used?

After all desired descriptive statistics are calculated, they are typically visually summarized into either a table or graph.

Tables:

A **table** is a set of data values arranged into columns and rows. Typically the columns encompass a broad data category, and the rows encompass another. Within each broad category, there are subcategories that determine how many columns and rows the table

consists of. Tables are used to both collect and summarize data. However, most of the time when tables are presented, they consist of summarized data, not raw data. Although tables allow summarized data to be presented in an orderly manner, most people prefer to translate tables into the more powerful data visualization tool, a graph.

Graphs:

A **graph** is a diagram showing the relation between variable quantities, typically of two variables, each measured along one of a pair of axes at right angles. Graphs can look like a chart or drawing. Most graphs use bars, lines, or parts of a circle to display data. However, there are sometimes when graphs are overlaid on top of maps to also display geographical location, or are even animated to be interactive.

Major graph type categories:

- **Circle/Pie** – A circular chart divided into slices to illustrate numerical proportion. In a pie chart, the arc length of each slice (and consequently its central angle and area), is proportional to the quantity it represents. While it is named for its resemblance to a pie that has been sliced, there are variations on the way it can be presented.
- **Line** – A type of chart which displays information as a series of data points called 'markers' connected by straight line segments. It is a basic type of chart common in many fields. It is similar to a scatter plot except that the measurement points are ordered in sequence (typically by their x-axis value) and joined with straight line segments. A line chart is often used to visualize a trend in data over intervals of time – a time series – thus the line is often drawn chronologically.

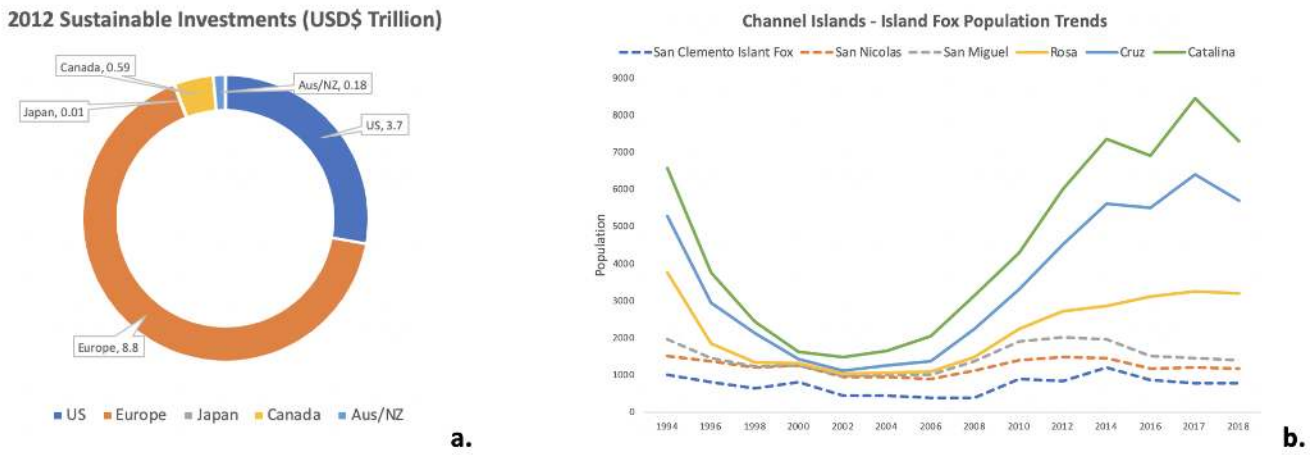


Figure 36.1.1: Examples of a circle/pie graph (a.) and a line graph (b.). Image created by Rachel Schleiger (CC-BY-NC).

- **Scatter plot** – Is a graph in which the values of two variables are plotted along the horizontal and vertical axes, the pattern of the resulting points revealing any correlation preset. The data are displayed as a collection of points, each having the value of one variable determining the position on the horizontal axis and the value of the other variable determining the position on the vertical axis.

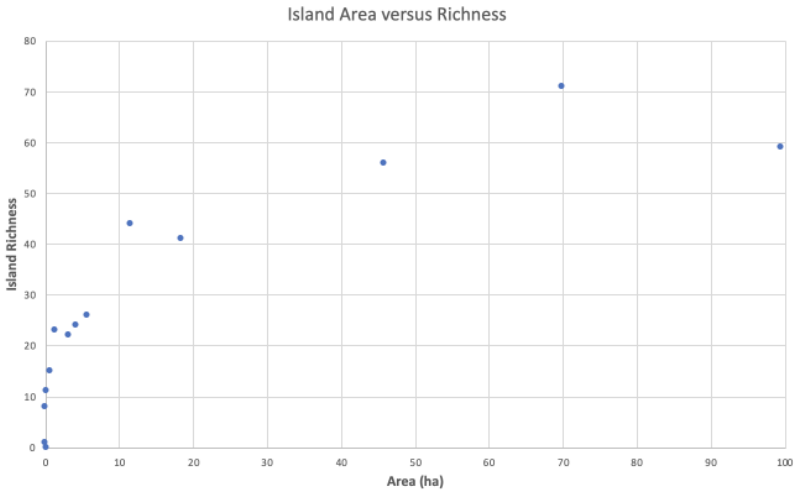


Figure 36.1.2: Example of a scatter plot. Image created by Rachel Schleiger (CC-BY-NC).

- **Bar** – A chart or graph that presents categorical data with rectangular bars with heights or lengths proportional to the values that they represent. The bars can be plotted vertically or horizontally.
- **Histogram** – Is an approximate representation of the distribution of numerical data. To construct a histogram, the first step is to "bin" (or "bucket") the range of values—that is, divide the entire range of values into a series of intervals—and then count how many values fall into each interval. The bins are usually specified as consecutive, non-overlapping intervals of a variable. The bins (intervals) must be adjacent (meaning there are not spaces between them like there are in bar graphs), and are often (but not required to be) of equal size. If the bins are of equal size, a rectangle is erected over the bin with height proportional to the frequency—the number of cases in each bin.

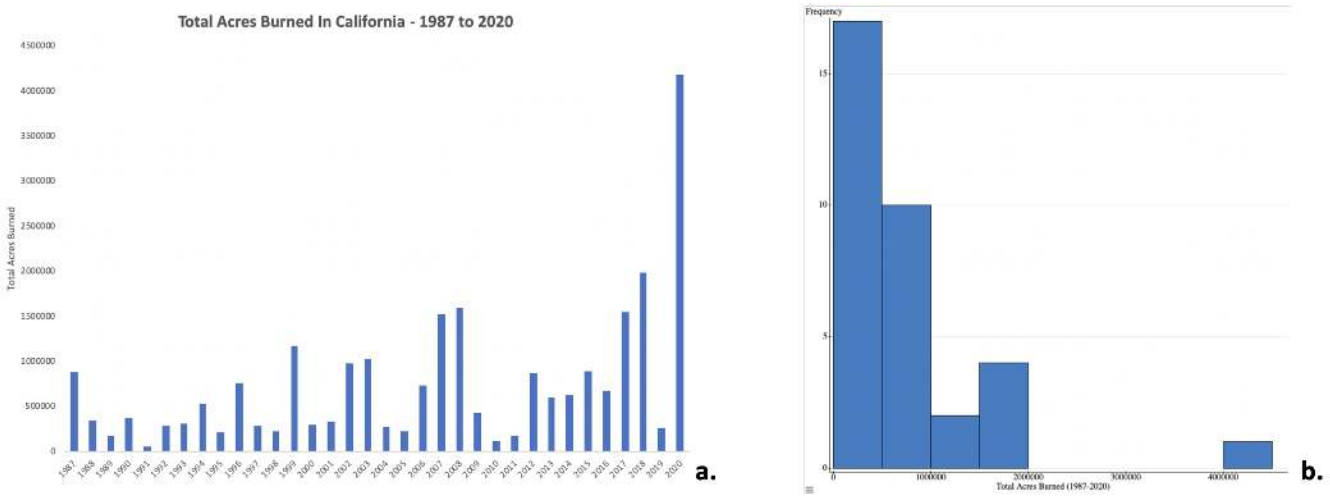


Figure 36.1.3: Examples of a bar graph (a.) and a histogram (b.) using the same dataset. Image created by Rachel Schleiger (CC-BY-NC).

Characteristics of effective graphical displays

> *The greatest value of a picture is when it forces us to notice what we never expected to see.*

Graphics *reveal* data. Indeed graphics can be more precise and revealing than conventional statistical computations."[3]

In his 1983 book *The Visual Display of Quantitative Information*, Edward Tufte defines 'graphical displays' and principles for effective graphical display in the following passage: "Excellence in statistical graphics consists of complex ideas communicated with clarity, precision, and efficiency.

Professor Tufte explained that users of information displays are executing particular *analytical tasks* such as making comparisons. The *design principle* of the information graphic should support the analytical task.[1] As William Cleveland and Robert McGill show, different graphical elements accomplish this more or less effectively. For example, dot plots and bar charts outperform pie charts.[12]

Graphical displays should:

- show the data
- induce the viewer to think about the substance rather than about methodology, graphic design, the technology of graphic production, or something else
- avoid distorting what the data has to say
- present many numbers in a small space
- make large data sets coherent
- encourage the eye to compare different pieces of data
- reveal the data at several levels of detail, from a broad overview to the fine structure
- serve a reasonably clear purpose: description, exploration, tabulation, or decoration
- be closely integrated with the statistical and verbal descriptions of a data set.

Additional Resource

Explore data visualizations at from data to viz.

1. *techatstate (7 August 2013). "Tech@State: Data Visualization - Keynote by Dr Edward Tufte". Archived from the original on 29 March 2017. Retrieved 29 November 2016 – via YouTube.*
2. *leveland, W. S.; McGill, R. (1985). "Graphical perception and graphical methods for analyzing scientific data". Science. **229** (4716): 828–33. Bibcode:1985Sci...229..828C. doi:10.1126/science.229.4716.828. PMID 17777913. S2CID 16342041.*
3. *Tufte, Edward (1983). The Visual Display of Quantitative Information. Cheshire, Connecticut: Graphics Press. ISBN 0-9613921-4-2. Archived from the original on 2013-01-14. Retrieved 2019-08-10.*

Attribution (Section A)

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B. What is a hypothesis and are there different kinds?

Biological (Scientific) hypothesis: An idea that proposes a tentative explanation about a phenomenon or a narrow set of phenomena observed in the natural world. This is the backbone of all scientific inquiry! As such it is important to have a solid biological hypothesis before moving forward in the scientific method (i.e. procedures, results, discussion). After the creation of a solid biological hypothesis, it can then be simplified into a statistical hypothesis (as defined below) that will become the basis for how the data will be analyzed and interpreted.

Statistical hypotheses: After defining a strong biological hypothesis, a statistical hypothesis can be created based on what you will predict will be the measured outcome(s) (dependent variable(s)). If a study has multiple measured outcomes there can be multiple statistical hypotheses. Each statistical hypothesis will have two components (Null and Alternative).

- **Null hypothesis** (Ho) –This hypothesis states that there is no relationship (or no pattern) between the independent and dependent variables.
- **Alternative hypothesis** (H1) – This hypothesis states that there is a relationship (or is a pattern) between the independent and dependent variables.

Independent versus dependent variables: For both biological and statistical hypotheses there should be two basic variables defined:

- **Independent (explanatory) variable** – It is usually what phenomena you think will affect the measure you are interested in (dependent variable).
- **Dependent (response) variable** – A dependent variable is what you measure in the experiment and what is affected during the experiment. The dependent variable responds to (depends on) the independent variable. In a scientific experiment, you cannot have a dependent variable without an independent variable.

Example

Yellow-billed Cuckoo nests were counted during breeding season in degraded, restored, and intact riparian habitats to see overall habitat preference for nesting sites increased with habitat health.

- Scientific hypothesis: Yellow-billed Cuckoo will have habitat preferences because of habitat health/status.
- Statistical hypotheses: (Ho) There will be no differences in number of nests between habitats with different health/status. (H1) There will be more nests in restored and intact habitats compared to degraded.
- Independent variable = Habitat health/status
- Dependent variable = Number of nests counted

C. How do you reach conclusions?

Finally, after defining the biological hypothesis, statistical hypothesis, and collecting all your data, a researcher can begin statistical analysis. A statistical test will mathematically "test" your data against the statistical hypothesis. The type of statistical test that is used depends on the type and quantity of variables in the study, as well as the question the researcher wants to ask. After computing the statistical test, the outcome will indicate which statistical hypothesis is more likely. This, in turn indicates to scientists what level of inference can be gained from the data compared to the biological hypothesis (the focus point of the study). Then a conclusion can be made based on the sample about the entire population. It is important to note that the process does not stop here. Scientists will want to continue to test this conclusion until a clear pattern emerges (or not) or to investigate similar but different questions.

D. Types of Basic Statistical Tests

Inferential statistics generally provide a test statistic, the degrees of freedom (related to the number of individuals in each sample) and a p-value. Significance (acceptance of the alternative hypothesis) is generally based on the p-value. Depending on the field, scientists will often use a cut-off of 0.01 or 0.05 to determine significance. If the test returns a p-value that is less than this value, the relationship is deemed significant.

- **Chi-Square – Are two categorical variables related?**
  - (e.g. do different habitats different in the numbers of species of each type?)
- **T-Test – Does the mean (continuous data) of one group (a categorical variable) differ from the mean of another group?**
  - (e.g. are oak trees taller than hickory trees, on average?)
- **ANOVA – Does the mean of several groups differ? A post-hoc test is used to run pairwise comparisons if so**
  - (e.g. does height differ across tree species, on average?)
- **Linear regression - Are two continuous variables linearly related?**
  - (e.g. do taller trees have a larger circumference?)

E. The "Magic" level of Significance

If $p \leq 0.05$ – accept alternative hypothesis

- There is less than 5% chance that the samples are from the same population
- There is a significant difference between the samples

If $p > 0.05$ – accept null hypothesis

- There is no significant difference between the samples

Attribution (Sections B - E)

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36.2: Reading - Statistical Analysis

A. Statistical Analysis - The t-test

Suppose that a researcher wishes to test if a certain kind of growth hormone will produce faster growth in mice. She injects 10 mice with the hormone and uses another 10 as a control. Three weeks later, she weighs the mice and discovers that the mean weight of mice that have received the injections is 12.05 g and the mean weight of control mice is 9.3 g. These values indicate that the mice receiving the hormone are heavier. Is her value of 12.05 significantly different than 9.3? Is it possible that the hormone has no effect, that the weight difference between the two groups is due to chance? This is like flipping a coin 10 times. You expect 5 heads and 5 tails but you might get 6 heads or 7 heads or perhaps 8 heads. Similarly, if the hormone does not work, you expect the mean for the two groups to be similar but it may not be exactly the same.

Mice Weight (grams) with and Without Hormone

Group 1 - Hormone - Weight (grams)	Group 2 – No Hormone - Weight (grams)
12.5	12
13	8.5
12	10
12	8
13	8
14	13.5
13	9
10.5	8.5
9.5	6.5
11	9
Mean = 12.05	Mean = 9.3

What is the chance that the two means would be as different as 12.05g and 9.3g if the hormone really did not work? Statistical tests test whether differences in the data are real differences or whether they are due to chance. In the example above, we test if the mean of group 1 is significantly different than the mean of group 2. The alternative is that the difference is due to chance or random fluctuations and the hormone did not cause additional weight gain. The test gives the probability that difference could be due to chance. If the probability that the difference is due to chance is less than 1 out of 20 (<0.05), then we conclude that the difference is real. If the probability is greater than 0.05, we conclude that the difference is not significant, it could be due to chance.

There are several tests available for testing means. A commonly used test for data that are normally distributed is the t-test.

Sara's Hypothesis is that newborn mice injected with the hormone will be heavier after 3 weeks of growth than mice without the hormone.

The calculations for the test can be performed by hand, but computer software can do them very quickly. To perform the test, the weight data for the two groups of mice above are entered into a t-test program.

The software reveals that $p = 0.0012$. The probability that the difference between the two means (12.05 and 9.3) is due to chance (random effects) is 0.0012 (or 12 out of 10,000). Because $p < 0.05$, we conclude that the two means are really different and that the difference is not due to chance. The researcher accepts her hypothesis that the hormone produces faster growth. If p had been greater than 0.05, we would reject her hypothesis and conclude that the two means are not significantly different; the hormone did not cause one group to be heavier.

The word "significant" has a slightly different meaning in statistics than it does in general usage. In a statistical test of two means, if the difference is not due to chance, we conclude that the two means are significantly different. In the example above, the mean weight of group 1 is significantly heavier than the mean weight of group 2.

Number of Tails

The number of tails in a test refers to the number of ways that the two groups can differ. The following hypothesis would lead us to perform a two-tailed test:

The mean weight of mice injected with the hormone will be ***different than*** the mean weight of the control mice.

This is two-tailed because the hypothesis proposes two possible outcomes. The hypothesis is true if the weight hormone mice is greater than the weight of control mice. The hypothesis is also true if the weight of hormone mice is less than the weight of control mice.

The following hypothesis would lead us to perform a one-tailed test:

The mean weight of mice injected with the hormone will be ***greater than*** the mean weight of the control mice.

The following hypothesis would lead us to perform a one-tailed test.

The mean weight of mice injected with the hormone will be ***less than*** the mean weight of the control mice.

This is a one-tailed test because the hypothesis proposes that there is only one possible outcome: the weight of the hormone mice will be less than the weight of the control mice.

Example 36.2.1: One-tailed test

A researcher wishes to learn if a certain drug slows the growth of tumors. She obtained mice with tumors and randomly divided them into two groups. She then injected one group of mice with the drug and used the second group as a control. After 2 weeks, she sacrificed the mice and weighed the tumors. The weight of tumors for each group of mice is below.

The researcher is interested in learning if the drug reduces the growth of tumors. Her hypothesis is: The mean weight of tumors from mice in group A will be less than the mean weight of mice in group 2.

Tumor Weight of Group A vs. Group B Mice

	Group A Treated with Drug	**Group B Control- Not Treated**
	0.72	0.71
	0.68	0.83
	0.69	0.89
	0.66	0.57
	0.57	0.68
	0.66	0.74
	0.70	0.75
	0.63	0.67
	0.71	0.80
	0.73	0.78
Mean =	**` 0.675**	**0.742**

A t-test can be used to test the probability that the two means do not differ. The alternative is that tumors from the group treated with the drug will not weigh less than tumors from the control group. This is a one-tailed test because the researcher is interested in if the drug ***decreased*** tumor size. She is not interested in if the drug ***changed*** tumor size.

The calculations involved in doing a t-test will not be discussed in this course but this is often covered in introductory statistics courses. A spreadsheet has been prepared to perform these calculations. The values from the table above are entered into the spreadsheet as shown below.

t-test				
Enter tails:	2	Enter 1 for a 1-tailed test. Enter 2 for a 2-tailed test.		
Enter type:	2	1 = paired test; 2 = equal variances; 3 = unequal variances		
Enter data in yellow area below:			P =	0.113499
Group 1	Group 2		t =	1.663627
42	43		Degrees of Freedom =	18.000000
45	51			
40	56		Mean (Group 1) =	43.5
37	40		Std. Deviation (Group 1) =	3.979112
41	32			
41	54		Mean Group 2) =	48
48	51		Std. Deviation (Group 2) =	7.571878
50	55			
45	50			
46	48			

The t-test shows that tumors from the drug group were significantly smaller than the tumors from the control group because $p < 0.05$. The researcher therefore accepts her hypothesis that the drug reduces the growth of tumors.

Example 36.2.2: two-tailed test

A researcher wishes to learn whether the pH of soil affects seed germination of a particular herb found in forests near her home. She filled 10 flower pots with acid soil (pH 5.5) and ten flower pots with neutral soil (pH 7.0) and planted 100 seeds in each pot. The mean number of seeds that germinated in each type of soil is below.

Number of Germinated Seeds: Acid vs. Neutral Soil

	Acid Soil pH 5.5	**Neutral Soil pH 7.0**
	42	43
	45	51
	40	56
	37	40
	41	32
	41	54
	48	51
	50	55
	45	50
	46	48
Mean =	**43.5**	**48**

The researcher is testing whether soil pH affects germination of the herb. Her hypothesis is: The mean germination at pH 5.5 is different than the mean germination at pH 7.0.

A t-test can be used to test the probability that the two means do not differ. The alternative is that the means differ; one of them is greater than the other. This is a two-tailed test because the researcher is interested in if soil acidity ***changes*** germination percentage. She does not specify if it increases or decreases germination. Notice that a 2 is entered for the number of tails below.

t-test				
Enter tails:	2	Enter 1 for a 1-tailed test. Enter 2 for a 2-tailed test.		
Enter type:	2	1 = paired test; 2 = equal variances; 3 = unequal variances		
Enter data in yellow area below:			P =	0.113499
Group 1	Group 2		t =	1.663627
42	43	Degrees of Freedom =		18.000000
45	51			
40	56	Mean (Group 1) =		43.5
37	40	Std. Deviation (Group 1) =		3.979112
41	32			
41	54	Mean Group 2) =		48
48	51	Std. Deviation (Group 2) =		7.571878
50	55			
45	50			
46	48			

The t-test shows that the mean germination of the two groups does not differ significantly because $p > 0.05$. The researcher concludes that pH does not affect germination of the herb.

Example 36.2.3

Suppose that a researcher wished to learn if a particular chemical is toxic to a certain species of beetle. She believes that the chemical might interfere with the beetle's reproduction. She obtained beetles and divided them into two groups. She then fed one group of beetles with the chemical and used the second group as a control. After 2 weeks, she counted the number of eggs produced by each beetle in each group. The mean egg count for each group of beetles is below.

Egg Count for Group 1 vs. Group 2 Beetles

	Group 1 fed chemical	**Group 2 not fed chemical (control)**
	33	35
	31	42
	34	43
	38	41
	32	
	28	
Mean =	**32.7**	**40.3**

The researcher believes that the chemical interferes with beetle reproduction. She suspects that the chemical reduces egg production. Her hypothesis is: The mean number of eggs in group 1 is less than the mean number of group 2.

A t-test(opens in new window) can be used to test the probability that the two means do not differ. The alternative is that the mean of group 1 is greater than the mean of group 2. This is a 1-tailed test because her hypothesis proposes that group B will have greater reproduction than group 1. If she had proposed that the two groups would have different reproduction but was not sure which group would be greater, then it would be a 2-tailed test. Notice that a 1 is entered for the number of tails below.

The results of her t-test are copied below.

t-test				
Enter tails:	1	Enter 1 for a 1-tailed test. Enter 2 for a 2-tailed test.		
Enter type:	2	1 = paired test; 2 = equal variances; 3 = unequal variances		
Enter data in yellow area below:			P =	0.004506
Group 1	Group 2		t =	3.42574
33	35	Degrees of Freedom =		8.000000
31	42			
34	43	Mean (Group 1) =		32.66667
38	41	Std. Deviation (Group 1) =		3.32666
32				
28		Mean Group 2) =		40.25
		Std. Deviation (Group 2) =		3.593976

The researcher concludes that the mean of group 1 is significantly less than the mean for group 2 because the value of $P < 0.05$. She accepts her hypothesis that the chemical reduces egg production because group 1 had significantly less eggs than the control.

Contributors and Attributions

- Michael J. Gregory, Ph.D. (Clinton Community College)

B. Statistical Analysis - The Chi-Square Test

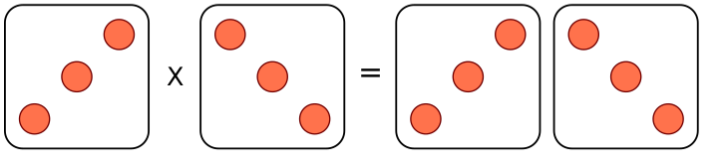
Mendel's Observations

Parental Cross	F_1 Phenotypes	F_2 Phenotypes	F_2 Ratios
Round x Wrinkled Seeds	All Round	5474 Round: 1850 Wrinkled	2.96:1
Yellow x Green Seeds	All Yellow	6022 Yellow: 2001 Green	3.01:1
Purple x White Flowers	All Purple	705 Purple: 224 White	3.15:1
Tall x Dwarf Plants	All Tall	787 Tall: 227 Dwarf	2.84:1

Probability: Past Punnett Squares

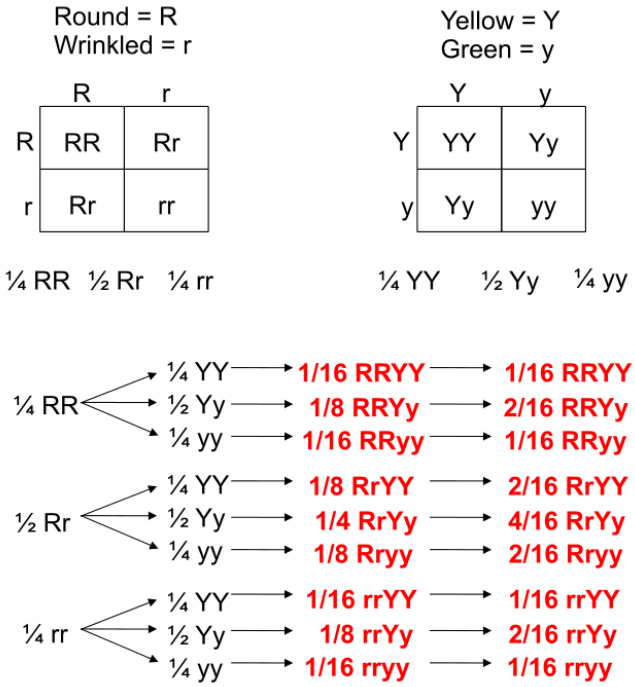
Punnett Squares are convenient for predicting the outcome of monohybrid or dihybrid crosses. The expectation of two heterozygous parents is 3:1 in a single trait cross or 9:3:3:1 in a two-trait cross. Performing a three or four trait cross becomes very messy. In these instances, it is better to follow the rules of probability. **Probability** is the chance that an event will occur expressed as a fraction or percentage. In the case of a monohybrid cross, 3:1 ratio means that there is a $\frac{3}{4}$ (0.75) chance of the dominant phenotype with a $\frac{1}{4}$ (0.25) chance of a recessive phenotype.

$$1/6 \text{ X } 1/6 = 1/36$$



A single die has a 1 in 6 chance of being a specific value. In this case, there is a $\frac{1}{6}$ probability of rolling a 3. It is understood that rolling a second die simultaneously is not influenced by the first and is therefore independent. This second die also has a $\frac{1}{6}$ chance of being a 3.

We can understand these rules of probability by applying them to the dihybrid cross and realizing we come to the same outcome as the 2 monohybrid Punnett Squares as with the single dihybrid Punnett Square.



This forked line method of calculating probability of offspring with various genotypes and phenotypes can be scaled and applied to more characteristics.

The Chi-Square Test

The χ^2 statistic is used in genetics to illustrate if there are deviations from the expected outcomes of the alleles in a population. The general assumption of any statistical test is that there are no significant deviations between the measured results and the predicted ones. This lack of deviation is called the **null hypothesis** ($\mathbf{H_0}$). X^2 statistic uses a distribution table to compare results against at varying levels of probabilities or **critical values**. If the X^2 value is greater than the value at a specific probability, then the null hypothesis has been rejected and a significant deviation from predicted values was observed. Using Mendel's laws, we can count phenotypes after a cross to compare against those predicted by probabilities (or a Punnett Square).

Critical values for χ^2 test

p-val / DF	0.1	0.05	0.01	0.005
1	2.706	3.84	6.64	7.88
2	4.605	5.99	9.21	10.60
3	6.251	7.82	11.35	12.94
4	7.779	9.49	13.28	14.86
5	9.236	11.07	15.09	16.75
6	10.65	12.59	16.81	18.55
7	12.02	14.07	18.48	20.28
8	13.36	15.51	20.09	21.96
9	14.68	16.92	21.67	23.59
10	15.99	18.31	23.21	25.19

In order to use the table, one must determine the stringency of the test. The lower the p-value, the more stringent the statistics. **Degrees of Freedom** (**DF**) are also calculated to determine which value on the table to use. Degrees of Freedom is the number of classes or categories there are in the observations minus 1. DF=n-1

In the example of corn kernel color and texture, there are 4 classes: Purple & Smooth, Purple & Wrinkled, Yellow & Smooth, Yellow & Wrinkled. Therefore, DF = 4 – 1 = 3 and choosing $p < 0.05$ to be the threshold for significance (rejection of the null hypothesis), the X^2 must be greater than 7.82 in order to be significantly deviating from what is expected. With this dihybrid cross example, we expect a ratio of 9:3:3:1 in phenotypes where 1/16th of the population are recessive for both texture and color while $\frac{9}{16}$ of the population display both color and texture as the dominant. $\frac{3}{16}$ will be dominant for one phenotype while recessive for the other and the remaining $\frac{3}{16}$ will be the opposite combination.

With this in mind, we can predict or have expected outcomes using these ratios. Taking a total count of 200 events in a population, 9/16(200)=112.5 and so forth. Formally, the χ^2 value is generated by summing all combinations of:

$$\frac{(Observed - Expected)^2}{Expected} \tag{36.2.1}$$

Chi-Square Test: Is This Coin Fair or Weighted? (Activity)

1. Everyone in the class should flip a coin 2x and record the result (assumes class is 24).
2. Fair coins are expected to land 50% heads and 50% tails.
   - 50% of 48 results should be 24.
   - 24 heads and 24 tails are already written in the “Expected” column.
3. As a class, compile the results in the “Observed” column (total of 48 coin flips).
4. In the last column, subtract the expected heads from the observed heads and square it, then divide by the number of expected heads.
5. In the last column, subtract the expected tails from the observed tails and square it, then divide by the number of expected tails.
6. Add the values together from the last column to generate the X^2 value.
7. Compare the value with the value at 0.05 with DF=1.
   - There are 2 classes or categories (head or tail), so DF = 2 – 1 = 1.
   - Were the coin flips fair (not significantly deviating from 50:50)?

Heads or Tails	Expected Number	Observed Number	$\frac{(\text{Observed-Expected})^2}{\text{Expected}}$
Heads	24		
Tails	24		
	$\chi^2 = \sum \frac{(\text{Observed-Expected})^2}{\text{Expected}} \rightarrow$		

Let’s say that the coin tosses yielded 26 Heads and 22 Tails. Can we assume that the coin was unfair? If we toss a coin an odd number of times (eg. 51), then we would expect that the results would yield 25.5 (50%) Heads and 25.5 (50%) Tails. But this isn’t a possibility. This is when the X^2 test is important as it delineates whether 26:25 or 30:21 etc. are within the probability for a fair coin.

Chi-Square Test of Kernel Coloration and Texture in an F_2 Population (Activity)

1. From the counts, one can assume which phenotypes are dominant and recessive.
2. Fill in the “Observed” category with the appropriate counts.
3. Fill in the “Expected Ratio” with either 9/16, 3/16 or 1/16.
4. The total number of the counted event was 200, so multiply the “Expected Ratio” x 200 to generate the “Expected Number” fields.

5. Calculate the $\frac{(Observed-Expected)^2}{Expected}$ for each phenotype combination
6. Add all $\frac{(Observed-Expected)^2}{Expected}$ values together to generate the X^2 value and compare with the value on the table where DF=3.
7. Do we reject the Null Hypothesis or were the observed numbers as we expected as roughly 9:3:3:1?
    - What would it mean if the Null Hypothesis was rejected? Can you explain a case in which we have observed values that are significantly altered from what is expected?

Phenotype	Expected Ratio (9/16, 3/16,1/16)	Expected Number (Total # x Ratio)	Observed Number	$\frac{(Observed-Expected)^2}{Expected}$
Purple & Smooth				
Purple & Wrinkly				
Yellow & Smooth				
Yellow & Wrinkly				
		$\chi^2 = \sum \frac{(Observed-Expected)^2}{Expected} \rightarrow$		

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CHAPTER OVERVIEW

37: Appendix C- Sample Lab Report

37.1: Rubric

37.2: Sample Lab Report- Sugar Size and Diffusion Through a Mock-Cell Membrane

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37.1: Rubric

The following rubric will be used to grade the lab reports:

	Excellent 5 points	Satisfactory 2.5 points	Unsatisfactory 0 points
Title Page	Contains title, student name, instructor name and section	Missing either instructor name or section	No title page
Formatting: typed, spacing	Typed and single spaced	Typed, but not single spaced	Not typed
Grammar and Spelling	No errors; contains complete sentences and no misspellings	A few minor errors in grammar and spelling	Several major errors in grammar and spelling
Formatting: headings	Each section has a heading as described in template	Some sections lack headings	No headings
Hypothesis	Predictions are clearly stated and written as a testable statement	Predictions/expected outcomes are not clearly stated	Not written as a testable statement
Materials	All equipment and materials described; identify variables, controls and constants	Materials incompletely described	No identification of variables, controls and constants
Procedure	Clear step-by step description	Description missing details making it difficult for another scientist to repeat experiment	Description missing so much detail it would be impossible to repeat
Results	Clearly written description of results comparing controls and variables	Results are presented but no comparison between controls and variables are made	No written description of results
Data tables, graphs or charts	Easy to interpret, clear labels, all data, including calculated averages, included	Disorganized (not easy to understand, missing labels) but **all** data included	Disorganized and or data clearly missing
Conclusion	Clearly explains acceptance or rejection of hypothesis using data to support conclusion; identifies sources of error	Accepts or rejects hypothesis but does not use data to explain why; or does not identify sources of error	Does not explain conclusion and does not identify sources of error
Total _____out of 50 points			

LICENSES AND ATTRIBUTIONS

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37.2: Sample Lab Report- Sugar Size and Diffusion Through a Mock-Cell Membrane

Introduction

Diffusion is the process in which a substance moves from an area of high concentration to an area of lower concentration. It is important for membranes to be semi-permeable. If membranes were universally permeable, they would not legitimately serve their purpose as membranes; certain substances need to be kept out of a cell, and others kept in. If membranes were not at all permeable, there would be no interface between the cell and its environment – effectively starving the cell. Membranes, being selectively permeable, allow in nutrients and other necessary substances, and also provide for the purging of cell waste.

This experiment investigates the permeability of cell membranes to various types of sugars: polysaccharides, disaccharides, and polysaccharides. Dialysis tubing is used to simulate a cell membrane; it is permeable to small molecules and water, but not to larger molecules.

Given the generally larger size of polysaccharides, it is hypothesized that starch will not pass through the dialysis tubing, and that iodine will pass through the membrane due to the small size of its molecules. Based on the trouble that some people have digesting lactose, it is predicted that it is a polysaccharide or disaccharide and will yield diffusion results similar to starch.

Methods

I) Permeability of cell model to starch

The 100ml graduated cylinder was used to measure out 50ml of tap water; the water was poured into a 250ml beaker. One teaspoon of corn starch was added to the beaker and stirred with a spoon. A 50ml beaker was filled with 50ml of tap water. A piece of dialysis tubing was placed into the beaker of water until it became soft and pliable. The tubing was then extracted from the beaker; one end was tied closed with dental floss, using a double-knot. The other end of the dialysis tubing was opened; a pipette was used to fill the dialysis tubing with the starch solution. Another piece of dental floss was used to tie the end of the dialysis tubing closed. A second 250ml beaker was filled halfway with tap water. Another pipette was used to add 15 drops of iodine to the beaker; the solution was mixed with a spoon. The filled dialysis tube was placed into the 250ml beaker so that the cornstarch mixture was submerged in the iodine water mixture. After 15 minutes had passed, results were observed and recorded.

II) Permeability of cell model to lactose

Part I: Determining the type of sugar being tested

The 100ml graduated cylinder was used to measure out 100ml of tap water; the water was poured into a 250ml beaker. Two teaspoons of the lactose was added to the water, and the solution was stirred with a spoon for thorough mixing. 50ml of the resultant solution was measured out using the 100ml graduated cylinder, and was reserved for Part II.

Twenty drops of Benedict's reagent were placed in a clean, empty test tube. Twenty drops of the lactose solution were added to the same test tube, and the solution was heated in a boiling water bath for 2 minutes. The results were then interpreted.

Twenty drops of Barfoed's reagent were placed in a clean, empty test tube. Twenty drops of lactose solution were added to the same test tube. The resultant solution was headed in a boiling water bath for 3.5 minutes. The results were then interpreted.

Part II: Permeability of cell model

This method is based on the premise of the unknown sugar being a disaccharide.

A 50ml beaker was filled with 50 ml tap water. A piece of dialysis tubing was placed in the beaker of water and left to soak until it became soft and pliable. The dialysis tubing was then removed from the beaker, and one end tied closed with a double-knotted piece of dental floss. The other end of the dialysis tubing was opened. The tubing was filled with lactose solution (set aside from Part I); a pipette was used to transfer the solution from the graduated cylinder to the tubing. A second piece of dental floss was used to tie the other end of the dialysis tubing closed. A second 250ml beaker was filled halfway full with tap water.

15 drops of iodine were added to the tap water in the beaker. The resulting solution was swirled with a spoon to mix it; the colors of the baggie solution and the beaker solution were noted. The dialysis tubing baggie was placed in the 250ml beaker so that the

lactose solution was submerged in the beaker solution, and left to sit undisturbed for 15 minutes. The color of the baggie solution was noted.

The baggie was removed from the beaker and samples of the beaker solution were transferred to separate, appropriately marked test tubes. 20 drops of Bendict's reagent were added to one test tube, and the tube heated for 2 minutes. The color of the resulting solution was noted. 20 drops of Barfoed's reagent were added to the second test tube, and the tube heated for 3.5 minutes. The color of the resulting solution was noted.

Results

Table 1: Starch experiment results

	Solution in baggie	Solution in Beaker
Starting color	Murky white	Clear yellow
Color after 15 minutes	Dark purple	yellow

In the starch experiment as seen in Table 1, the starch solution inside of the dialysis baggie was initially a murky white color. The solution in the beaker, external to the baggie was a clear yellow color. After 15 minutes of submersion in the beaker solution, the baggie had turned a dark purple color. The beaker solution remained clear and yellow.

In Part I of the lactose experiment, the lactose solution was initially a dark brown color. Benedict's reagent is pale blue in color. Lactose, mixed and heated with the Benedict's reagent, yielded a solution of a murky yellow-brown color. Barfoed's reagent, like Benedict's reagent, is pale blue in color. Lactose, mixed and heated with the Barfoed's reagent, yielded a pale blue solution.

Table 2: Lactose experiment results

	Solution in baggie	Solution in Beaker
Starting color	brown	yellow
Color after 15 minutes	yellow	(? Not given)

In Part II of the lactose experiment, as seen in Table 2, the lactose solution inside of the dialysis baggie was initially dark brown in coloration. The iodine and water solution in the beaker was a clear yellow color. A Benedict's test on the beaker solution after the experiment yielded a dark brown liquid; a Barfoed's test on the beaker solution after the experiment resulted in a clear blue liquid.

Discussion

Permeability of cell model to starch

The starch solution inside of the dialysis baggie went from a murky white color to dark purple; iodine from the beaker solution must have diffused into the dialysis baggie, reacting with the starch solution and producing the "positive" dark-purple result, confirming the presence of a polysaccharide inside of the baggie. The beaker solution remained a clear yellow color throughout the experiment; it can hence be inferred that no polysaccharide was present in the beaker solution at the end of the experiment, and in turn, that no starch diffused out of the baggie and into the beaker solution during the 15-minute soaking.

The experimental hypothesis for this section was correct; starch was unable to diffuse through the cell model, however, iodine was able to diffuse through the cell model. The discrepancy in permeability is due to the difference in the sizes of iodine and starch molecules.

Permeability of cell model to lactose

The Benedict's test control on lactose yielded a solution that was a murky yellow-brown color; this indicated the presence of a mono- or di- saccharide. The Barfoed's test control on lactose yielded a solution that was pale blue in color, without any red precipitate; this indicates that no monosaccharaides were present, and in turn, that lactose is a disaccharide. The solution inside of the dialysis tubing changed color in the course of the experiment; this implies that iodine diffused into the dialysis tubing and

reacted with the lactose solution. The resulting clear yellow color indicates that there were no polysaccharides present inside of the dialysis tubing.

A negative Benedict's test is of blue coloration; a test on the beaker solution after the experiment is a very dark red-orange-brown color that looks similar to the original lactose in the tubing. A Barfoed's test on the post-experiment beaker solution was a clear light blue; no monosaccharaides diffused out into the beaker solution, but this result was irrelevant. The Benedict's test revealed that lactose was able to diffuse out of the dialysis baggie, into the beaker solution. If the cell model is reliable, it appears that lactose is able to diffuse in and out of cells.

The experimental hypothesis for this section appears to have been wrong; the cell model was permeable to lactose.

Overall, the cell model has demonstrated impermeability to large molecules such as polysaccharides, and permeability to smaller molecules such as disaccharides and iodine molecules. Since the model was permeable to a disaccharide, it would be reasonable to infer that the model will be permeable to monosaccharaides, as they are even smaller in size than disaccharides. Further testing with a variety of disaccharides should be done, to determine whether lactose is unique or whether the cell model is permeable to all disaccharides.

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