Lab Manual for Biology Part I

LAB MANUAL FOR BIOLOGY PART I

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PREFACE

The Creation of This Book

This textbook was created as part of the Interactive OER for Dual Enrollment project, facilitated by LOUIS: The Louisiana Library Network and funded by a $2 million Open Textbooks Pilot Program grant from the Department of Education.

This project supports the extension of access to high-quality post-secondary opportunities to high school students across Louisiana and beyond by creating materials that can be adopted for dual enrollment environments. Dual enrollment is the opportunity for a student to be enrolled in high school and college at the same time.

The cohort-developed OER course materials are released under a license that permits their free use, reuse, modification and sharing with others. This includes a corresponding course available in MoodleNet and Canvas Commons that aligns with the lecture textbook and that can be imported to other Learning Management System platforms. For access/questions, contact Affordable Learning Louisiana.

If you are adopting this textbook, we would be glad to know of your use via this brief survey.

Adaptation Statement

"Lab Manual for Biology Part I" has been revised and remixed for the LOUIS: The Louisiana Library Network and Open Textbooks Pilot Program grant from the Department of Education by Stephanie Aamodt, Jennifer Blanchard, Ruby Broadway, Hope Clay, Christian Clement, Waneene Dorsey, Sarah Hunter, Illya Tietzel, and Peter Yaukey, with editors Emily Frank, Elizabeth Joan Kelly, and Maletta Payne. This work retains the original Creative Commons license set forth at the chapter level by the original authors, unless otherwise noted in the text.

This book is an adaptation of multiple sources:

- The OpenStax College Biology for AP Courses Lab Manual Student Version. This text is licensed as CC BY 4.0.
- The OpenLab at City Tech's NYCCT General Biology 1 Lab. This text is licensed as CC BY-NC-SA 4.0.
- Foundations of Biology Lab Manual (Georgia Highlands College) by Jacqueline Belwood, Brandy Rogers, Jason Christian. This text is licensed as CC BY 4.0.

We would like to wholeheartedly thank these authors for their tremendous work in this area of open educational resources.

The following changes were made to this book as a whole:

- Some chapters were removed and all chapter numbers were updated to make this a concise resource to align with Biology I Lecture and Lab for Science Majors.
- Minor text edits for grammar, punctuation, and clarification were made throughout.
- Minor edits involving fonts, spacing, and other formatting
- Added Key Terms sections.

The following chapter sections were updated to address specific topics:

- Lab 1 Lab Safety, Scientific Method, and Measurements: adapted from OpenStax Lab 1 Lab Safety, Scientific Method, and Measurements. Separated Lab Safety into standalone lab (Hope Clay)
- Lab 2 Introduction to Microscopy: merged OpenStax Lab 2: Introduction to Microscopy with OpenLab Microscope: Basics; Scientific Method; Reporting in Science (Waneene Dorsey)
- Lab 3 Macromolecules in Common Food: adapted from OpenStax Lab 3: Macromolecules in Common Food (Peter Yaukey)
- Lab 4 Prokaryotic and Eukaryotic Cells: adapted from OpenStax Lab 4: Prokaryotic and Eukaryotic Cells (Sarah Hunter)
- Lab 5 Subcellular Structures: adapted from OpenStax Lab 5: Subcellular Structures
- Lab 6 Diffusion and Osmosis: adapted from OpenStax Lab 6: Diffusion and Osmosis with OpenLab Week 7: Membranes and Biological Transport: Diffusion and Osmosis (Waneene Dorsey)
- Lab 7 Factors Affecting the Enzymatic Activity of Lactase: adapted from OpenStax Lab 7: Affecting the Enzymatic Activity of Lactase (Ruby Broadway)
- Lab 8 Cellular Respiration: adapted from OpenStax Lab 8: Cellular Respiration (Stephanie Aamodt)
- Lab 9 Fermentation in Yeast: adapted from OpenStax Lab 9: Fermentation in Yeast (Jennifer Blanchard)
- Lab 10 Plant Pigments: merged OpenStax Lab 10: Plant Pigments with OpenLab Week 2: Chromatography
- Lab 11 The Light Reaction of Photosynthesis: adapted from Openstax Lab 11: The Light Reaction of Photosynthesis
- Lab 12 Mitosis and Meiosis: merged Openstax Lab 13: Mitosis and Meiosis with OpenLab Week 11: Cell Division (Christian Clement)
- Lab 13 Mendelian Genetics: merged OpenStax Lab 14: Mendelian Genetics with OpenLab Week 12: Genetics (Illya Tietzel)

- Lab 14 Plant Transpiration: adapted from OpenStax Lab 19: Plant Transpiration
- Lab 15 Strawberry DNA: adapted from Foundations of Biology Lab Manual LAB ACTIVITY: DNA EXTRACTION FROM STRAWBERRIES (Ruby Broadway)

Review Statement

This textbook and its accompanying course materials went through at least two review processes:

- Peer reviewers, coordinated by Jared Eusea, River Parish Community College, used an online course development standard rubric for assessing the quality and content of each course to ensure that the courses developed through Interactive OER for Dual Enrollment support online learners in that environment. The evaluation framework reflects a commitment to accessibility and usability for all learners.
    - Reviewers
        - Andrea Leonard
        - Francesca Mellieon-Williams
        - Esperanza Zenon
        - Iris Henry
- The Institute for the Study of Knowledge Management in Education (ISKME) collaborated with LOUIS to review course materials and ensure their appropriateness for dual enrollment audiences. Review criteria were drawn from factors that apply across dual enrollment courses and subject areas, such as determining appropriate reading levels, assessing the fit of topics and examples for high school DE students; applying high-level principles for quality curriculum design, including designing for accessibility, appropriate student knowledge checks, and effective scaffolding of student tasks and prior knowledge requirements, addressing adaptability and open educational practices, and principles related to inclusion and representational social justice.
    - Reviewers
        - Jennifer Simon
        - Latoya T Paul
        - Emily Jackson-Osagie

SAFETY CONTRACT

1. Do not:
    a. be late for class. You will miss important instructions.
    b. eat or drink in the laboratory
    c. ingest any reagents or chemicals used in the laboratory
    d. pour chemicals down the sink unless instructed otherwise
2. Dispose of laboratory materials as instructed. Take note of the location of:
    a. Regular trash can
    b. Biohazard bag
    c. Sharps container for disposal of glass slides and small sharp objects
3. Take note of the location of the:
    a. Fire extinguisher
    b. Eye wash and emergency shower
    c. Emergency power shut-off button
    d. Location of security phone
    e. Safety glasses cabinet
4. Report all spills, unsafe conditions, or accidents to the instructor.
5. Wash your hands before leaving the laboratory.
6. Keep work area neat and organized. Clean up when done with the laboratory exercise.
7. Push in your chair before you leave.

"I understand all of the safety procedures and information presented and I have been given an opportunity to ask questions concerning this safety information."

Name:

Date:

Lab Time:

LAB 1: LAB SAFETY, SCIENTIFIC METHOD, AND MEASUREMENTS

LAB SAFETY

Learning Objectives

After completing the lab, the student will be able to:

1. Explain how to safely work in the lab.

Activity 1: Pre-Assessment

1. Name one piece of lab safety equipment. What is it used for?
2. What is one hazard of the lab, and why it is hazardous?
3. Discuss the answers to questions 1 and 2 with the class.

Safety Precautions

- Take care as you move around the lab.
- Wear all appropriate safety equipment.

For this activity, you will need the following:

- Access to the internet or a downloaded version of the safety video
- Lab safety equipment
- Lab safety agreement

Step 1: Locate the following safety equipment and supplies in the lab: first-aid kit, emergency exits, shower, fire extinguisher, eye-wash, waste containers (broken-glass waste), safety glasses bin, gloves, and any other safety equipment and supplies in the lab.

Step 2: Draw a diagram of the lab showing the location of the safety equipment and supplies.

Step 3: Watch the lab safety video. Search online for *Lab Techniques & Safety: Crash Course Chemistry #21*

One or more interactive elements has been excluded from this version of the text. You can view them online here: https://louis.pressbooks.pub/generalbiology1lab/?p=35#oembed-1

Step 4: Review the personal lab safety equipment shown in Figure 1.1.

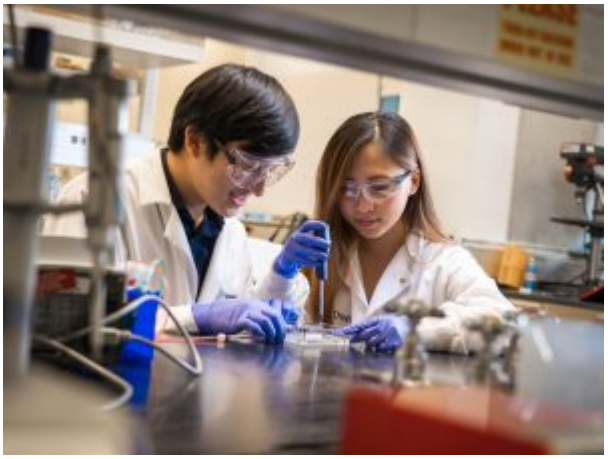


Figure 1.1: Students working in lab. Source: UCSD Jacobs School of Engineering – David Baillot. Licensed CC 3.0 – Jacobs School of Engineering, UC San Diego

Step 5: Record and sign your lab's safety agreement to confirm you agree to follow the rules of the lab.

Assessments

1. Describe what you would do in each of the following situations.
    1. There is a chemical spill in the lab.
    2. A fire starts in the lab.
    3. A beaker breaks in the lab.
2. List personal safety equipment needed to work in the lab.
3. Name one rule that you learned from the video.

SCIENTIFIC METHOD AND MEASUREMENTS

Learning Objectives

After completing the lab, the student will be able to:

1. Use the scientific method.

Activity 2: Pre-Assessment

1. When doing an experiment, why is it beneficial to alter only one experimental condition at a time?
2. How can you ensure that your experiment results aren't influenced by any outside conditions?
3. Discuss the answers to questions 1 and 2 with the class.

Activity 2: The Scientific Method

Safety Precautions

- Inform your teacher immediately of any broken glassware, as it could cause injuries.
- Clean up any spilled fluids to prevent other people from slipping.
- Wash your hands with soap and water after completion of the activity.

For this activity, you will need the following:

- Water
- Sugar or salt
- Beakers

- Ice
- Hot plate
- Thermometer

For this activity, you will work *in pairs*.

Structured Inquiry

Step 1: **Hypothesize/Predict:** Examine the materials available in the lab. Think of a question you could examine about the available materials (e.g., does sugar dissolve faster in warm water than in ice-cold water?). Formulate a clear, specific **hypothesis** that you could test during the lab period. Record your hypothesis in the data table.

Step 2: **Student-Led Planning:** Design an experiment to test your hypothesis. Set up a data table in your notebook to record your data. Write out the steps of your experiment, and devise positive and negative controls. Discuss your plan with your teacher before proceeding.

Step 3: After your teacher approves, execute your experiment and record the data in your data table.

Step 4: **Critical Analysis:** Does your data support your hypothesis? Why or why not? What methods could you use to improve your results? Discuss with your partner, and then write your answers in your notebook.

Guided Inquiry

Step 1: Hypothesize/Predict: Redesign the experiment you previously performed in the Structured Inquiry. Create a more specific hypothesis, or create a follow-up hypothesis that you can test based on the results of the Structured Inquiry.

Step 2: Student-Led Planning: Create a plan for your experiment, including controls and data tables. As before, your teacher should approve your plan before you start your experiment.

Step 3: After your teacher approves, execute your experiment and record the data in your data table.

Step 4: Critical Analysis: Does your data support your hypothesis? Why or why not? How did you improve your experiment over what you did in the Structured Inquiry? How could you further improve your results? Discuss with your partner, and then write your answers in your notebook.

Assessments

1. What is the purpose of the scientific method?
2. You observe that plants sitting in one location in your house grow faster than plants sitting in another location. Create a testable hypothesis to test why this occurs. Then, describe an experiment that would

test your hypothesis that includes controls.

MEASUREMENT

Learning Objectives

After completing the lab, the student will be able to:

1. Measure weight and volume.

Activity 3: Pre-Assessment

1. What are the conversions for the following measurements?
    1. 50 ml = _____ L
    2. 20 g = _____ mg
    3. 700 µl = _____ ml
    4. 1 gal = _____ pt
    5. 2000 ml = _____ L
2. Visit the weighing station. What is the weight of a weighing boat? Some balances have a method of subtracting it by pressing the "zero" or "tare" button. Weigh three items. Record the results in your notebook.
3. Discuss the answers to questions 1 and 2 with the class.

Activity 3: Measurement

In the lab, you will need to use instruments to make your measurements. There are instruments to use for each type of substance (liquid or solid). Some of the instruments to measure volume are graduated cylinders (shown in Figure 1.2) and pipettes (shown in Figure 1.3 and Figure 1.4). Some instruments to measure solids are balances and scales (a scale is shown in Figure 1.5). You may also need to adjust the instrument or container you are using based on the amount of substance you need to measure.

Figure 1.2: Graduated Cylinders

Figure 1.3: Pipettes

Figure 1.4: Pipettes

Figure 1.5: Scale

Also, when recording your measurements in a data table, it is important to be aware of significant figures. The number of significant figures is the number of digits needed to express the accuracy of a calculation. The rules for significant figures are presented in Figure 1.5. To use these rules, let's look at an example. An experiment requires you to measure the weight of 10 marbles. The scale shows a weight of 32.46547g. All the digits in this measurement are significant figures. However, in the number 32.465470, how do we know if the last 0 is significant or not? There are three rules on how to determine significant figures:

- Non-zero digits are always significant.
- Any zeros between two significant digits are significant.
- A final zero or trailing zeros in the decimal portion only are significant.

So, based on this rule, the final zero in 32.465470 is significant, as is the first zero in 32.046570. However, the zero in 0.324657 would *not* be significant because it is simply a placeholder.

Safety Precautions

- Inform your teacher immediately of any broken glassware, as it could cause injuries.
- Clean up any spilled fluids to prevent other people from slipping.
- Wash your hands with soap and water after completion of the activity.

For this activity, you will need the following:

- A balance
- A weighing boat
- A graduated cylinder
- Weighable objects

For this activity, you will work *in pairs*.

Structured Inquiry

Step 1: Create a data table for your measurements, and show your calculations in your notebook.

Step 2: Hypothesize/Predict: Examine one of the objects. Predict the object's mass, and write the predicted mass in your notebook.

Step 3: Student-Led Planning: Listen to your teacher's instructions on how to use the balance. Discuss with your partner how you should determine the weight of the object and how many significant figures your measurement should have. Write your ideas in your notebook.

Step 4: Determine the weight of the object using the scale. Repeat the measurement twice more, and record all results in your notebook.

Step 5: Critical Analysis: How much did your measurements vary among the three trials? Were you able to accurately measure to the amount of significant figures stated in Step 3? Why or why not? Could you have improved your methods to get more accurate measurements or obtain a greater number of significant figures? Why or why not? Discuss with your partner, and then write your answers in your notebook.

Guided Inquiry

Step 1: Hypothesize/Predict: Volume is a measure of how much space matter occupies, and it is used to calculate density (d) where m is the mass of the object and v is the object's volume.

$$d = \frac{m}{v}$$

Pick another object available in your lab and predict whether it has a higher or lower density than the object you used during the Structured Inquiry. Write your prediction in your notebook.

Step 2: Student-Led Planning: How can you calculate the density of your two objects? Devise a plan for finding the density of both objects and present it to your teacher for approval. Create the data tables you

need and decide how many times you should measure each object as well as how many significant figures you will use.

Step 3: After your teacher approves, take the measurements you need to calculate the density of both objects. Write your final densities in your notebook.

Step 4: Critical Analysis: How did the object's size influence its mass and its volume? What would you have to do to increase the mass, volume, and density of an object? How could you increase the accuracy of your measurements? Discuss with your partner, and then write your answers in your notebook.

Assessments

1. Why does the weight of the weighing boat need to be subtracted when measuring the weight of an object sitting in the weighing boat?
2. What is the most likely instrument used to measure the following volumes?
    1. 200 ml
    2. 50 µl
    3. 1 L
3. Calculate the density of an object that is 3 g and that displaces water in a graduated cylinder by 3 ml.

KEY TERMS

hypothesis
suggested explanation for an observation, which one can test

LAB 2: INTRODUCTION TO MICROSCOPY

PARTS OF A MICROSCOPE

Learning Objectives

After completing the lab, the student will be able to:

1. Explain or describe the parts of a light compound microscope.
2. Determine the total magnification of each objective lens.

Activity 1: Pre-Assessment

1. A microscope has two sets of lenses. What do you think is the purpose of each lens?
2. Why do you think scientists use microscopes? Think of useful functions for the microscope and discuss your ideas with your partner.

The Use of a Microscope

The hidden world becomes visible with the aid of a microscope. A microscope is used to see items and structures that the human eye cannot visualize. Microbes, cell structures, and minute particulate materials are examples of such objects. The microscopic examination of one-celled organisms as well as the cells and tissues of multicellular organisms are all part of biological research. To properly utilize the microscope, you must first learn the operational modes of each part. In this laboratory exercise, you will learn to use and identify the parts of a compound light microscope.

Activity 1: Parts of a Microscope

A microscope magnifies the image of an object through a series of lenses. The **condenser lens** focuses the light from the microscope's lamp onto the specimen. The light then passes through the object and is refracted by

the objective lens. The **objective lens** is the more powerful lens of a microscope and is closest to the object. The light then travels to the **ocular lens**, which focuses the image onto the user's eye. Usually, the power of the ocular lens is fixed for a given microscope.

Different microscopes can magnify objects hundreds, thousands, or even millions of times. **Magnification** refers to how much larger the image is compared to the original object. As magnification increases, the image becomes larger. The total magnification is the power of the objective multiplied by the power of the ocular lenses. **Resolution** refers to the ability of a microscope to distinguish two points on the image. As resolution increases, objects that are closer together appear as separate points. **Contrast** refers to the ability of a microscope to distinguish an object from its background. The higher the contrast, the greater the difference in intensity between an object and the background.

Safety Precautions

- Handle microscopes and lenses with care.
- Do not drop or crush slides. Alert your teacher immediately to any broken glass so it can be properly disposed.

For this activity, you will need the following:

- Compound microscope
- Slide with the letter *e*; if you do not have premade slides, cuttings from a newspaper can be taped to a slide.

For this activity, you will work *in pairs*.

Structured Inquiry

Step 1: When moving a microscope, grasp the neck firmly with one hand and place your other hand under the microscope's base. Do not bang the microscope on the desk. Once you have set down your microscope, turn the revolving nosepiece so that the lowest power objective is pointed at the stage.

Step 2: Throughout this lab, handle slides by their edges and do not touch the specimen area to avoid smudges. Place the slide with the letter *e* on the stage. Make sure it is oriented so that you can read the letter *e* correctly with the naked eye while standing at the microscope.

Step 3: You will now observe the slide containing the letter *e.* Center the specimen on the stage and look through the ocular lens. Adjust the coarse and fine focus knobs until the letter comes into focus. Be careful while focusing so that the slide does not touch the lenses.

Step 4: Hypothesize/Predict: From your observations of the letter *e*, what do you think is the function of each part of the microscope? Write the function of each in your notebook.

Step 5: Student-Led Planning: Using your textbook and other resources, research each part of the microscope. Identify each part of the microscope in Figure 2.1. Label each part of the microscope in Table 2.1. Write the function of each part in Table 2.1. Parts and Function of a Light Compound Microscope.

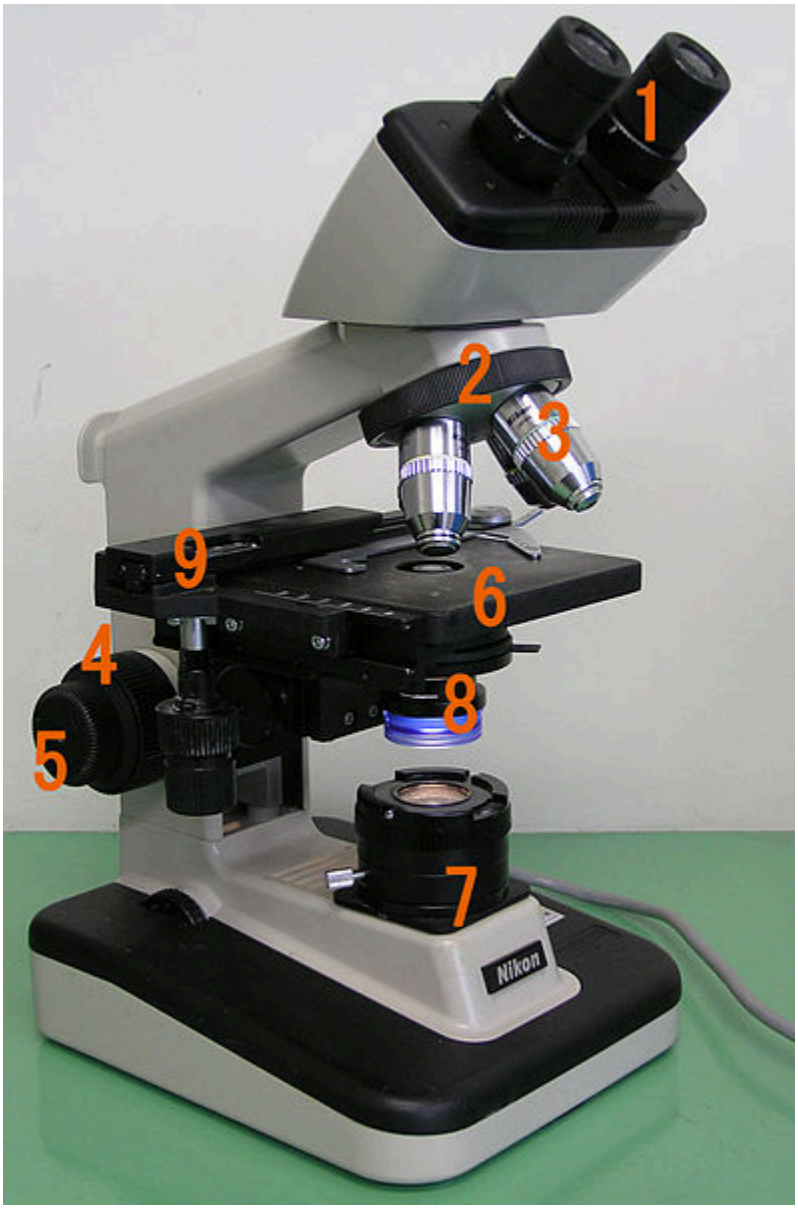


Figure 2.1: The parts of the microscope. By GcG(jawp) and under the Public Domain. https://commons.wikimedia.org/wiki/File%3AOptical_microscope_nikon_alphaphot.jpg

Table 2.1: Parts and Function of a Light Compound

Microscope

Part	Function
1. Ocular lens (eyepiece)	
2. Revolving nosepiece	
3. Objective lens	
4. Coarse adjustment knob	
5. Fine adjustment knob	
6. Stage	
7. Lamp	
8. Condenser	
9. Mechanical stage	

Learn the parts of the microscope (activity)

- Visit the BioNetwork's Virtual Microscope
- Click on "Explore"
- Click on parts "?" to learn their name and function

Step 6: Critical Analysis: You place a sample on the stage of a microscope, and you look through the ocular lens. All you see is black in the field of view. What would you adjust to fix this problem?

Step 7: Microscope Care and Maintenance: When you are finished using a microscope, make sure the lowest power objective is in place and take the slide off the stage. Use only lens paper to wipe the lenses if they are dirty. Support the microscope by the base when carrying it as described in Step 1. If you are continuing with the other activities in this lab, keep the microscope out.

Guided Inquiry

Step 1: Find the light source on the microscope. Turn on the light source and trace the path of light from the source to the ocular lens.

Step 2: Hypothesize/Predict: Knowing the function of each part of the microscope, draw how the light path of the microscope bends and changes as it travels from the light source to the observer on Figure 2.1.

Step 3: Student-Led Planning: Using your textbook and other resources, research how light travels through the microscope. Use a piece of paper to block the light path through each component of the microscope to confirm that it is part of the light path.

Step 4: Critical Analysis: Sometimes, scientists want to study living cells that are kept in dishes. The dishes are very deep and filled with media. The cells are on the bottom of the plate. In order to see these cells on the microscope, the microscope has to be inverted so that the objective lens is below the sample instead of above it. Draw a diagram of how you would design the light path of an inverted microscope.

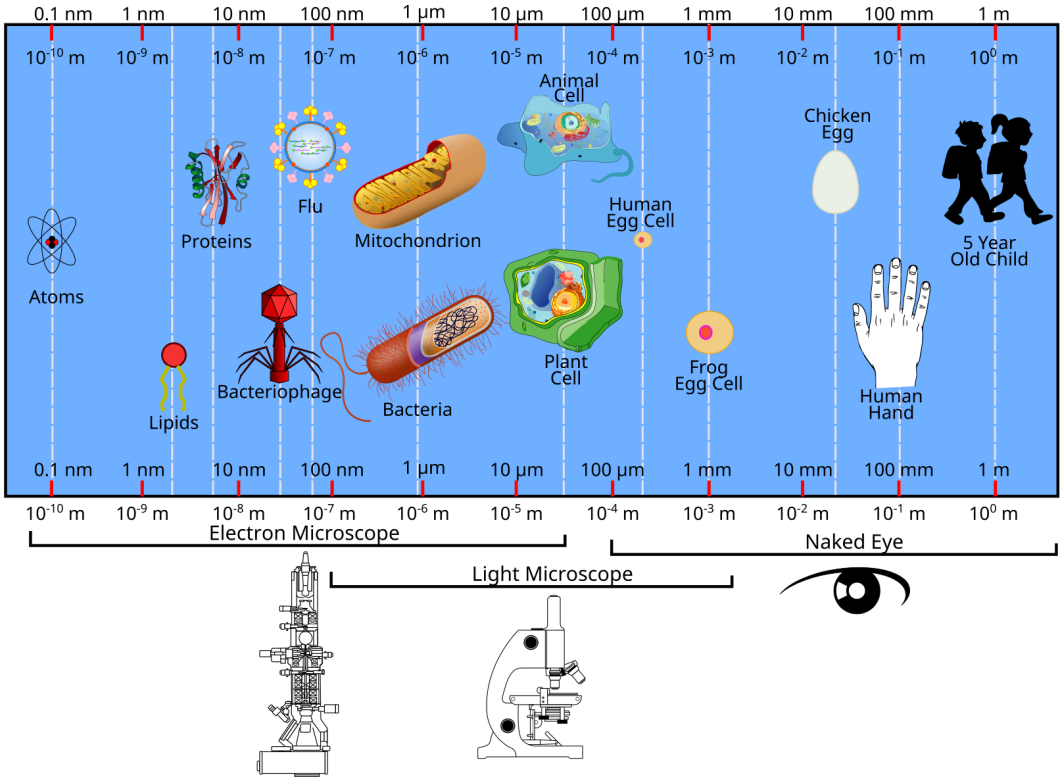


Figure 2.2 Comparative sizes of different objects. Credit: Jeremy Seto and derived from works by IgniX, Gringer, Jiver, Ninjatacoshell, Ali Zifan (CC-BY-SA) (CUNY Lab: Microscope)

Magnification[1]

Magnification is the process of enlarging the appearance of an object. We calculate the magnification of an object by indicating the fold change in size. So if something appears to be double the size of the real item, then it is obviously magnified 2X. Because there is a magnification by the eye-piece (**ocular lens**), as well as the **objective lenses**, our final magnification of an item is the product of those two lenses.

1. The Magnification activity is adapted from Microscopy in Biology OER, a site sponsored by the Ursula Schwerin Library to select and curate resources for use in General Biology 1 and originally authored and curated by Jeremy Seto, Department of Biological Sciences – New York City College of Technology. It is licensed CC-BY-NC-SA.

The lowest magnification objective lens (usually 4X or 5X) is referred to as a **scanning lens**. There is also usually a low-power lens at 10X and a higher magnification lens at 40X. There may be a higher magnification lens at 100X, but these usually require oil to function properly and are often reserved for microbiology labs.

What is the power of the ocular lens?

We can calculate that as:

$\text{Magnification}_{\text{total}} = \text{Magnification}_{\text{objective}} \text{ X } \text{Magnification}_{\text{ocular}}$

With this in mind, fill in the following table:

	Objective Magnification	Ocular Magnification	Total Magnification
Scanning			
Low Power			
High Power			
Oil Immersion	100X		

Assessments

1. What are three precautions you must take to prevent damage to the microscope?
2. A scientist uses a 40× objective to observe his specimen. He has a 10× ocular lens. What is the total magnification of the object?
3. A magnifying glass is also used to magnify objects. How is a compound microscope different from a magnifying glass?
4. Draw how Figure 2.3 would appear under a compound microscope.

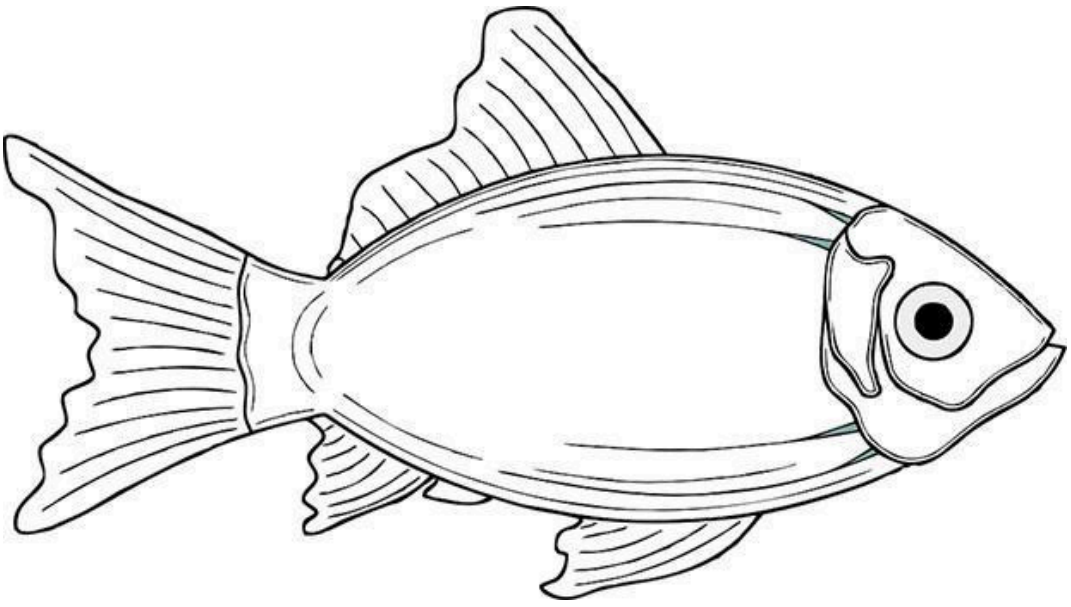


Figure 2.3: This illustration shows a simple line drawing of a fish facing right.

MAGNIFICATION

Learning Objectives

After completing the lab, the student will be able to:

1. Determine the total magnification of each objective lens.
2. Explain or describe the field of view.

Activity 2: Pre-Assessment

1. So far you have only viewed a slide at the lowest power of the microscope. How do you think the field of view will change as you move to higher powers on the microscope?
2. The microscope makes small organisms appear larger so that scientists can more carefully observe them. How can you measure the size of small organisms on the slide of a microscope?

Activity 2: The Field of View

When you first place a specimen on the microscope's stage, the image you see in the ocular lens will likely appear blurry. This is because light rays from the object are not reaching your eye at the same time. **Focusing** is used to improve the image. Focusing moves the stage up and down, which changes the point at which the light rays from the object converge. When the stage is at a position so that the light rays exactly converge on the retina of your eye, the image will appear sharp and clear.

When focusing the specimen, you must be careful! The distance between the objective and the specimen is very small, and it is possible to crush the specimen against the lens if you move the stage too far. The proper distance between the specimen and the object is called the **working distance**.

When you look at a specimen through the microscope's lenses, you will likely not see the entire specimen at one time. Because the microscope enlarges the image of the object, only a small portion of the object will be

visible at any given time. The **field of view** refers to the portion of the object that is seen through the ocular lenses (see Figure 2.3).

Safety Precautions

- Handle microscopes and lenses with care.
- Do not drop or crush slides.

For this activity, you will need the following:

- Compound microscope
- Slide with the letter *e*; if you do not have premade slides, cuttings from a newspaper can be taped to a slide
- Slide with a stage micrometer or a clear millimeter ruler taped across the center of the slide.

For this activity, you will work *in pairs*.

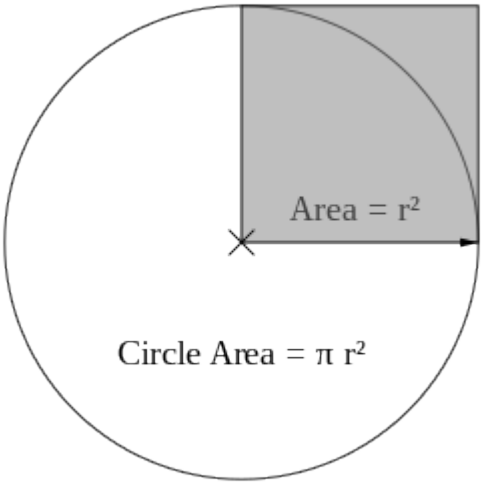


Figure 2.3: Field of View (FOV). Licensed CC-BY-NC-SA by Jeremy Seto in Biology OER

Structured Inquiry

Step 1: Turn the revolving nosepiece of the microscope so that the lowest power objective is pointed at the stage.

Step 2: Place the slide with the letter *e* on the stage. Make sure it is oriented so that you can read the letter *e* correctly with the naked eye while standing at the microscope.

Step 3: Hypothesize/Predict: How do you think the letter *e* will appear under different magnifications? Draw your predictions in your lab notebook.

Step 4: Student-Led Planning: Change the magnification by turning the revolving nosepiece. Draw your observations in your lab notebook.

Step 5: Critical Analysis: How do your observations compare to your predictions? Why do you suppose the letter *e* looks different under the microscope and at different magnifications?

The Letter "E"[1]

1. Visit the BioNetwork's Virtual Microscope
    1. Click on "Explore"
    2. Click the sample box "?"
    3. Click "Sample Slides"
    4. Click "Letter E"
2. The slide is oriented so the "e" is right side up
    1. *What do you observe with the image under the microscope?*
    2. The image is blurry so pull focus
3. Switch between scanning, low power and high power
    1. Draw the "e" at scanning, low and high magnification (Figure 2.4)

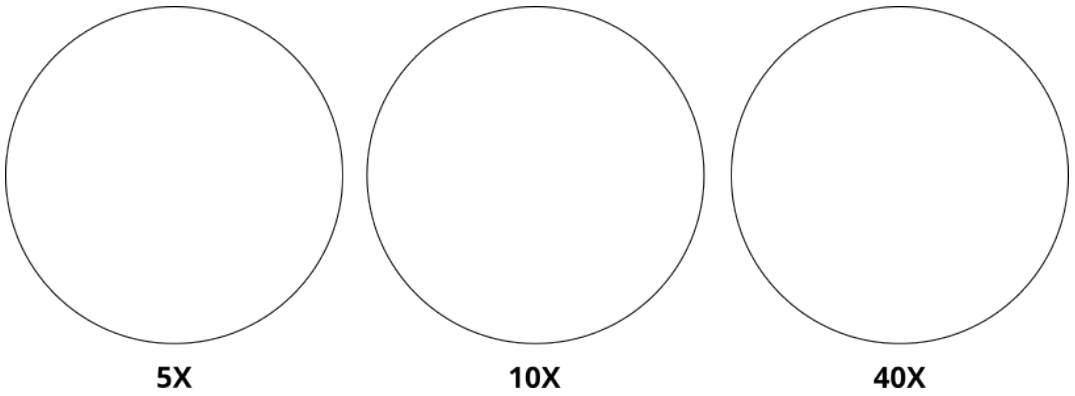


Figure 2.4: Magnification at 5x, 10x, and 40x. Licensed CC-BY-NC-SA by Jeremy Seto in Biology OER

Guided Inquiry

Step 1: Hypothesize/Predict: From your observations of the letter *e,* hypothesize how large the field of view is under the low-power objective. To do this, measure the letter *e*, in millimeters, and then calculate how many letter *e*'s would fit in the field of view. From that, hypothesize the size of the field of view, in mm. Will the size of

1. The Letter "E" activity is adapted from Microscopy in Biology OER, a site sponsored by the Ursula Schwerin Library to select and curate resources for use in General Biology 1 and originally authored and curated by Jeremy Seto, Department of Biological Sciences – New York City College of Technology. It is licensed CC-BY-NC-SA.

the field of view change when you change magnifications? Based on the change in magnification, hypothesize the size of the field of view under the high-power objective.

Step 2: Student-Led Planning: With your partner, formulate a plan to measure the field of view using either the stage micrometer or the slide with the attached ruler. Observe the micrometer or ruler under the microscope to understand how it works before measuring. Then take three measurements of the field of view under low power. Repeat under high power. Write your results in your notebook.

Step 3: Critical Analysis: How did your estimated size of the field of views from Step 1 compare to the measurements you made using the micrometer or ruler? How does the size of the field of view change as the magnification changes?

Assessments

1. When you take a picture on your phone, does the field of view get larger or smaller as you increase the magnification? How does this compare to the microscope? Explain.
2. As you increased in power on the microscope, the image became darker. Why do you suppose this is so?

DEPTH OF FIELD AND WET MOUNT OF SPECIMENS

Learning Objectives

After completing the lab, the student will be able to:

1. Explain or describe the depth of field.
2. Construct a temporary wet mount when given a slide, coverslip, and specimen.

Activity 3: Pre-Assessment

1. When you focused the slides in the previous activities, what part of the microscope was moving? Why do you think this changes the focus?
2. Review the definition of life. What are characteristics found in all living things?
3. **Discuss the answers to questions 1 and 2 with the class.**

Activity 3: Depth of Field and Wet Mount of Specimens

As you work with a microscope, you will notice that even when an object is in focus, you can continue to adjust the focus and see new things in the lenses. This is because most specimens are very thick. As you move up and down with the stage, different planes of the object come into focus. The **depth of field** refers to how thick each plane is. It tells you how much of a specimen is in focus at any given time. Although you may think that a higher depth of field is more useful because it allows you to see more objects, most scientists try to make the depth of field as narrow as possible. This prevents light from other focal planes from interfering with the observations.

Depth of field[1]

1. Examine the slide of colored threads under scanning power so the cross-point of the threads is at the center of the field (Figure 2.5)
2. Raise the magnification to the low-power objective
    1. What do we notice about the threads and the focus?
    2. How can we explain this observation with respect to the threads?
    3. Close the diaphragm to allow a pinpoint of light through the slide. What effect does this have on the image?

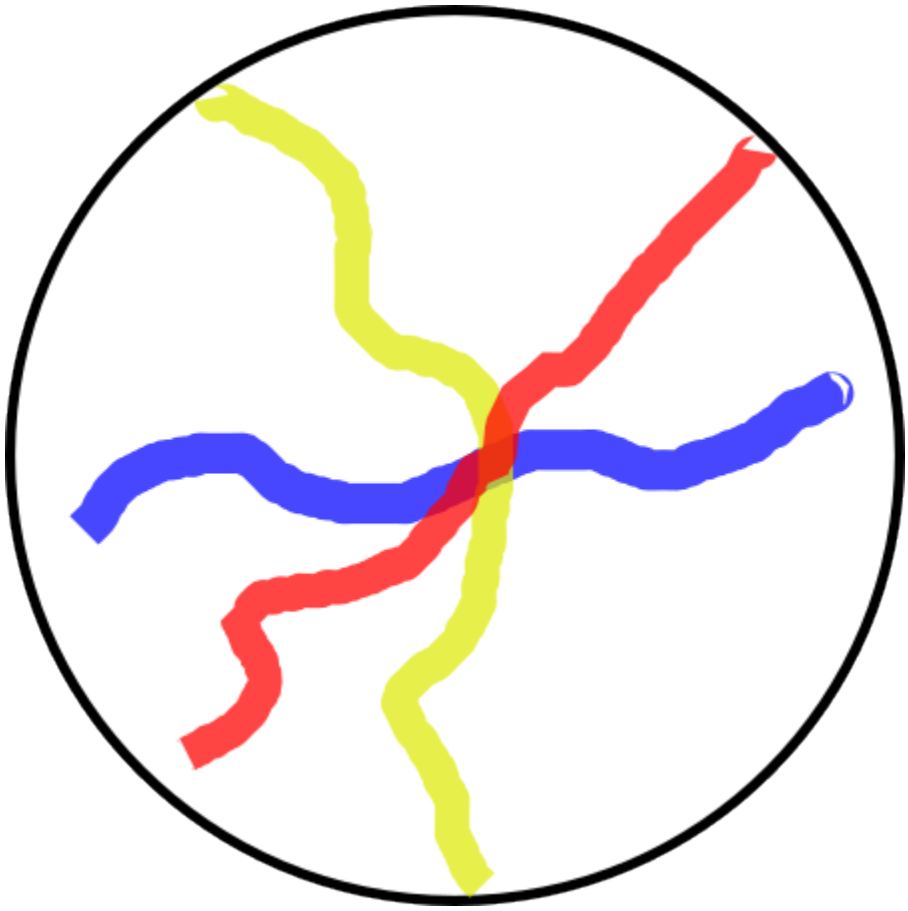


Figure 2.5: Colored threads with cross-points at the center of the field of view. Licensed CC-BY-NC-SA by Jeremy Seto in Biology OER

1. The Depth of field activity is adapted from Microscopy in Biology OER, a site sponsored by the Ursula Schwerin Library to select and curate resources for use in General Biology 1 and originally authored and curated by Jeremy Seto, Department of Biological Sciences – New York City College of Technology. It is licensed CC-BY-NC-SA.

Examining Cells

The specimens you use on your microscope can either be wet-mounted or dry-mounted. A **wet-mount** refers to living tissues that are placed on a slide with an aqueous solution to keep them wet. Usually, a coverslip is placed on top of the specimen to flatten the specimen onto the slide. A **dry-mount** refers to preserved tissue that has been fixed and stained on a slide. This technique allows you to preserve specimens for a long time, and it also allows you to add chemicals to increase the contrast of a specimen from its background.

Safety Precautions

- Handle glass slides with care.
- Dispose of specimens as necessary.
- Dispose of coverslips in a broken-glass container.

For this activity, you will need the following:

- Compound microscope
- Slides with crossed colored fibers or crossed strands of hair
- Clean slides
- Coverslips
- *Elodea*, onion skin, pond water, or other samples

For this activity, you will work *in pairs*.

Structured Inquiry

Step 1: Hypothesize/Predict: Look at the slide with crossed fibers or hair. How do you think you can use the microscope to determine which fiber is closest to the top of the slide and which fiber is closest to the bottom?

Step 2: Place the slide with crossed fibers or hair on the stage. Center the slide and focus it.

Step 3: Student-Led Planning: Turn the fine focus knob up and down. With your partner, decide how to determine which fiber is on top and which fiber is on the bottom of the slide. Record your results in your notebook.

Step 4: Critical Analysis: Take a look at the slide again without the microscope. With your naked eye, do you think the results of your analysis make sense?

Guided Inquiry

Step 1: Hypothesize/Predict: Based on the microscopy you have performed so far, what would be the challenges of viewing living organisms under the microscope?

Step 2: Student-Led Planning: You will now observe pond water or samples of living organisms provided by your teacher. Place a small drop of water from near the bottom of the sample jar on a slide. Place a cover slip at an angle so that one edge touches the drop. Slowly lower the coverslip onto the specimen. Avoid trapping air bubbles under the coverslip.

Record drawings of your observations in your lab notebook. Make sure to record the magnification for each drawing.

Step 3: Critical Analysis: What were some of the differences you observed between your specimens? What were some of the challenges in viewing living organisms that move under the microscope? Be prepared to present your findings to the class.

Procedure

1. Choose a prepared slide of a Protist (*Euglena*, *Amoeba*, *Paramecium*)
2. Prepare a wet mount of a drop of pond water and place a cover slip over the drop (Figure 2.6)
3. Swab the inside of your cheek
    1. Roll the swab across a slide
    2. Drop some methylene blue onto the slide
    3. Place a coverslip over the drop
    4. Visualize and draw your cheek cells
4. Document your observations by drawing the cells and by using your phone to snap an image (Figure 2.7).

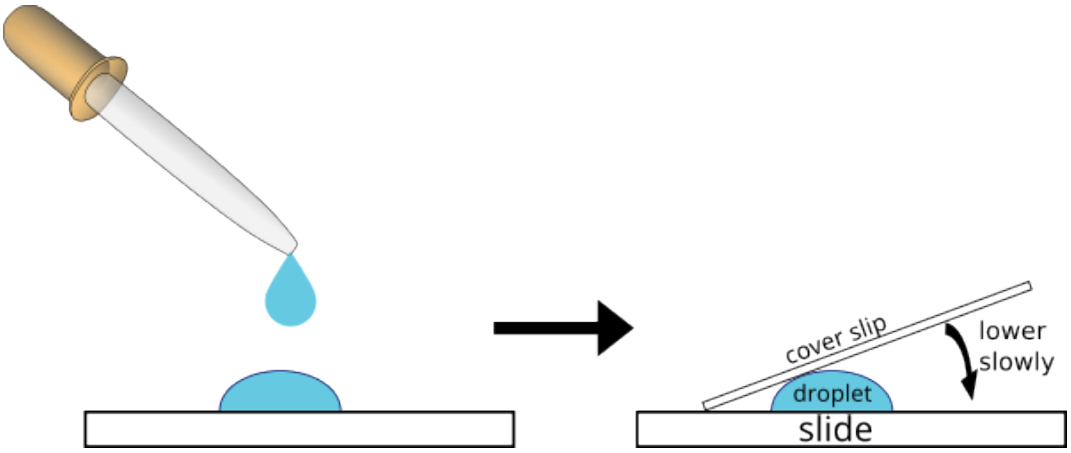


Figure 2.6: Wet mount preparation. Licensed CC-BY-NC-SA by Jeremy Seto in Biology OER

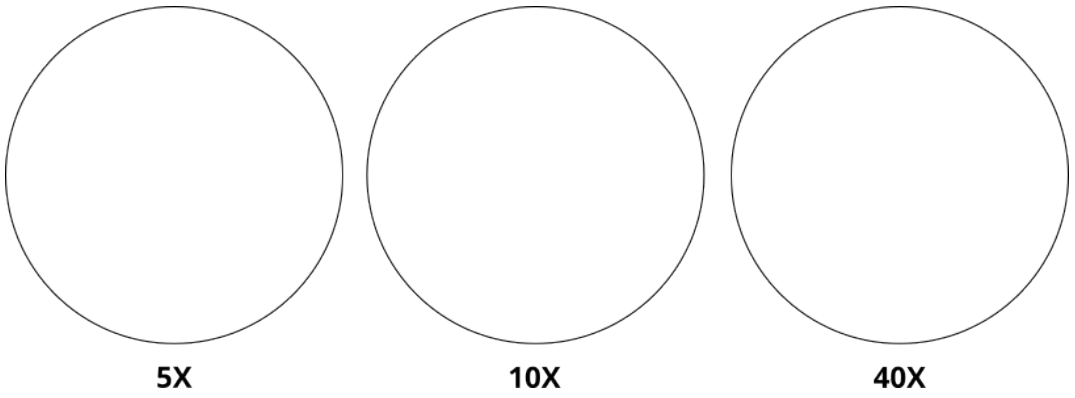


Figure 2.7: Magnification at 5x, 10x, and 40x. Licensed CC-BY-NC-SA by Jeremy Seto in Biology OER

Assessments

1. A microscope can focus on many fields of different depths. However, thick samples have to be sliced into very thin slices in order to be viewed under the microscope. Why do you suppose this is the case?
2. *Elodea* plants appear as solid green objects to the naked eye, but appear mostly clear under a microscope. Explain this phenomenon.
3. A scientist takes a sample from a pond and examines it under their microscope. He thoroughly examines the wet mount and finds no living thing on the slide. He concludes that the pond is unable to support life. Is this a valid conclusion? Why or why not?
4. Before the cell theory was developed, two previous theories were prevalent as different historical theories. Explain how the invention of the microscope would lead to the replacement of both of these theories with the cell theory:
    1. The Miasma Theory, where diseases were thought to be spread by miasma, a poisonous, foul-smelling vapor that can travel through the air.
    2. The Theory of Spontaneous Generation, where living things could be generated from non-living matter, such as how maggots seem to appear spontaneously on meat left out in the open.

KEY TERMS

condenser lens
: focuses the light from the microscope's lamp onto the specimen

contrast
: the ability of a microscope to distinguish an object from its background

depth of field
: area visible through the microscope

dry mount
: preserved tissue that has been fixed and stained on a slide

field of view
: how thick each plane is. It tells you how much of a specimen is in focus at any given time

magnification
: focuses the image onto the user's eye

objective lens
: focuses the light from the microscope's lamp onto the specimen

ocular lens
: focuses the image onto the user's eye

resolution
: the ability of a microscope to distinguish two points on the image. As resolution increases, objects that are closer together appear as separate points

wet mount
: living tissues that are placed on a slide with an aqueous solution to keep them wet.

working distance
: the proper distance between the specimen and the object

LAB 3: MACROMOLECULES IN COMMON FOOD

MACROMOLECULES IN COMMON FOOD

Learning Objectives

After completing the lab, the student will be able to:

1. Detect the presence of macromolecules.
2. Explain how the macromolecules found in a particular food provide evidence about their energy resources.

Pre-Assessment

1. A chemical indicator called Benedict's solution turns from blue to brick red in the presence of certain monosaccharides. Would you expect it to turn red in the presence of glucose? Of sucrose?
2. A chemical mixture containing copper sulfate undergoes a color change when it binds to the nitrogen atoms in certain macromolecules. What types of macromolecules can be detected using this solution?
3. Discuss the answers to questions 1 and 2 with the class.

Detecting Reducing Sugars, Starch, Proteins, and Lipids

The three major macromolecules that make up the bulk of our foods are carbohydrates, lipids, and proteins. Carbohydrates and proteins often consist of many smaller structural units, called **monomers**, linked together into long chains, called **polymers**. Before investigating the presence of these macromolecules in food, it is important to first perform what is called a positive control and negative control standards test for each macromolecule. Your **positive control** samples will contain the macromolecules glucose, starch, egg white protein, and vegetable oil. These samples will provide you with a reference for identifying each of the representative macromolecules in your food samples. Your **negative control** will be distilled water, which should provide results for the absence of the macromolecule in your food sample.

To begin your investigation, you will conduct a sequence of four tests for investigating macromolecules. In

test 1, you will use a procedure called the **Benedict's test** to confirm the presence of glucose, which is a monosaccharide found in many types of food. For test 2, you will use a solution of iodine to determine whether the polysaccharide starch is present in a solution. For test 3, you will use the **Biuret test** to identify if a solution contains protein. Then, in test 4, lipids will be detected using a very simple test called the **paper spot test**.

For this activity, you will work *in pairs*.

To expedite the lab activity, one team member should carry out the carbohydrates (glucose and starch) activities while the other team member does the tests on the lipids and the proteins.

Safety Precautions

- Safety goggles must be used throughout the duration of the laboratory activity.
- It is highly recommended that you wear rubber gloves and a lab apron when handling any of the chemicals.
- Immediately inform your teacher of spills containing the test solutions. The acids and bases in the solutions can harm your skin.
- Immediately inform your teacher of any broken glassware, as it could cause injuries.
- Clean up any spilled water or other fluids to prevent other people from slipping.
- Immediately wash your hands if they directly come in contact with the test solutions. It is important to wash your hands after the laboratory activity to avoid any possible contamination of other surfaces with chemical residues that might be on your hands.

For this activity, you will need the following:

- Eight test tubes (one test tube per sample being tested)
- 10 ml graduated cylinder
- Grease pencil or washable marker
- One test tube rack
- One test tube holder
- Eight sample droppers or pipettes (do not mix pipettes from one solution to another)
- One thermometer
- Hot plate or microwave
- 250 ml beaker
- Brown paper bag
- Scissors
- Small ruler
- Hair dryer or lamp

Chemicals

- Distilled water
- Benedict's solution
- 1 percent copper sulfate solution or Biuret reagent
- 10 percent sodium hydroxide
- Lugol's solution or iodine-potassium iodide solution
- 5 percent glucose solution
- 2 percent starch solution
- 1 percent albumin solution or raw liquid egg white
- Vegetable oil
- A selection of common foods (contrasting in their reputation of healthiness)

Structured Inquiry

Calibrating the test tubes:

Before beginning this activity, it is important to calibrate (mark increments of measurement upon) your test tubes to ensure accurate and consistent test results. To do this, you will need the test tube rack, seven test tubes, the 10 ml graduated cylinder, and a marker or grease pencil. For this laboratory activity, it is best to calibrate your test tubes in 1 ml increments. The maximum volume you will measure is 5 ml.

Step 1: Collect eight test tubes, a test tube rack, the grease pencil or marker, and a 10 ml graduated cylinder.

Step 2: Write out a procedure for calibrating your eight test tubes.

Step 3: Get your procedure approved by your teacher before you continue with the calibration.

Step 4: Use the grease pencil or marker provided by your teacher to draw the calibration lines.

Step 5: Explain how estimating the quantities of a solution in an uncalibrated test tube could affect the results of an experiment. Record your answers in your laboratory notebook.

Carbohydrates:

As mentioned earlier, you will test for glucose and starch. In this investigation, explain which samples are the negative controls and which are the positive controls.

Use the following steps to test for the presence of glucose and starch.

Step 1: Place four clean test tubes in the test tube rack and use the grease pencil or marker to number each test tube as shown here.

Test tube number	1	2	3	4
Test solution	Distilled water	Glucose test solution	Distilled water	Starch test solution

Table 3.1

Step 2: Hypothesis/Predict: Predict which of the tubes will show a positive result for the Benedict's test and which will show a negative result. Write your prediction in a table for recording the data from the tests.

Step 3: Which tubes represent the positive and negative controls for this experiment? Explain why in your notebook.

Step 4: Add approximately 150 ml of water into the 250 ml beaker. Use the hot plate or microwave to heat the water to about 80^{o}C.

Step 5: Add 1 ml of distilled water to test tube 1. Then add 1 ml of the 5 percent glucose solution to test tube 2.

Step 6: Observe the appearance of each solution and record your observations in your table.

Step 7: Add 2 ml of the Benedict's solution to test tube 1 and to test tube 2. Observe the color of each solution and record your observations in your table.

Step 8: Place test tubes 1 and 2 in the beaker of heated water for 5 minutes. If using a hot plate, turn off the heat after removing the test tubes.

Step 9: Use a test tube holder to remove test tubes 1 and 2 and allow them to cool in the test tube rack. Observe the color of each solution and record your observations in your data table.

Step 10: Add 1 ml of distilled water to test tube 3. This will be your negative control standard for the starch test.

Step 11: Add 1 ml of the 2 percent starch solution to test tube 4. This will be your positive control standard.

Step 12: Observe the appearance of each solution and record your observations in your data table.

Step 13: Add 8 drops of the Lugol's solution or potassium iodide solution to test tube 3 and to test tube 4. Observe the color of each solution and record your observations in your data table. It may be useful to take photographs of your results.

Step 14: Cleanup:

- Empty test tubes 1 and 2 into a container designated for copper wastes or hazardous wastes.
- Empty test tubes 3 and 4 into a container designated for iodine wastes or hazardous wastes.
- Rinse out all of the test tubes with soap and water. Make sure to rinse out all of the soap from the test tubes.
- Dispose of the water from the 250 ml beaker into a sink only after it has cooled.
- Place the test tubes upside down in a test tube rack to dry.

Step 15: Critical Analysis: Discuss with your partner if your results match the expected results shown in

Figure 3.1 and Figure 3.2. If your result did not match those in these figures, what could be some reasons for your results not matching?

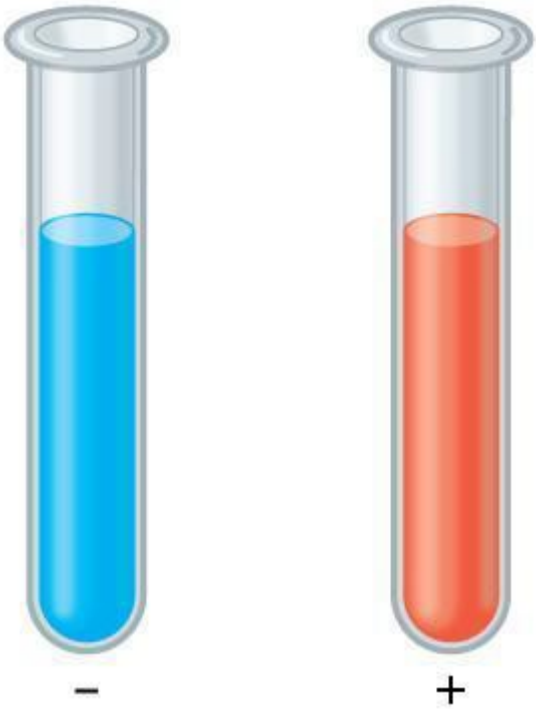


Figure 3.1: Benedict's test results.

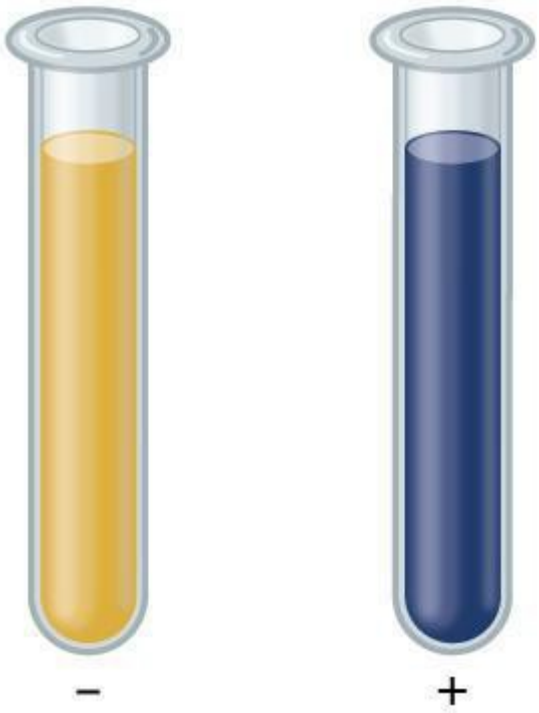


Figure 3.2: Iodine test results.

Proteins:

Step 1: Place three clean test tubes in the test tube rack and use the grease pencil or marker to number each test tube as shown here.

Test tube number	**1**	**2**	**3**
Test solution	Distilled water	Dilute protein solution	Concentrated protein solution

Table 3.2

Step 2: Hypothesis/Predict: Predict which of the tubes will show a positive result for the Biuret test and which will show a negative result. Write your prediction in a table for recording the data from the tests. Which tubes represent the positive and negative controls for this experiment? Explain why in your notebook

Step 3: Add 2 ml of distilled water to test tube 1. Then add 1 ml of distilled water and 1 ml of the 1 percent albumin solution or raw egg white to test tube 2.

Step 4. Add 2 ml of 1 percent albumin solution or raw egg white to test tube 3.

Step 5: Observe the appearance of each solution and record your observations in your data table.

Step 6: Add 2 ml of the 10 percent sodium hydroxide to each test tube and carefully swirl the tubes to mix the solution.

Step 7: Add five drops of the 1 percent copper sulfate solution or Biuret reagent to each test tube. Carefully swirl the tubes to mix the solution.

Step 8: Observe the color of each solution and record your observations in your data table. It may be useful to take photographs of your results.

Step 9: Cleanup:

- Empty all test tubes into a container designated for copper wastes or hazardous wastes.
- Rinse out all of the test tubes with soap and water. Make sure to rinse out all of the soap from the test tubes.
- Place the test tubes upside down in a test tube rack to dry.

Step 10: Discuss with your partner whether your results match the expected results shown in Figure 3.3. Does the color of the positive test more closely match your 50% albumin sample, or your 100% albumin? Why do you think? Record your answers in your laboratory notebook.

Figure 3.3: Biuret results.

Lipids:

As discussed in the introduction, you will use a strip of brown paper to indicate the presence of lipids in a solution.

In this investigation, explain which samples are the negative controls and which are the positive controls. Why is it important to perform a negative control and a positive control before using these tests to analyze foods for lipids? Write your answers in your laboratory notebook.

Use the following steps to test for the presence of lipids.

Step 1: Design a table for recording the data from the lipid test. You should show your table to the teacher before proceeding with the activity.

Step 2: Place 3 clean test tubes in the test tube rack and use the grease pencil or marker to number each test tube as shown here.

Test tube number	**1**	**2**	**3**
Test solution	Distilled water	Water mixed with vegetable oil	Vegetable oil

Table 3.3

Step 3: Use the scissors to cut a piece of the brown paper bag into a 7 cm × 15 cm rectangle. At each 5 cm

interval along the long side of this rectangle, draw a line using the grease pencil so that you have three equal boxes. Label the cut sections of the bag according to Figure 3.4.

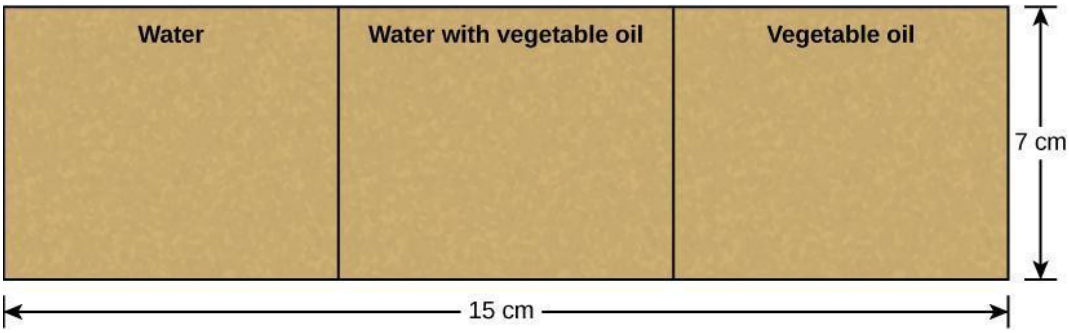


Figure 3.4: Labeled section of brown paper bag for lipid test.

Step 4: Add 1 ml of distilled water to test tube 1.

Step 5: Add 1 ml of distilled water and 1 ml of vegetable oil to test tube 2.

Step 6: Add 1 ml of vegetable oil to test tube 3.

Step 7: Use a pipette to collect the distilled water from test tube 1. Transfer the liquid to the brown paper bag. Add enough of the liquid to make a spot 1 cm in diameter on the section of the brown paper bag labeled *Water*.

Step 8: Gently swirl test tube number two until the solution is uniform. Quickly, use a pipette to collect the solution.

Step 9: Add enough of the liquid to make a spot 1 cm in diameter on the section of the brown paper bag labeled *Water mixed with vegetable oil*.

Step 10: Use a pipette to collect the vegetable oil from test tube 3. Transfer the liquid to the brown paper bag. Add enough of the liquid to make a spot 1 cm in diameter on the section of the brown paper bag labeled *Vegetable oil*.

Step 11: Record your results in your table. It may be useful to take photographs of your results.

Step 12: Critical Analysis: Discuss with your partner whether your results match the expected results shown below in Figure 3.5. Does the positive test result for lipids look more like your 50% oil or 100% oil result? Why do you think? Record your answers in your laboratory notebook.

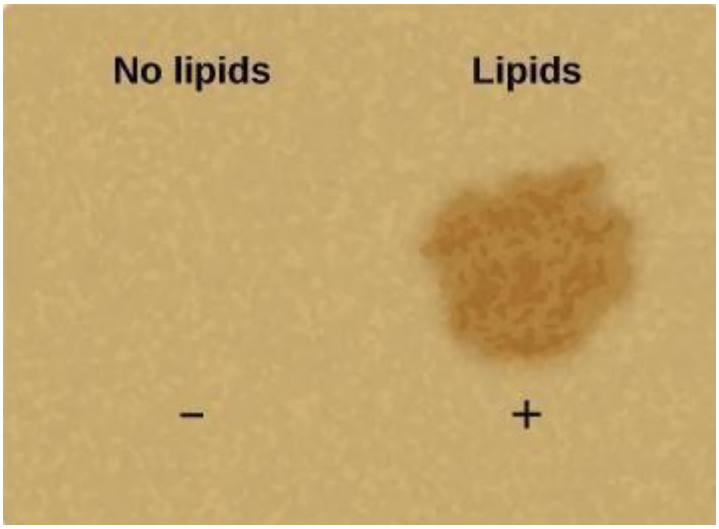


Figure 3.5: Paper spot test for lipids.

Guided Inquiry

Step 1: Hypothesize/Predict: Explain the types of nutrient macromolecules you would expect to find in food grouping 1. Do the same assessment for food grouping 2. Write your ideas in your laboratory notebook.

Step 2: Student-Led Planning: Write out the steps you will need to analyze the macromolecule content of the foods. Select only one food from each grouping. Remember to keep in mind how you will work safely in the lab and how you will dispose of the lab wastes. Submit your experimental procedure to your teacher. Once your teacher approves of your procedure, perform your experiment and record your data in an appropriate format. Write your results in your laboratory notebook.

Step 3: Critical Analysis: Which of the foods had the best balance of different macromolecules?

Assessments

1. A student needs to calibrate an unmarked beaker in order to mix a food sample into 100 ml of distilled water and 50 ml of dilute acid. The student is provided with a 1 ml pipette, a 5 ml pipette, a 50 ml graduated cylinder, and a 100 ml flask. The student selects the 5 ml pipette to use in making markings at 50 ml increments on the beaker.

    - Did the student carry out the most accurate way for calibrating the beaker? Why or why not?
    - Which would be the correct apparatus for calibrating a test tube in two 1 ml increments?

2. You are doing a study on crop nutrition. The study is investigating the effects of nitrogen fertilizer concentration on the protein content of plants. Design a simple experiment for carrying out this investigation.
3. Use the image of the Benedict's test Figure 3.6 to help you design an experiment to compare the glucose

levels of different foods.

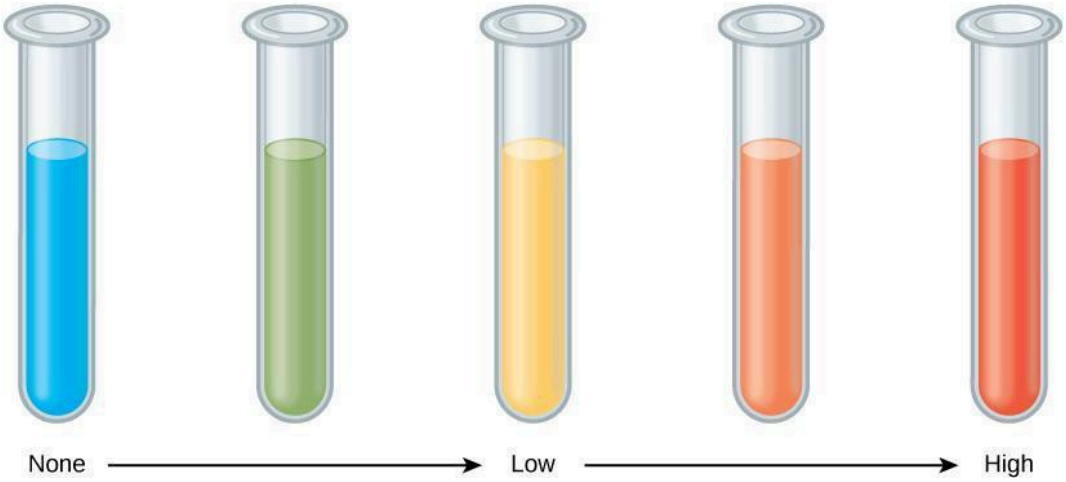


Figure 3.6: Benedict's test results for glucose concentration.

KEY TERMS

Benedicts Test
: a laboratory test used to detect the presence of simple carbohydrates

Biuret Test
: a laboratory test used to detect peptide bonds, and thus proteins

monomers
: smallest unit of larger molecules that are polymers

negative control
: a group in an experiment that receives a treatment that is expected to have no effect, to be used as a standard of comparison with future results.

paper spot test
: a simple laboratory test for the presence of lipids

polymers
: chain of monomers that covalent bonds link; polymerization is the process of polymer formation from monomers by condensation

positive control
: a group in an experiment that receives a treatment, to provide an example of what a positive result of that treatment would look like, to be used as a standard of comparison with future results.

LAB 4: PROKARYOTIC AND EUKARYOTIC CELLS

OBSERVATION OF ANABAENA OR NOSTOC

Learning Objectives

After completing the lab, the student will be able to:

1. Make wet mounts of bacteria, plant, and animal cells and view them under the microscope.
2. Observe and identify differences between cells and cell structures under low and high magnification and record your observations.

Activity 1: Pre-Assessment

1. What is the difference between the cells of a bacterium and the cells of your own body?
2. Compare and contrast the structures of prokaryotic and eukaryotic cells.
3. Discuss the answers to questions 1 and 2 with a partner and then the class.

Activity 1: Observation of Anabaena or Nostoc

Prokaryotes, unicellular organisms lacking a nucleus, include cyanobacteria (formerly blue-green algae. This name is now considered inaccurate because algae are eukaryotes). Cyanobacteria, like those shown in Figure 4.1, are **photoautotrophs**—organisms that carry out **photosynthesis** by using light energy, water, and carbon dioxide from the air and converting to sugars, and providing oxygen to the atmosphere as a waste product. Cyanobacteria contain pigments capable of capturing light energy but do not contain chloroplasts. Cyanobacteria are single-celled organisms, but some can form colonies with differentiated cell types. For example, some species can form specialized cells called **heterocysts**—structures containing enzymes that can take atmospheric nitrogen (**nitrogen fixation**) from the air and convert it into usable molecules for DNA, RNA, and protein synthesis. Nitrogen fixation involves converting nitrogen gas (N2) from the atmosphere to ammonia (NH3). Ammonia is a form of nitrogen that can be used to build other molecules, including DNA, RNA, and proteins. Oxygen, a waste product of photosynthesis, interferes with a key enzyme in nitrogen

fixation. Thus, only a few cells in a colony (about 1 in 10) become heterocysts. Resources, such as ammonia and sugars from photosynthesis, are then shared between cells. These cells range from 1–40 micrometers in size. Not all bacteria can carry out photosynthesis. The majority of bacteria are heterotrophic and they live virtually everywhere on Earth. These cells are much smaller, ranging from 0.5–8 micrometers. Eukaryotic cells have many more features and organelles and range in size from 10–500 micrometers.

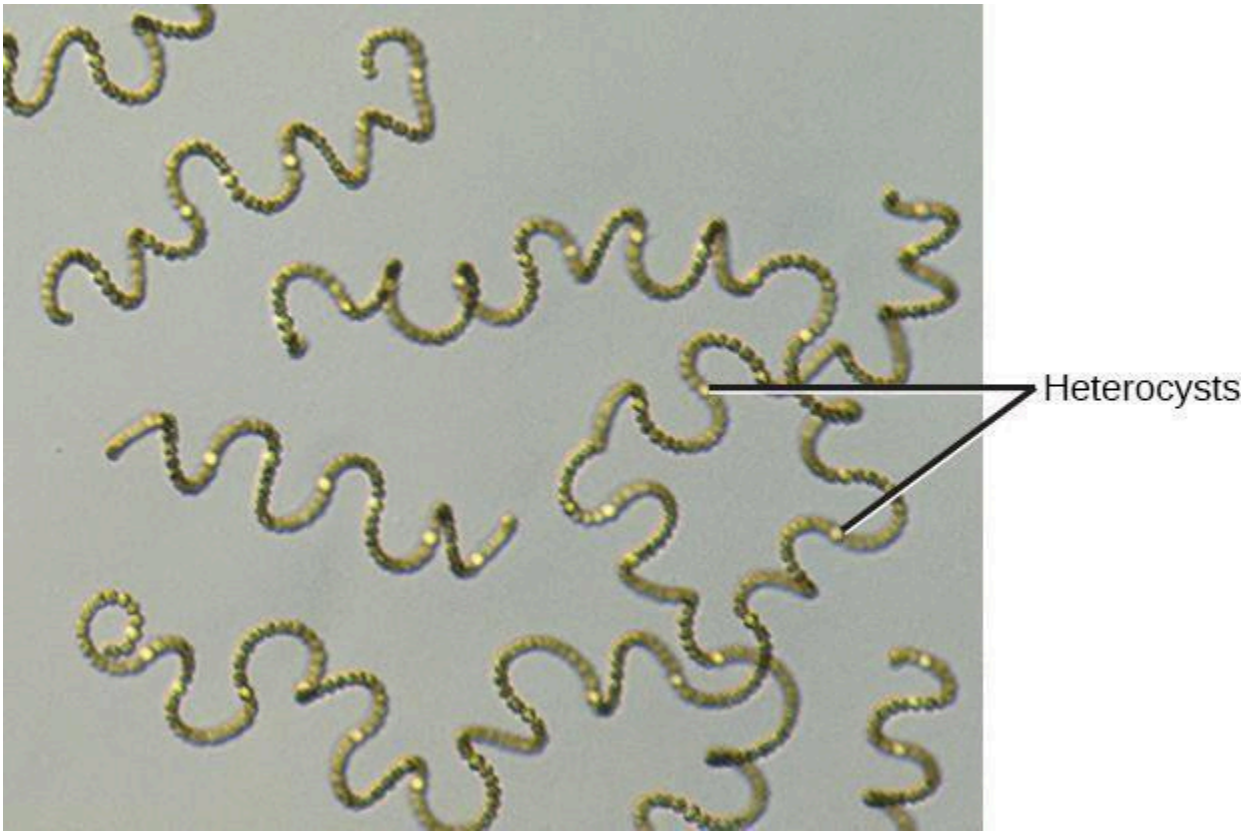


Figure 4.1: Cyanobacteria Nostoc with larger heterocysts.

Safety Precautions

- Be careful when handling glass slides; the edges may be sharp.
- Observe proper use of the microscope; avoid handling the electric cord with wet hands.
- Do not use the coarse adjustment knob of the microscope when using the high and oil immersion objective
- Inform your teacher immediately of any broken glassware, as it could cause injuries.
- Wash your hands with soap and water after handling live organisms.

For this activity, you will work *in pairs*.

For this activity, you will need the following:

- Light compound microscope (10×, 40×, 100×)
- Lens paper
- Prepared slide of *Anabaena* or *Nostoc* or images of *Anabaena* or *Nostoc*
- Special slide with micron ruler or clear millimeter ruler (you can photocopy ruler on overhead transparency, then cut and tape to microscope slide)

Structured Inquiry

Step 1: Estimate the size of the **field of view** at all the magnifications of your microscope by placing a clear millimeter ruler on the stage of the microscope. This will allow you to estimate the sizes of cells. Convert your millimeter estimates to micrometers for this activity.

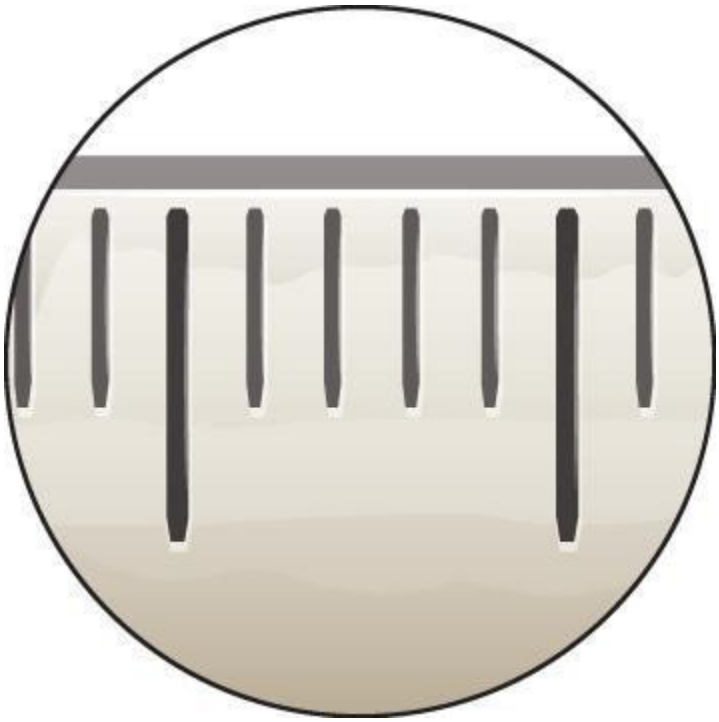


Figure 4.2: Example of a millimeter ruler taped to a microscope slide.

Step 2: Hypothesize/Predict: In your notebook predict (draw) what you would expect to see in the microscope. How big do you predict the cells will be? What features do you expect to see? Do you expect to see organelles or a cell wall?

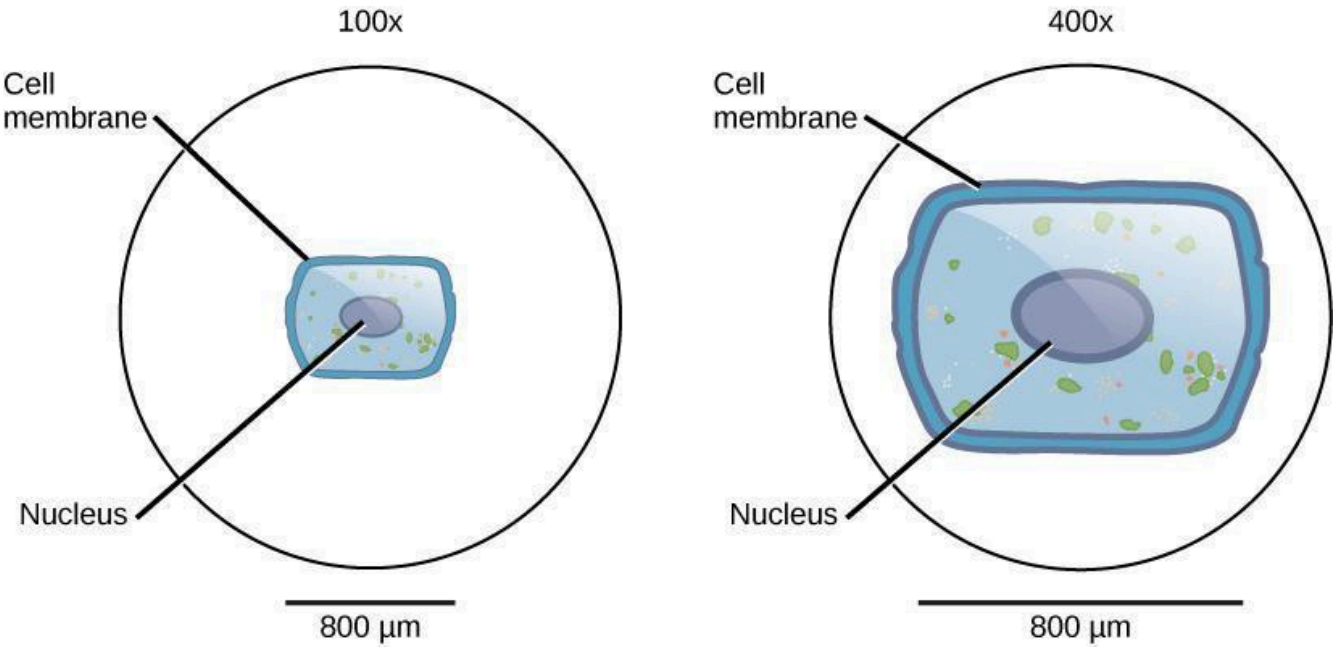


Figure 4.3: Example of a student drawing including labels and magnification.

Step 3: In your notebook, create a detailed drawing, with a sharp pencil, of the structure of the cyanobacterium. An example of a detailed drawing is seen in Figure 4.3. Record the estimated size of the cells at the magnification used. Use color in your drawings if appropriate. Identify the colors used and label any obvious structures. Note the shapes or organization of the cells.

Step 4: Critical Analysis: Think about the cell types you observed. Do your observations match your expectations? Given that the cyanobacteria are photosynthetic, was the color what you expected? Why? Did you expect the cells to have organelles? Did they? Did they have a cell wall? Did you find heterocysts? Discuss with your partner and write your answers in your notebook.

Assessments

1. Create a diagram of a general prokaryotic cell and a general eukaryotic cell. Label the cell structures that differ between the two cell types.
2. How would internal membrane-bound structures, such as chloroplasts and mitochondria, allow chemical reactions to occur more efficiently in cells?

COMPARING PLANT CELLS

Learning Objectives

After completing the lab, the student will be able to:

1. Make wet mounts of bacteria, plant, and animal cells and view them under the microscope.
2. Observe and identify differences between cells and cell structures under low and high magnification and record your observations.
3. Explain how and why microscope stains are used when viewing cells under the microscope.

Activity 2: Pre-Assessment

1. What new structures would you observe in *Elodea* cells that are not present in a cyanobacterium cell?
2. Which of those structures would you expect to observe in an onion skin cell? Can you explain why some structures will be present in an *Elodea* cell but not in an onion epidermal cell?
3. Discuss the answers to questions 1 and 2 with a partner and with the class.

Activity 2: Comparing Plant Cells

Plant cells are **eukaryotic**; they are larger than prokaryotic cells and have subcellular organelles. Like bacteria, they have a **cell wall** to help keep the cell rigid—in plants, the cell wall is composed of a complex carbohydrate called *cellulose*. Plant cells, like that shown in Figure 4.4, also have a **nucleus** with DNA and a **large central vacuole** full of water and other important substances for maintaining life, such as carbohydrates, non-nutrients, and wastes, and help maintain cell pressure. Many plant cells also have chloroplasts with chlorophyll where photosynthesis occurs and/or other membrane-bound plastids, such as amyloplasts that store starch or chromoplasts that store pigments.

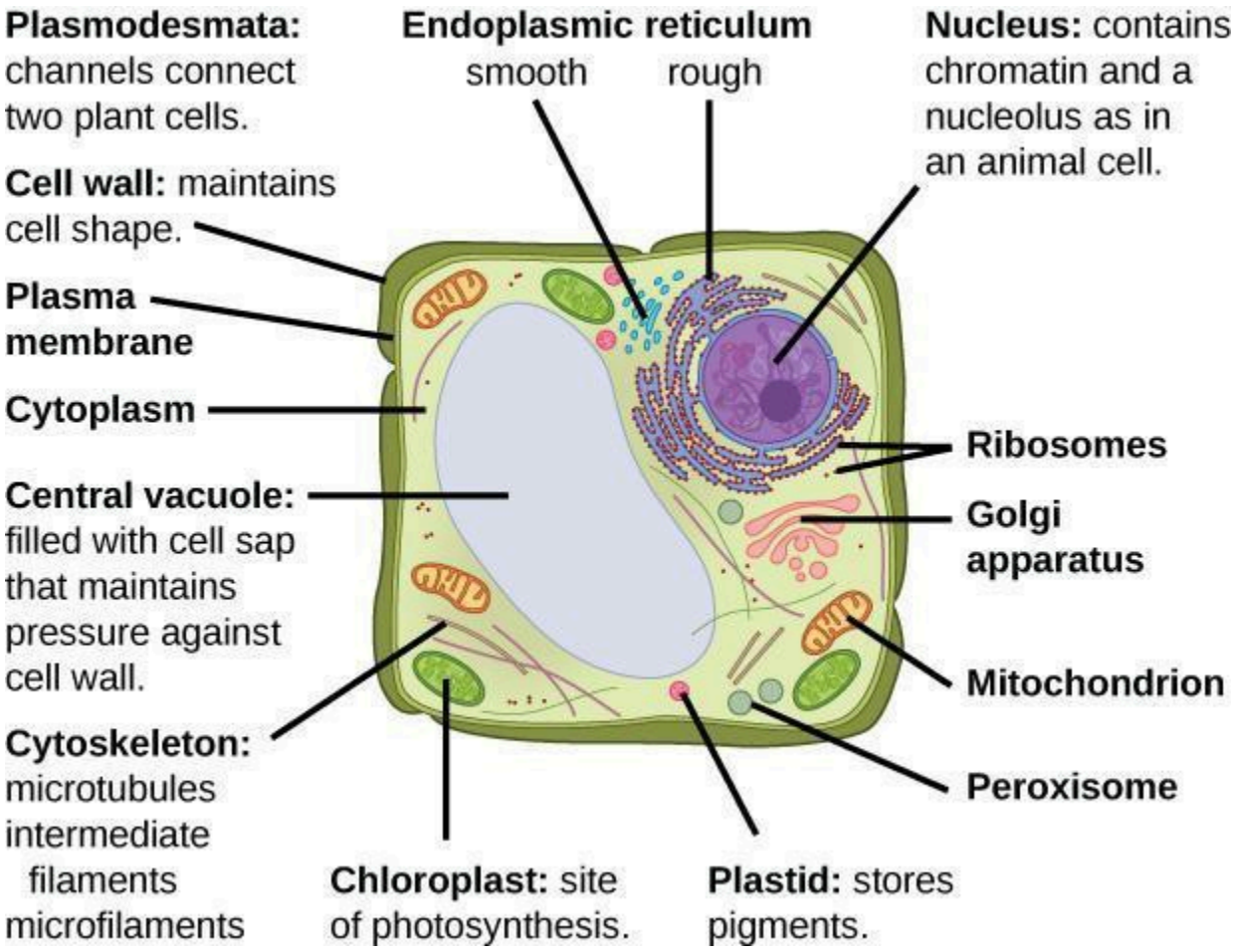


Figure 4.4: Structures found in a typical plant cell.

Safety Precautions

- Be careful when handling glass slides; the edges may be sharp.
- Dispose of used cover slips in a glass disposal box.
- Observe proper use of the microscope; avoid handling the electric cord with wet hands.
- Do not use the coarse adjustment knob of the microscope at high and oil immersion objectives.
- Inform your teacher immediately of any broken glassware, as it could cause injuries.
- Wash your hands with soap and water after handling live organisms.

For this activity, you will need the following:

- *Elodea anacharis*, moss, or *Spirogyra* green algae
- Light compound microscope
- Clean microscope slides and cover slips

- Lens paper
- Water and dropper
- Yellow onion
- Potato
- Forceps
- Iodine solution

For this activity, you will work *in pairs.*

Guided Inquiry

Step 1: Hypothesize/Predict: What features do you expect to see in the plant cell *Elodea* and the onion skin under low and high magnification? Draw and label your prediction in your notebook.

Step 2: Student-led Planning: Prepare a wet mount of *Elodea,* moss, or Spirogyra by removing a small piece with forceps, placing it on the middle of the slide, and adding a small drop of water. Place one edge of the coverslip in the water and drop onto the specimen to remove air bubbles. To prepare a wet mount of onion, remove a thin piece of the onion skin with forceps, add one drop of iodine solution to act as a stain, and place a coverslip on top of the specimen. To prepare a wet mount of potato, scrape potato cells onto the slide, add iodine and a coverslip. Record your observations as drawings. Use color if present, label the magnification, and estimate the size of the cells in your notebook. Each partner is expected to prepare one sample. Each person should view, draw, state the size and magnification, and label each sample.

Step 3: Critical Analysis: What differences are you expecting to see between *Elodea,* onion, and potato? What similarities? If you used iodine as a stain, did that reveal any other differences in the plants? Why would these organisms have anything in common based on your prediction in Step 1?

Assessments

1. Were the features you predicted to see in *Elodea* cells and the onion cell visible at low and high magnification? Which structures in Figure 4.4 can you identify in the *Elodea* cells? What about the onion cells? Why do you think this is the case?
2. What are the similarities and differences between a cyanobacterium and a plant cell?

OBSERVE ANIMAL CELLS AND IDENTIFY THEIR COMPONENTS

Learning Objectives

After completing the lab, the student will be able to:

1. Make wet mounts of bacteria, plant, and animal cells and view them under the microscope.
2. Observe and identify differences between cells and cell structures under low and high magnification and record your observations.
3. Explain how and why microscope stains are used when viewing cells under the microscope.

Activity 3: Pre-Assessment

1. Answer the following question in your notebook: How do plant cells and animal cells differ? Why would these differences likely evolve in plant and animal cells?
2. Answer the following question in your notebook: What microscope techniques could help us see more structures within cells?
3. Discuss the answers to questions 1 and 2 with a partner and then the class.

Activity 3: Observe Animal Cells and Identify Their Components

Animal cells are eukaryotic and possess subcellular components in common with the plant cells you observed in Activity 2. Organelles that plant and animal cells share in common include the nucleus, **Golgi apparatus**, **mitochondria**, **ribosomes**, and the **endoplasmic reticulum.** These are all participants in protein synthesis. An illustration of an animal cell is shown in Figure 4.5. There are some exceptions to these general components. For example, mature red blood cells (**RBC**) which have ejected their nuclei to have more room

for **hemoglobin**, the protein that carries oxygen around the body. One of the easiest eukaryotic cells to obtain in the lab is the **squamous epithelial** cell found in the tissue lining the internal surface of your cheek. These cells are arranged in a flat layer and are easy to remove and observe.

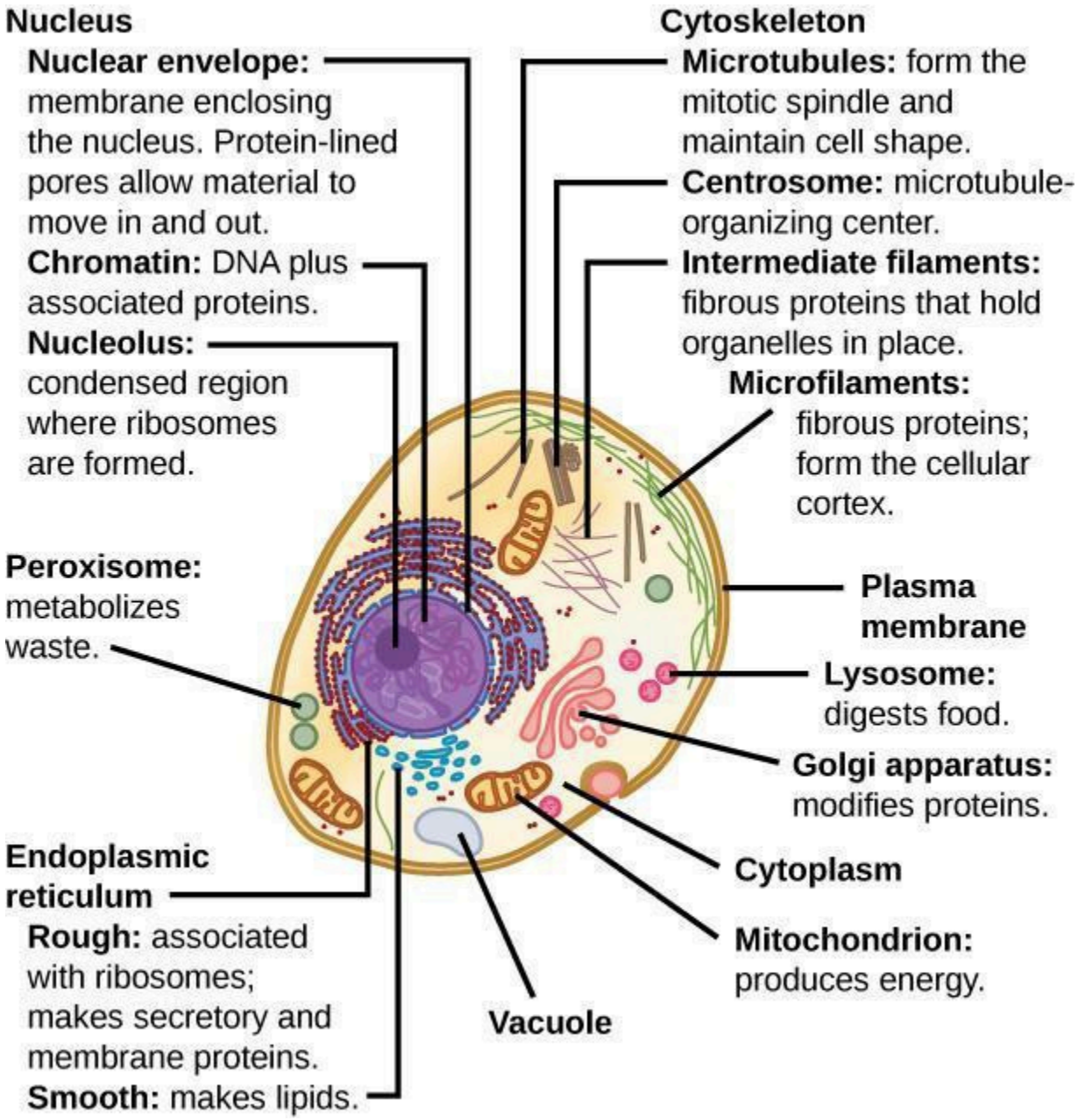


Figure 4.5: Diagram of the parts of an animal cell.

Safety Precautions

- Be careful when handling glass slides; the edges may be sharp.
- Dispose of used cover slips in a glass disposal box.

- Observe proper use of the microscope; avoid handling the electric cord with wet hands.
- Do not use the coarse adjustment knob of the microscope at high and oil immersion objectives.
- Inform your teacher immediately of any broken glassware as it could cause injuries.
- Used cotton swabs are considered biohazard; dispose of swabs in the biohazard trash container as soon as you have used them.
- Methylene blue is a dye; be cautious not to ingest methylene blue.
- Wash your hands with soap and water after handling live organisms.

For this activity, you will need the following:

- Prepared slide of red blood cells
- Light compound microscope
- Clean microscope slide, cover slip
- Clean cotton swab or toothpick
- 0.5–1 percent methylene blue solution
- Dropper or pipette
- Small squares of paper towels

For this activity, you will work *in pairs.*

Guided Inquiry

Step 1: Hypothesize/Predict: Predict the different features you expect to see in the animal cell versus the plant cell. Predict the differences you will see between animal cells and prokaryotic cells under low and high magnification. Include in your prediction the size differences between a cyanobacterium, plant cells, and animal cells. Create a table in your notebook to draw and label your predictions in your notebook.

Step 2: Student-led planning: Observe the red blood cell prepared slide. Record your observations (draw and label any visible parts, use color if visible, include magnification and size of cells) in your notebook. Both partners should view, draw, state the size and magnification, and label each sample.

Step 3: Prepare your cheek cell slides as shown in Figure 4.6 and Figure 4.7 and outlined below.

- Take a clean cotton swab or toothpick and gently scrape the inside of your mouth.
- Smear the cotton swab or toothpick on the center of the microscope slide for 2 to 3 seconds.
- Add a small drop of methylene blue solution (a dye) and place a coverslip on top.
- Remove any excess solution by allowing a paper towel to touch one side of the coverslip.
- View the slide at all magnifications.
- Record your observations as drawings. Use color if present, label the magnification, and estimate the size

of the cells in your notebook. Record your observations (drawings, color if present, labels, magnification, and size of cell) in your notebook.

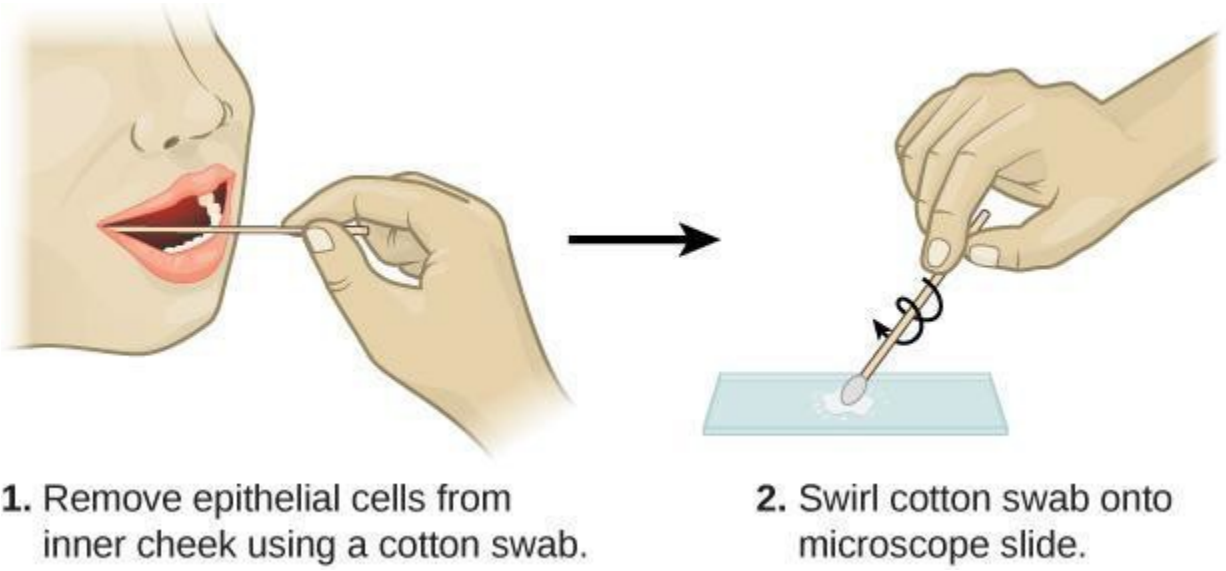


Figure 4.6: How to prepare a cheek swab.

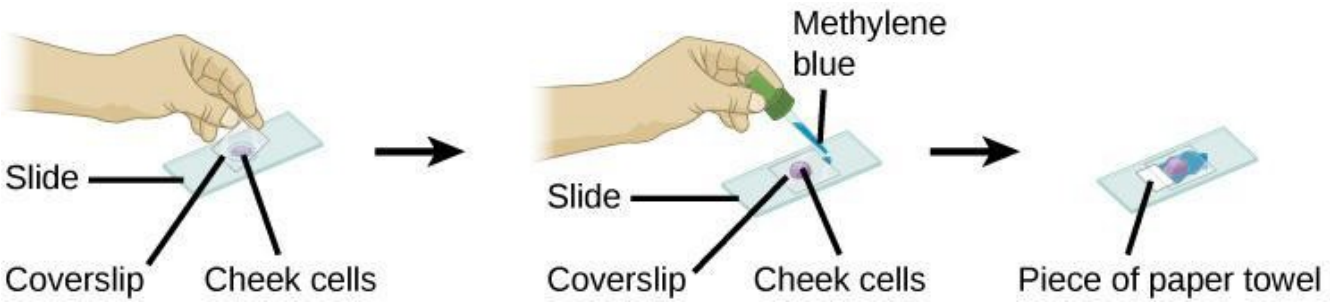


Figure 4.7: How to stain cheek cells with methylene blue dye.

Step 4: Critical Analysis: Were differences observed between the RBC and the cheek cell? What does the methylene blue stain reveal in the cheek cell? There should be small blue dots visible on the cheek cells much smaller than the nuclei.

Hypothesize what those blue dots might be. How does the animal cell compare to the plant cells in Activity 2 and the cyanobacteria in Activity 1? Record the answers to these questions in your notebook.

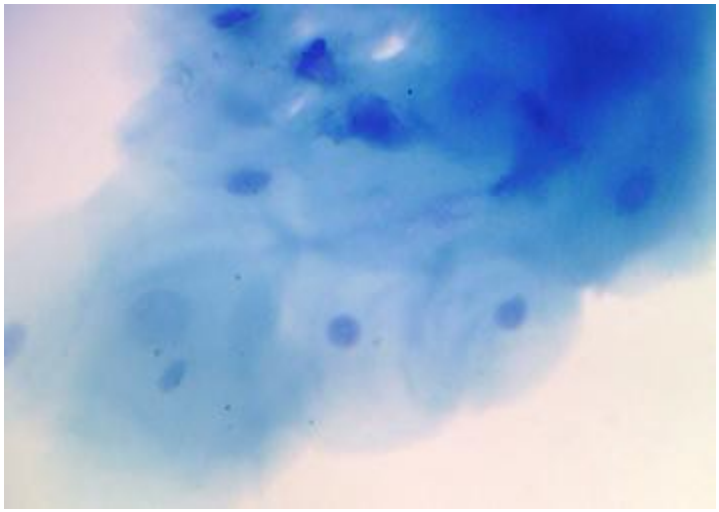


Figure 4.8: Cheek cells stained with methylene blue dye.

Assessments

1. Based on the staining technique you performed in this activity, how could you distinguish stained prokaryotic cells from stained eukaryotic cells?
2. What do all cells have in common, whether prokaryotic or eukaryotic? What major differences would you expect to find?
3. Identify whether the following images (Figure 4.9a, Figure 4.9b, and Figure 4.9c) show an animal cell, a plant cell, or a prokaryote cell. Explain how you know the difference.

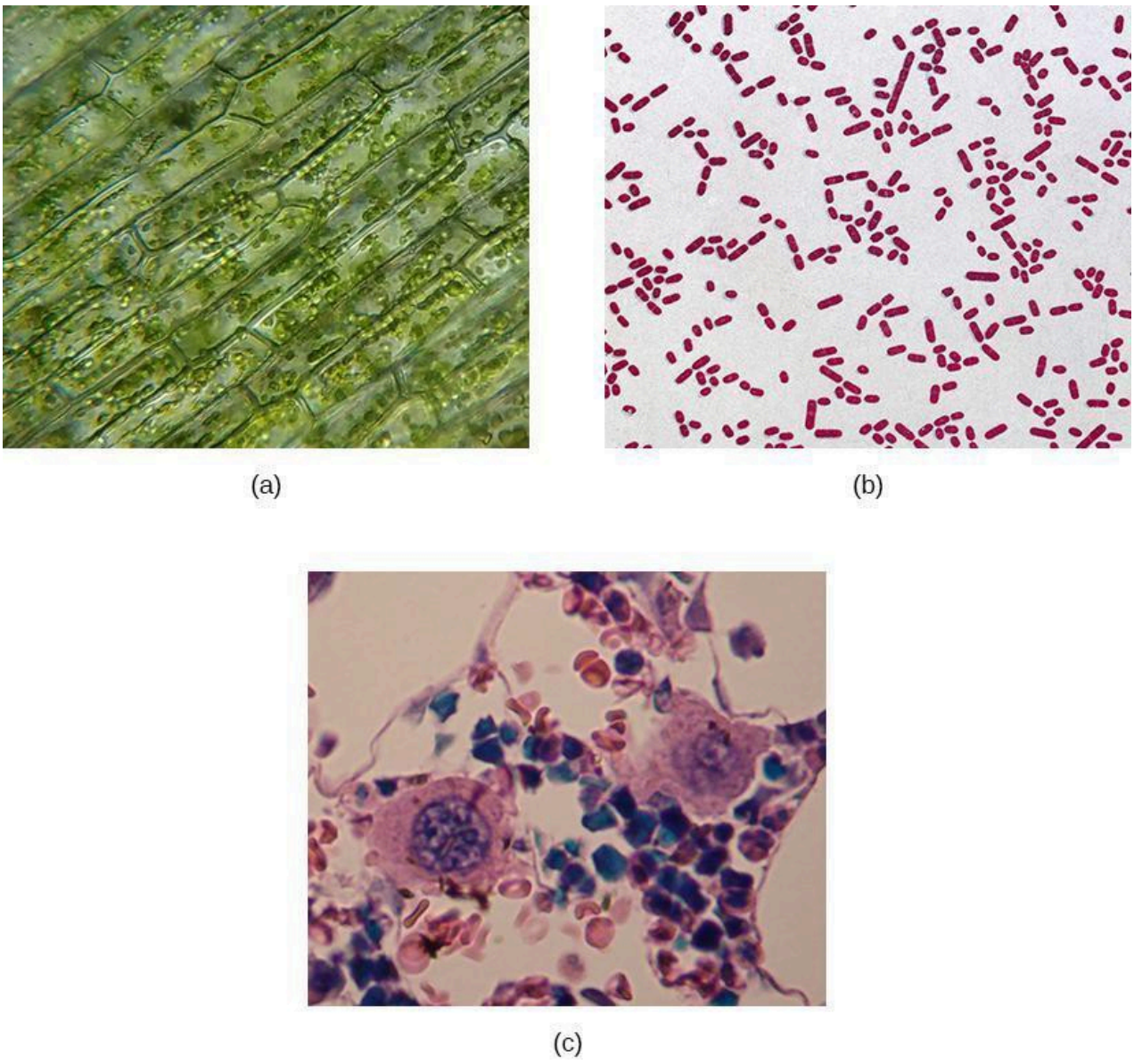


Figure 4.9: This figure shows three photos of different cell types. The photo in part (a) shows green cells with smaller organelles within. The photo in part (b) shows numerous tiny oval-shaped cells. The photo in part (c) shows a complex arrangement of different types of cells, some with a nucleus.

KEY TERMS

cell wall
rigid cell covering comprised of various molecules that protects the cell, provides structural support, and gives shape to the cell

central vacuole
large plant cell organelle that regulates the cell's storage compartment, holds water, and plays a significant role in cell growth as the site of macromolecule degradation

endoplasmic reticulum (ER)
series of interconnected membranous structures within eukaryotic cells that collectively modify proteins and synthesize lipids

eukaryotic cell
cell that has a membrane-bound nucleus and several other membrane-bound compartments or sacs

field of view
area visible through the microscope

Golgi apparatus
eukaryotic organelle comprised of a series of stacked membranes that sorts, tags, and packages lipids and proteins for distribution

nucleus
cell organelle that houses the cell's DNA and directs ribosome and protein synthesis

photoautotrophs
organisms that carry out photosynthesis

photosynthesis
chemical process that uses light energy, water, and carbon dioxide to produce sugar and oxygen

prokaryote
unicellular organism that lacks a nucleus or any other membrane-bound organelle

red blood cells
small, disc-shaped anucleate cells filled with the protein hemoglobin in the blood that transport oxygen throughout the body

ribosome
cellular structure that carries out protein synthesis

squamous epithelial cells
thin, flat cells that are arranged in layers on body surfaces

LAB 5: SUBCELLULAR STRUCTURES

CHROMOPLASTS AND AMYLOPLASTS

Learning Objectives

After completing the lab, the student will be able to:

1. Prepare wet mounts of carrot slices and observe chromoplasts.
2. Prepare wet mounts of potato slices and observe before and after staining with iodine.

Activity 1: Pre-Assessment

1. Why would pigments make visualizing subcellular structures easier under the light microscope?
2. How could you tell the difference between a lipid-soluble pigment and a water-soluble pigment when viewed immersed in water on a slide under a light microscope?
3. **Discuss the answers to questions 1 and 2 with a partner (think, pair, and share) and then the class.**

Activity 1: Chromoplasts and Amyloplasts

All plastids originate from proplastids that can differentiate into several mature types of plastids, as shown in Figure 5.1, similar to the idea of a stem cell. **Chloroplasts** ("green bodies"; Figure 5.2) are an example of a mature plastid that contains **pigments**, water insoluble lipid molecules that can be found in membranes and are capable of absorbing light energy. These start the process of photosynthesis. You will examine a few different types of plastids in this lab. The two plastids that are examined in this activity are **chromoplasts** and **amyloplasts**, shown in Figure 5.3 and Figure 5.4, respectively. Chromoplasts, or *colored bodies*, are mature plastids that are used primarily for the synthesis of pigments and their storage. Chromoplast pigments are found in many plant parts such as flowers, roots, and aging leaves. The purpose of these plastid pigments is to attract pollinators to the plant and to advertise to different animals that eat the fruit (such as mammals, birds, insects, and reptiles) and then disperse the seeds, generally with a bit of fertilizer. These pigments are also

found in root vegetables such as carrots and yams. Amyloplasts, meaning *starch bodies*, are a type of leucoplast, or *white body*, that are colorless; these plastids synthesize glucose into starch, store starch, and help the plant detect gravity using **statoliths**.

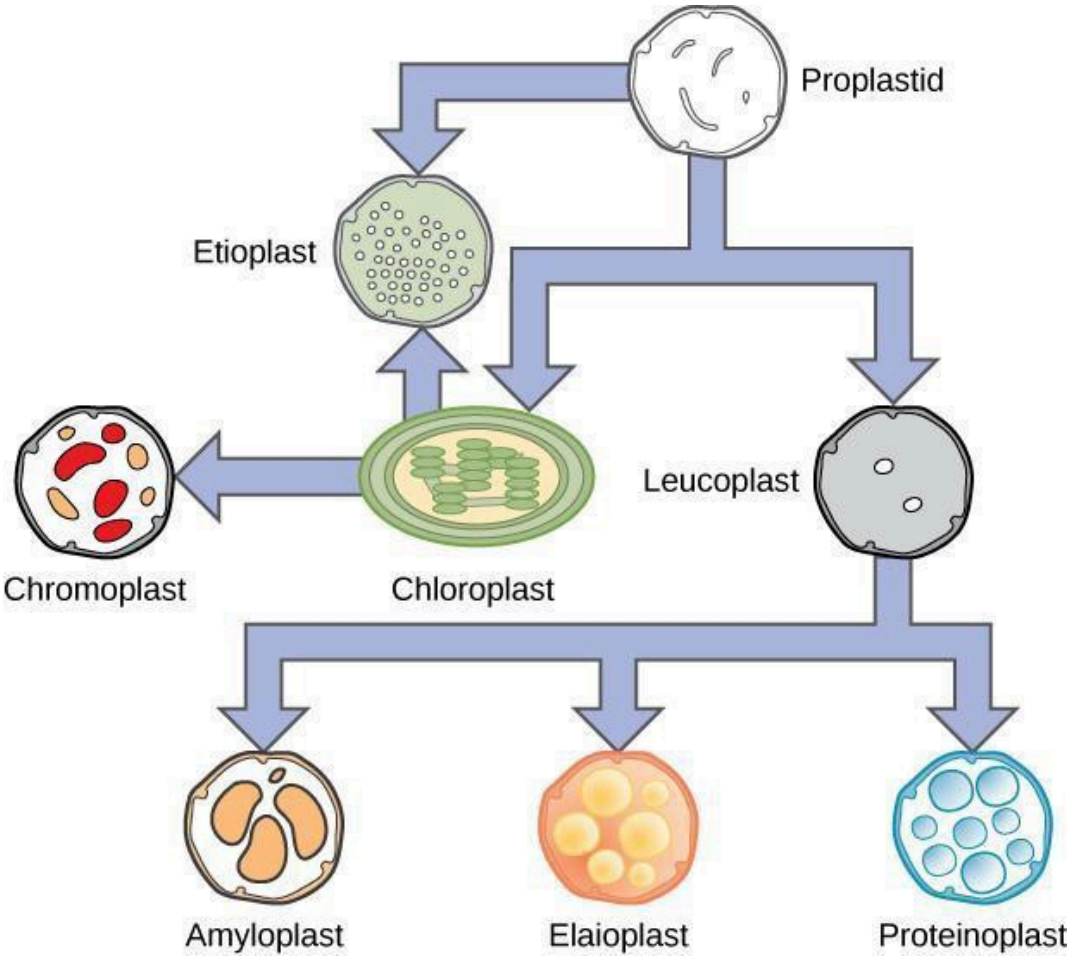


Figure 5.1: Protoplasts are the stem for several other plastids.

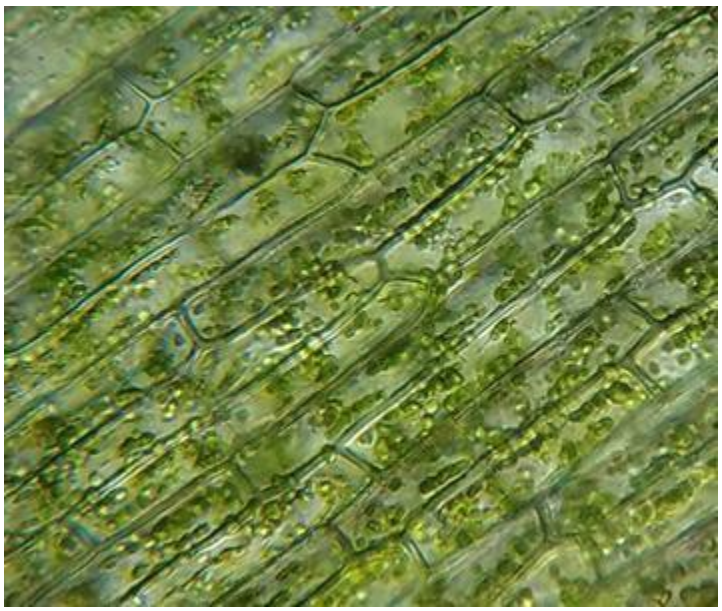


Figure 5.2: A micrograph of chloroplasts within plant cells

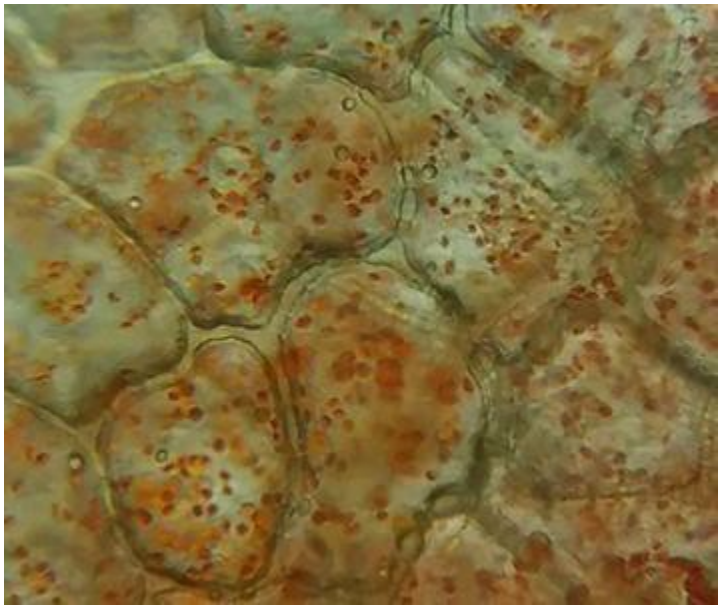


Figure 5.3: A micrograph of chromoplasts within plant cells

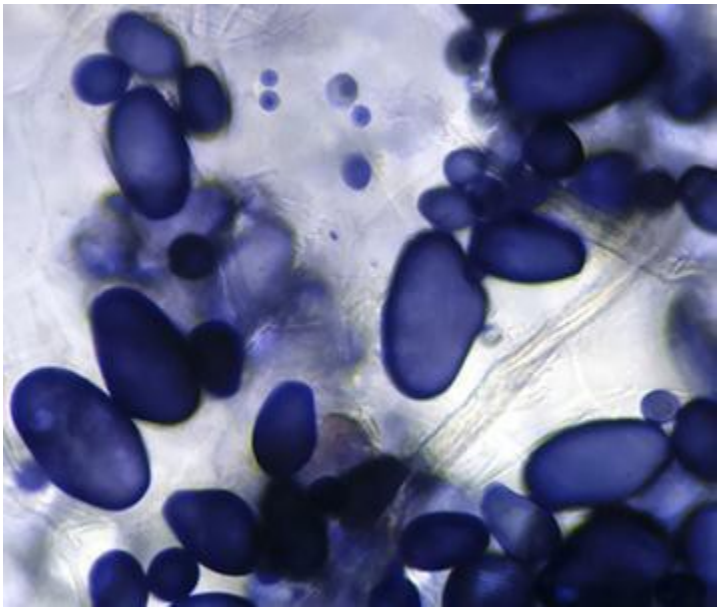


Figure 5.4: A micrograph of amyloplasts within plant cells

Safety Precautions

- Be careful handling glass slides, the edges may be sharp.
- Cut away from your body. Sectioning of potato and carrots involves using a single-edged razor blade, use care to avoid cuts.
- Observe proper use of the microscope; avoid handling the electric cord with wet hands.
- Do not use the coarse adjustment knob of the microscope at higher magnifications.
- Inform your teacher immediately of any broken glassware, as it could cause injuries.
- Dispose of biological stains according to teacher instructions and local regulations.
- Wash your hands with soap and water after handling biological stains.

For this activity, you will need the following:

- Clean microscope slides and coverslips
- Single-edge razor blade
- Carrot and potato
- Iodine stain (or Lugol's stain) in dropper bottle
- Paper towels

For this activity, you will work *in pairs*.

Structured Inquiry

Step 1: Make extremely thin slices of the carrot. The slice should be as transparent as possible. Place the carrot slice on a clean microscope slide with a coverslip. If the coverslip does not lie flat, the slice is too thick. Observe under middle (100×) and high power (400×) and draw and label what you observe in your notebook, and record the magnification. Note—you do not need to add a drop of water before placing the coverslip.

Step 2: Hypothesize/Predict: In your notebook predict (draw) what subcellular components you expect to see in the microscope with a potato slice. Will the addition of a biological stain (a dye that stains cellular structures) help visualize subcellular structures?

Step 3: Repeat Step 1 with the potato slice. Do not add iodine. Observe under high power (100×) and then draw and label what you observe in your notebook, recording the magnification.

Step 4: Add a drop of iodine stain to the edge of the coverslip. Grasp a small piece of paper towel and dab it to the opposite side of the coverslip to draw the iodine through the potato slice, as illustrated in Figure 5.5. Observe the slice under high power and draw and label what you observe in your notebook, recording the magnification.

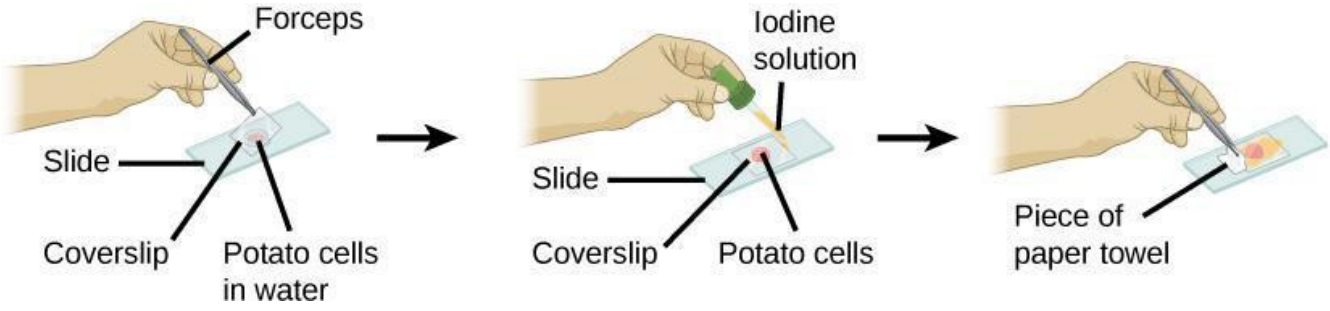


Figure 5.5: Process for making a wet mount cell of potato tissue

Step 5: Critical Analysis: Are the predictions you made in Step 2 supported by your data and observations? Why or why not? Are there any methods you could use to improve your results? Discuss with your partner and then write your answers in your notebook.

Assessments

1. How do your drawings of the microscope slides compare to the internet images in Figure 5.2, Figure 5.3, and Figure 5.4?
2. Iodine (or Lugol's solution) is not a very water-soluble stain. What type of molecules do you think the iodine solution is staining in the potato slice (polar or nonpolar)?
3. Based on the information provided above, what components of the cell are likely to have this characteristic?

CENTRAL VACUOLE AND ANTHOCYANINS

Learning Objectives

After completing the lab, the student will be able to:

1. Make wet mounts of red onion skin.
2. Describe the effect of pH on red onion skin.

Activity 2: Pre-Assessment

1. How do plants and animals capture energy that they use to survive?
2. Describe aspects of the morphological (physical structure) and behavioral characteristics of plants and animals that reflect their different means of acquiring energy.
3. Discuss the answers to questions 1 and 2 with a partner and then the class.

Activity 2: Central Vacuole and Anthocyanins

Plant cells are eukaryotic; they have subcellular organelles. Like bacteria, they have a **cell wall** to help keep the cell rigid—although in plants the cell wall is composed of a complex carbohydrate called cellulose. Plant cells also have a **nucleus** with DNA and a **central vacuole** (see Figure 5.6) full of water and other important substances (such as carbohydrates, non-nutrients, wastes) for maintaining life and to help maintain cell pressure.

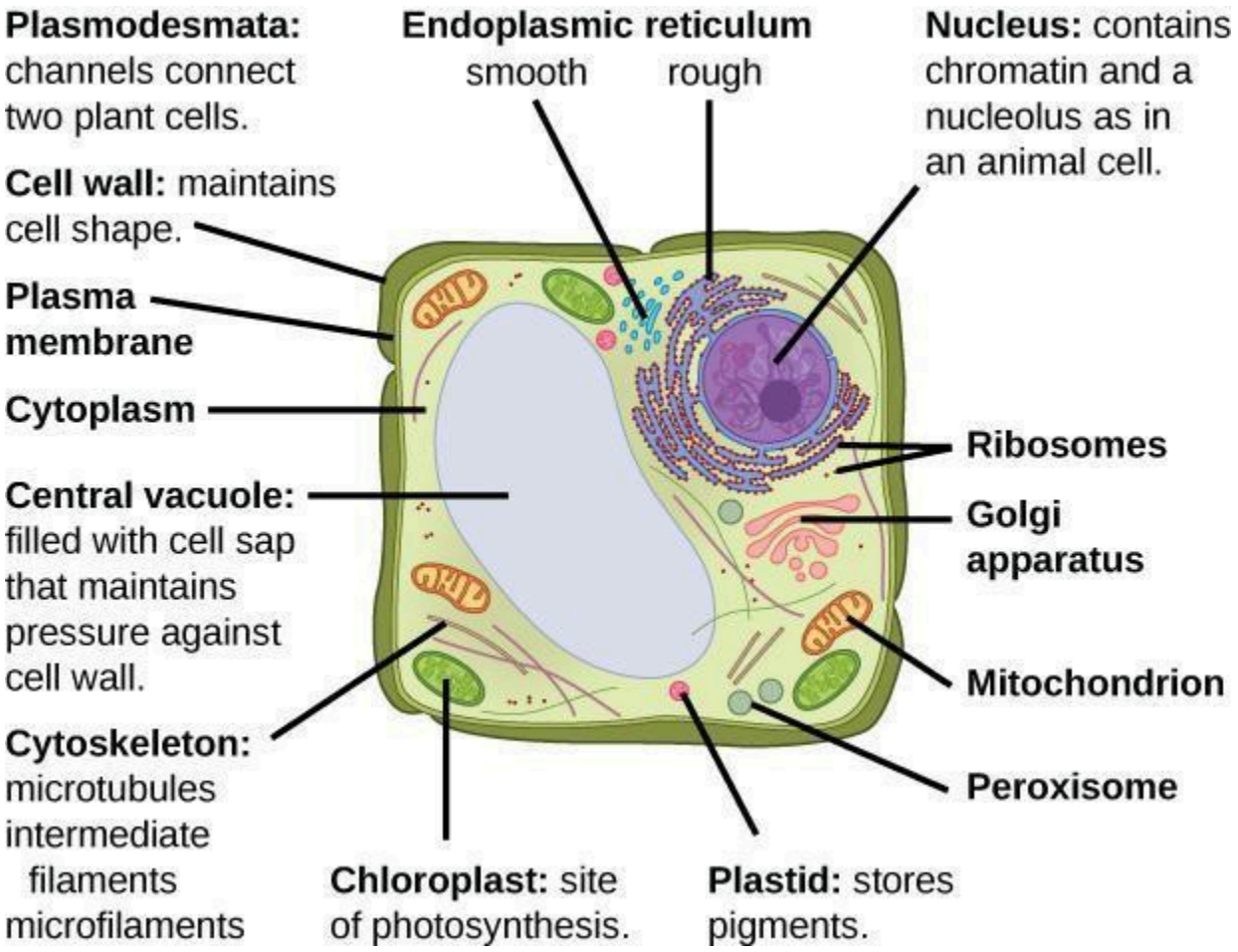


Figure 5.6: Structures of a generalized plant cell.

The second major type of pigment found in plastids is water soluble. These pigment molecules, called **flavonoids**, are stored in the central vacuole of plants. The specific pigment found in the central vacuole that is examined in this activity is called **anthocyanin** (meaning *flower blue*); the addition of a sugar group to its structure makes it water soluble. If cations (positively charged ions or polyatomic ions) are added or removed from the anthocyanin structure the pigment color will change, showing the anthocyanins are sensitive to pH changes. Anthocyanins are found in most plant tissues as well as algae and some bacteria. Anthocyanins are hypothesized to protect plant tissues from harmful UV radiation and are also used as camouflage from herbivores.

Safety Precautions

- Be careful handling glass slides; the edges may be sharp.
- Observe proper use of the microscope; avoid handling the electric cord with wet hands.
- Do not use the coarse adjustment knob of the microscope at higher magnifications.

- Inform your teacher immediately of any broken glassware, as it could cause injuries.
- Wear goggles when handling pH solutions.
- If any pH solutions get on your hands, flush with water to remove.
- Dispose of pH solutions according to teacher instructions and local regulations.
- Wash your hands with soap and water after handling pH solutions.

For this activity, you will need the following:

- Red onion
- Clean microscope slides and cover slips
- Water and dropper
- Forceps
- pH solutions in dropper bottles (pH 3.0, pH 7.0, and pH 8.5)
- Notebook to observe and draw features of plant cells. For this activity, you will work *in pairs*.

Structured Inquiry

Step 1: Hypothesize/Predict: What subcellular features do you expect to see in red onion cells? Will the central vacuole, nucleus, and plastids (including chromoplasts) be obvious? What do you hypothesize will happen to the red onion cells in various pH solutions? Record your ideas and hypothesis in your notebook.

Step 2: Student-led planning: Prepare your plant cell slides as shown in Figure 5.7. Each partner is expected to prepare one sample. Each of you should view, draw, state the size and magnification, and label each sample. To prepare the onion skin, cut the onion in quarters, take the outer red peel of an onion section, and place it on the microscope slide.

Experiment with a few different cuts to determine the best technique to get the thinnest possible slice. Spread the onion skin on the microscope slide, put one small drop of water, and top with a coverslip. Record your observations (drawings, color if present, labels, magnification, and size of cell) in your notebook, keeping in mind your hypotheses from above.

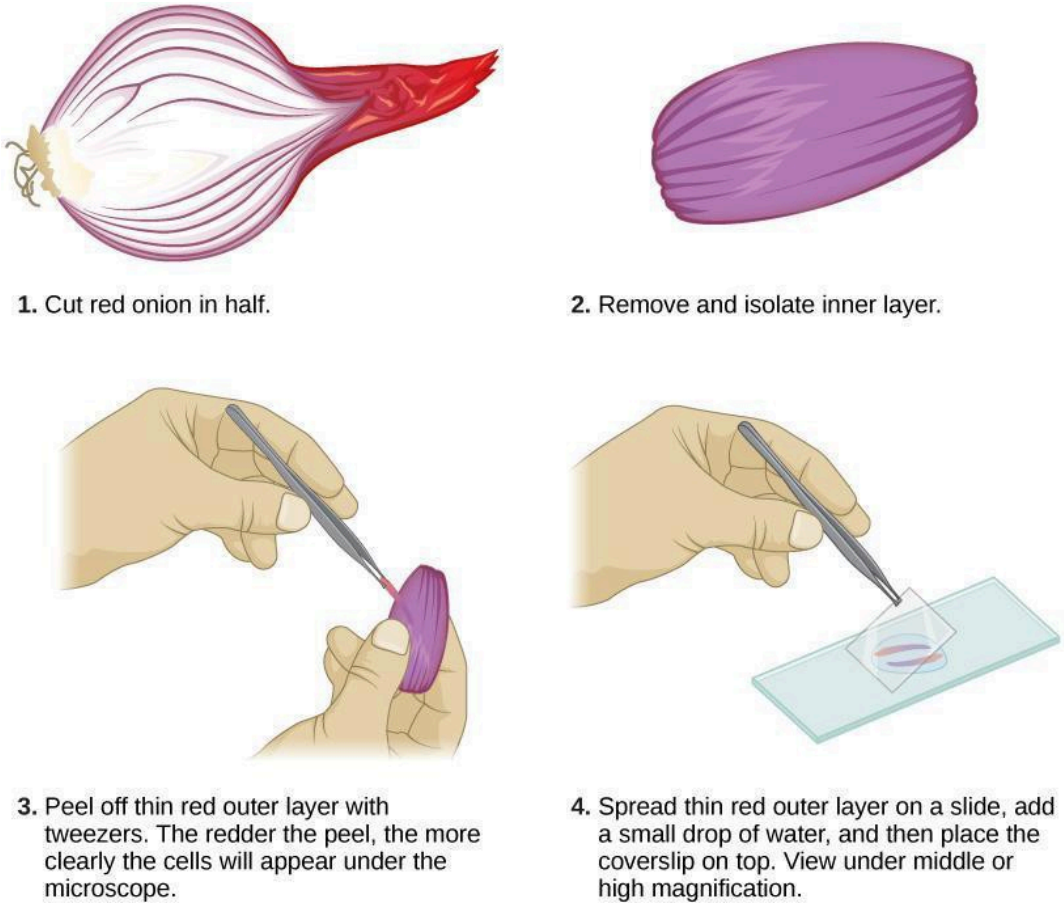


Figure 5.7: Process for preparing red onion wet mounts

Step 3: Critical Analysis: Were the predictions you made about what subcellular structures would be visible in an unstained specimen supported by your data (observations)? Why or why not? Are there any methods you could have used that would improve your results? Discuss with your partner and then write your answers in your notebook.

Guided Inquiry

Step 1: Hypothesize/Predict: A change in pH can often alter the function of macromolecules and cell structures. How do you think a change in pH will affect the pigment of the red onion cells? Write one hypothesis each describing how an increase and a decrease in pH would affect the onion cell pigment.

Step 2: Student-led planning. Your teacher has provided you with solutions of different pH. Discuss, with your partner, how you will examine each onion skin under the different pH solutions (e.g., How long should you observe each slide? How many times should you repeat your observations?). Record your plan in your notebook and create date tables in which to record your results.

Step 3: Carry out the experiment you designed in Step 2, preparing your slides using the technique in Figure 5.7 Create drawings in your notebook to record all of your observations.

Step 4: Critical analysis: Did the results you predicted match what you observed as the pH changed? In a

paragraph, describe how increasing and decreasing pH affected the red onion cells. Include an explanation of why you think pH affected the cells in the way you observed. Discuss your analysis with your partner and write it in your notebook.

Assessments

1. How were you able to find the vacuole of the red onion cells? What should you be looking for in the cell? Could you see individual chromoplasts?
2. Red wines range in color from pink, red, and even violet-blue. If red grapes are used to make red wine, what part of the grape would explain that coloration? Predict the pH of wine based on the color.

OBSERVATION OF SUBCELLULAR STRUCTURES IN ANIMAL TISSUES

Learning Objectives

After completing the lab, the student will be able to:

1. Observe Nissl bodies in a neuron as an example of endoplasmic reticulum.
2. Observe striated muscle as an example of cytoskeleton thin filaments.
3. Visualize mitochondria using a biological stain.

Activity 3: Pre-Assessment

1. What structures would you expect to find in plant cells but not animal cells?
2. Do you expect that the subcellular structures will be easy to see? Why or why not?
3. Discuss the answers to questions 1 and 2 with a partner (think, pair, and share) and then the class.

Activity 3: Observation of Subcellular Structures in Animal Tissues

Animal cells (Figure 5.8) are eukaryotic and possess subcellular components in common with the plant cells you observed earlier. Some examples include nuclei (contains DNA, controls cell function), **Golgi apparatus** (sorting, packing, and modification of proteins), **mitochondria** (energy production from organic molecules such as glucose), **ribosomes** (translation of messenger RNA into proteins), and the **endoplasmic reticulum** (folding of proteins and manufacture of lipids). Animal cells are predominantly colorless. Exceptions include the red of hemoglobin in red blood cells, red myoglobin in striated muscle, and melanin in skin cells. Thus, specific biological stains are required to make visible cellular features under a light microscope. Janus Green B stain is effective for viewing mitochondria, chromosomes, and the rough endoplasmic reticulum.

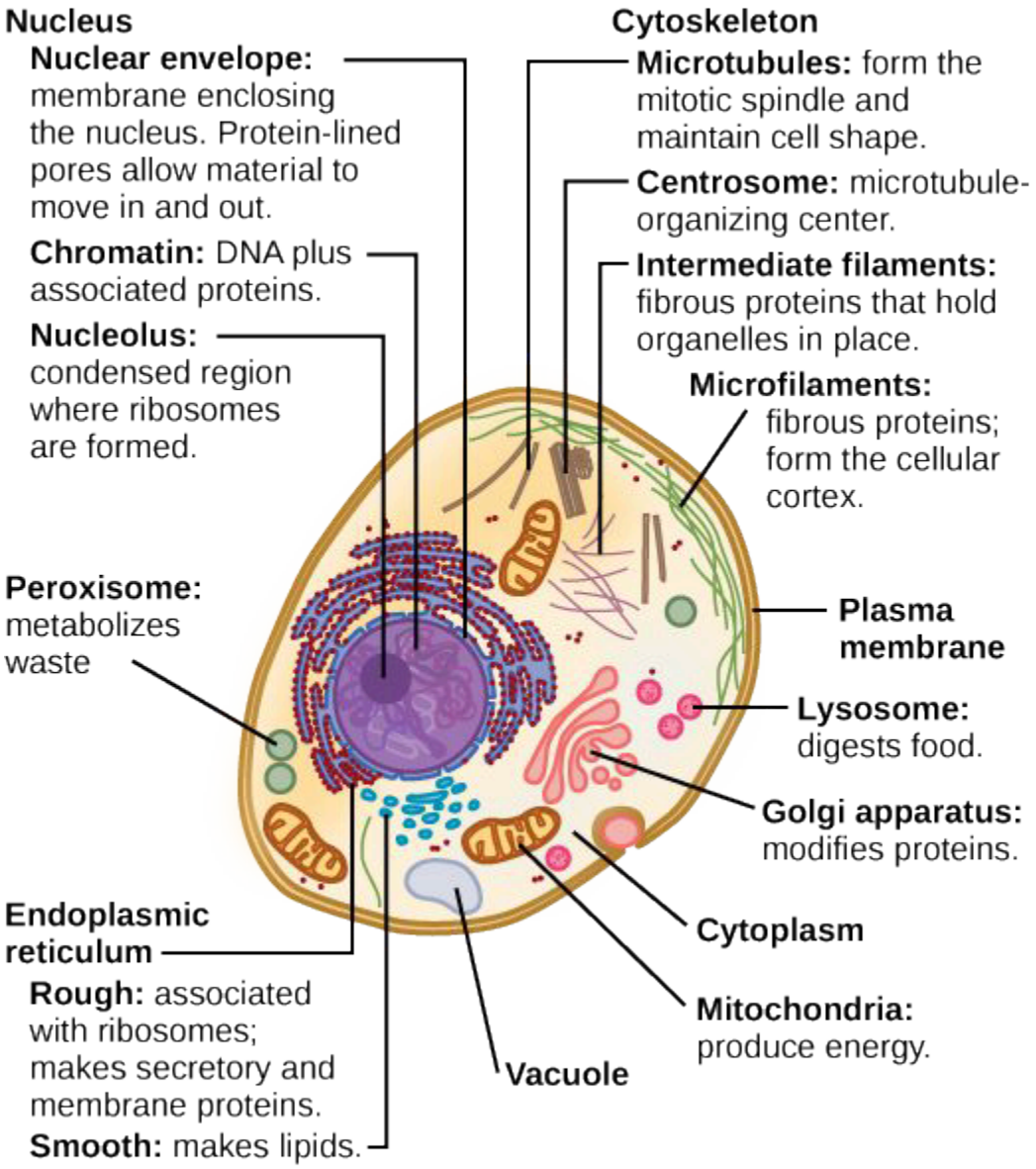


Figure 5.8: Structures of a generalized animal cell

Nissl bodies, shown in Figure 5.9, present in neurons can be stained and allow you to see the rough endoplasmic reticulum and rosettes of free ribosomes. The **cytoskeleton** includes thin and thick filaments, which are involved in cell shape, movement, and muscle contractions.

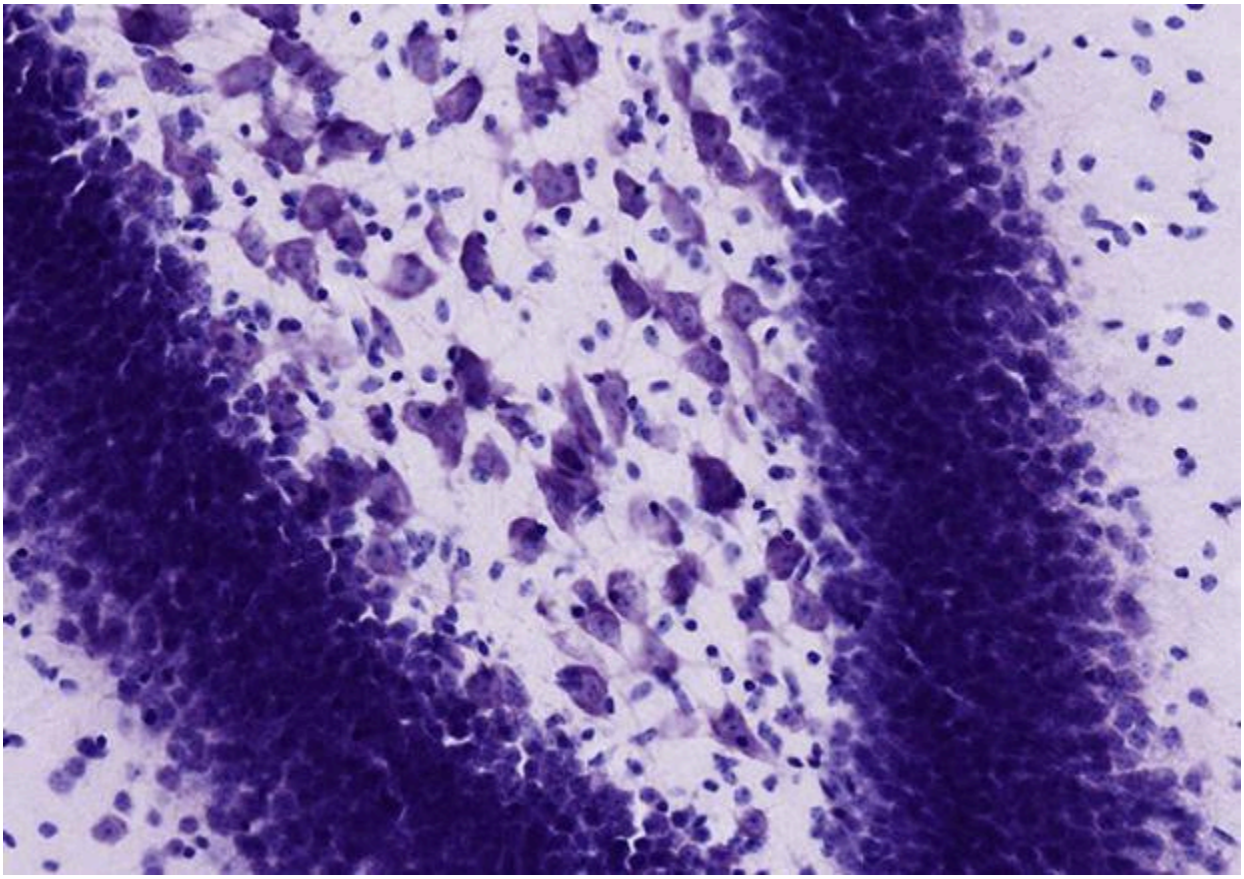


Figure 5.9: In this cross section from a rodent brain, cells containing Nissl bodies are stained purple.

Safety Precautions

- Be careful handling glass slides; the edges may be sharp.
- Observe proper use of the microscope; avoid handling the electric cord with wet hands.
- Do not use the coarse adjustment knob of the microscope at higher magnifications.
- Inform your teacher immediately of any broken glassware, as it could cause injuries.
- Wear goggles when using staining solutions.
- Dispose of staining solutions according to teacher instructions and local regulations.
- Wash your hands with soap and water after handling live organisms and biological stains.

For this activity, you will need the following:

- Prepared slides of Nissl bodies and striated muscle
- *Elodea anacharis*
- Clean microscope slides, cover slips
- Janus Green B stain

- Dropper
- Small squares of paper towels
- Notebook to observe and record data. For this activity, you will work *in pairs*.

Structured Inquiry

Step 1: Hypothesize/Predict: Predict how big the structures of the Nissl bodies, thin and thick filaments, nuclei, and mitochondria will appear differently when you look at them under the microscope. Which structures do you think you can see without staining?

Step 2: Observe the prepared slides and record (draw, label any visible parts, use color if visible, record magnification and size of cells) in your notebook.

Step 3: Critical analysis: How visible were the Nissl bodies, thin and thick filaments, and mitochondria before and after staining? Write your answers in your notebook.

Guided Inquiry

Step 1: Hypothesize/Predict: Janus Green B stains more when more oxygen is present. Given the function of mitochondria, predict how a biological stain would help you visualize mitochondria. Record your prediction in your notebook.

Step 2: Prepare a wet mount microscope slide with *Elodea anacharis*. Experiment with a few different cuts to determine the best technique to get the thinnest possible slice. Observe your prepared slide, record (draw, label any visible parts, use color if visible, record magnification and size of cells) in your notebook. Each partner should prepare a slide and observe and report on both slides.

Step 3: Each student should prepare a second wet mount, this time with Janus Green B stain, as in Figure 5.9. Observe over a period of time. Record your observations, draw, label any visible parts, use color if applicable, and record magnification in your notebook.

Step 4: Critical Analysis: Were the structures smaller or larger than you thought? There should be small blue dots visible on the *Elodea* cells—much smaller than the chloroplasts. Hypothesize what those blue dots might be. Record the answers to these questions in your notebook.

Assessments

1. Write a description that would allow another student to distinguish stained mitochondria, nuclei, and features of the rough endoplasmic reticulum from each other through a microscope.

KEY TERMS

amyloplasts
plastids that synthesize and store starch

anthocyanin
a type of flavonoid pigment

cell wall
rigid cell covering comprised of various molecules that protects the cell, provides structural support, and gives shape to the cell

central vacuole
rigid cell covering comprised of various molecules that protects the cell, provides structural support, and gives shape to the cell

centrosome
region in animal cells made of two centrioles that serves as an organizing center for microtubules

chloroplasts
organelle in which photosynthesis takes place

chromatin
protein-DNA complex that serves as the chromosomes' building material

chromoplasts
plastids used in synthesizing and storing pigments

cytoplasm
entire region between the plasma membrane and the nuclear envelope, consisting of organelles suspended in the gel-like cytosol, the cytoskeleton, and various chemicals

cytoskeleton
protein fiber network that collectively maintains the cell's shape, secures some organelles in specific positions, allows cytoplasm and vesicles to move within the cell, and enables unicellular organisms to move independently

endoplasmic reticulum
series of interconnected membranous structures within eukaryotic cells that collectively modify proteins and synthesize lipids

flavonoids
a class of biological pigments that is water soluble and found in plant vacuoles.

Golgi apparatus
eukaryotic organelle comprised of a series of stacked membranes that sorts, tags, and packages lipids and proteins for distribution

intermediate filaments
cytoskeletal component, comprised of several fibrous protein intertwined strands, that bears tension, supports cell-cell junctions, and anchors cells to extracellular structures

Janus Green B Stain
A stain used for microscopic viewing of certain components within cells, including mitochondria

lysosome
organelle in an animal cell that functions as the cell's digestive component; it breaks down proteins, polysaccharides, lipids, nucleic acids, and even worn-out organelles

microfilaments
the cytoskeleton system's narrowest element; it provides rigidity and shape to the cell and enables cellular movements

microtubules
the cytoskeleton system's widest element; it helps the cell resist compression, provides a track along which vesicles move through the cell, pulls replicated chromosomes to opposite ends of a dividing cell, and is the structural element of centrioles, flagella, and cilia

Mitochondrion
cellular organelles responsible for carrying out cellular respiration, resulting in producing ATP, the cell's main energy-carrying molecule

Nissl bodies
granules in the cytoplasm of nerve cells, visible by dying in microscopy

nucleolus
darkly staining body within the nucleus that is responsible for assembling ribosome subunits

nucleus
cell organelle that houses the cell's DNA and directs ribosome and protein synthesis

nuclear envelope
double-membrane structure that constitutes the nucleus' outermost portion

peroxisome
small, round organelle that contains hydrogen peroxide, oxidizes fatty acids and amino acids, and detoxifies many poisons

pigment
a compound which imparts color to tissues or cells

plasma membrane
phospholipid bilayer with embedded (integral) or attached (peripheral) proteins, and separates the cell's

internal content from its surrounding environment

plasmodesmata

channel that passes between adjacent plant cells' cell walls, connects their cytoplasm, and allows transporting of materials from cell to cell

plastid

a class of membrane-bound organelles (chloroplasts, amyloplasts, etc.) performing various functions within cells

ribosomes

cellular structure that carries out protein synthesis

rough endoplasmic reticulum (RER)

region of the endoplasmic reticulum that is studded with ribosomes and engages in protein modification and phospholipid synthesis

smooth endoplasmic reticulum (SER)

region of the endoplasmic reticulum that has few or no ribosomes on its cytoplasmic surface and synthesizes carbohydrates, lipids, and steroid hormones; detoxifies certain chemicals (like pesticides, preservatives, medications, and environmental pollutants), and stores calcium ions

statoliths

inclusions in plant cells, such as starch grains, used in responding to gravity

striated muscle

a type of muscle tissue that appears striped in microscopy

vacuole

membrane-bound sac, somewhat larger than a vesicle, which functions in cellular storage and transport

LAB 6: DIFFUSION AND OSMOSIS

DIFFUSION

Learning Objectives

After completing the lab, the student will be able to:

1. Explain or define the term diffusion.
2. Explain how different media affect the rate of diffusion.

Activity 1: Pre-Assessment

1. What happens when an air freshener is sprayed in a corner? What is the name of the process that causes the molecules to move?
2. Do you think that the rate of the air freshener molecules moving would change if the room temperature was warmer or colder? Why or why not?
3. Discuss the answers to questions 1 and 2 with the class.

Activity 1: Diffusion

The movement of molecules from a higher concentrated area to a wider and less concentrated area is referred to as **diffusion**. For example, you can smell the aroma of food flowing through the atmosphere as you walk towards a cafeteria. Molecules collide with each other and are in constant motion because of their kinetic energy. This activity propels molecules to move where there is a less concentrated area. Therefore, the net movement of molecules is always from a tightly concentrated area to a less tightly packed area. Osmosis is the process of water diffusion through a selectively permeable membrane. In body systems, various constituents such as gases, liquids, and solids are dissolved in water when they flow through the cell membrane from a highly concentrated place to a less concentrated area in bodily systems. In a solution, the dissolved substance is called the solute and the substance in which the solute is dissolved is called the solvent.

Diffusion is the movement of molecules from an area where the molecule is highly concentrated to an area

of low concentration, as illustrated in Figure 6.1. The rate of diffusion is dependent upon the temperature of a system, molecular size, and the medium through which diffusion is occurring (i.e., semi-solid, liquid, air). In this activity, we will be observing the diffusion of a dye through a beaker of water and through agar (a gelatinous substance), diffusion as a function of temperature, and diffusion as a function of molecular weight.

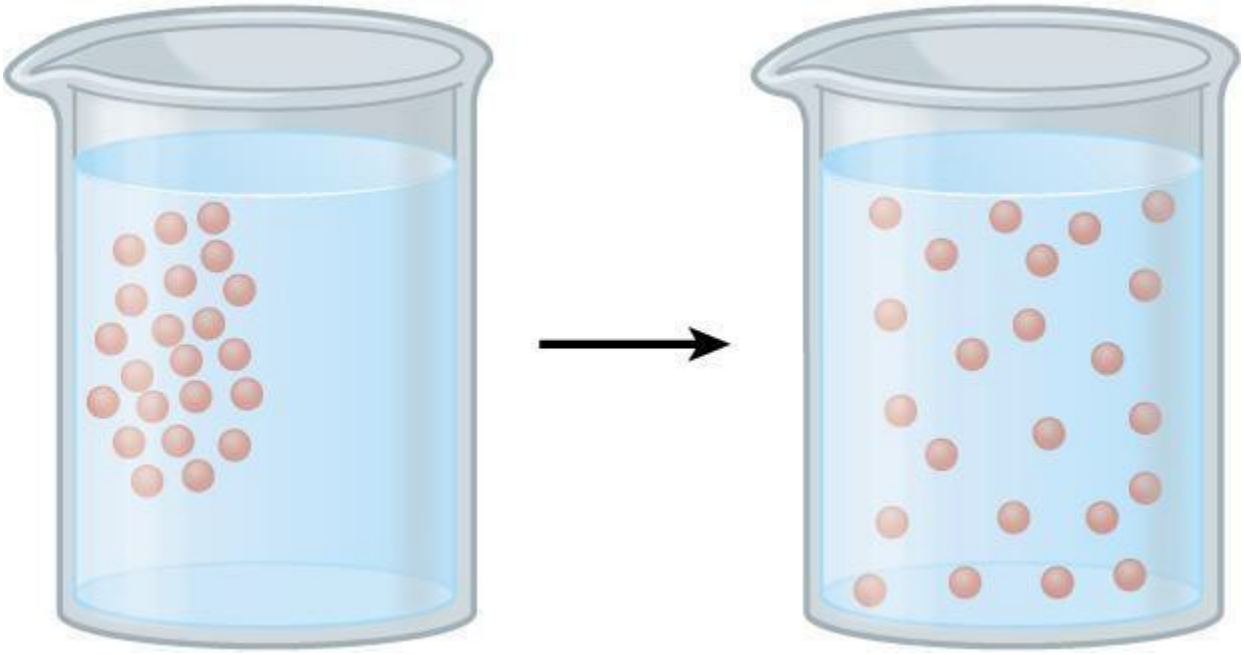


Figure 6.1: In diffusion, molecules move from areas of high concentration to areas of low concentration.

Safety Precautions

- Inform your teacher immediately of any broken glassware, as it could cause injuries.
- Clean up any spilled water or other fluids to prevent other people from slipping.
- Be careful with the dye as it can stain your clothes, and it should not be ingested.
- Wash your hands with soap and water after completion of the activity.

For this activity, you will need the following:

- Three 250 mL beakers
- Water
- Food coloring
- Agar plates
- Potassium permanganate
- Congo red

- Methylene blue
- Thermometer
- Incubator
- Refrigerator
- Clock or timer

For this activity, you will work in *groups of four*.

Structured Inquiry

Step 1: Measure 200 mL of room temperature water in a beaker. Put three drops of food coloring into the water. Time how long it takes for the dye to completely diffuse throughout the water. Record the time and describe in your notebook what you observe. Create a data table for your observations.

Step 2: Hypothesize/Predict: Predict what would happen to the rate of diffusion if you had beakers with both very hot and very cold water in them. Add your predictions to the data table you created in step 1.

Step 3: Student-led Planning: Determine how diffusion of the food color would be affected when the water is either very hot or very cold. Use a thermometer and record the temperature for each. Use a timer to measure how long it takes for complete diffusion to occur in all scenarios.

Step 4: Critical Analysis: Create a graph that shows how the diffusion rate is affected because of temperature change. Are the predictions you made in step 2 supported by your data? Why or why not? What methods could you use to improve your results? Discuss with your group and then write your answers in your notebook.

Guided Inquiry

Step 1: Gather four agar plates and the three dyes, provided by your instructor, that differ in molecular size: Congo red (mol. wt. 696.66 g/mol), methylene blue (319.85 g/mol), and potassium permanganate (mol. wt. 158.03).

Step 2: Hypothesize/Predict: How would the rate of diffusion of a molecule through a gel compare to its rate of diffusion through water? How would the rate of diffusion differ between molecules of different molecular sizes? Write your ideas in your notebook.

Step 3: Student-led planning: Use 1 plate for determining how molecular size affects diffusion using the 3 dyes. Determine how best to measure movement of the dye in an agar plate. Be sure to keep the dyes far enough apart so that they do not touch once they start diffusing. Get your instructor's approval before proceeding with the experiment. Measure the distance that the dye spreads in 20-minute intervals for 1 hour.

Step 4: Examine the effect of temperature on the rate of diffusion for 1 dye of your choosing. With your

group, determine 3 temperatures that would be appropriate. Measure the diameter of the dye spread for each. Write the results in your notebook.

Step 5: Critical Analysis: Rank all 3 dyes in terms of diffusion rate. What was the relationship between diffusion rate and molecular size? What is the relationship between temperature and diffusion rate? Discuss your answers with your group and write them in your notebook.

Assessments

1. In a system, there is a concentration of molecules. However, on the outside, there is little to no concentration of this particular molecule. In which direction would the molecules be moving more so than the other direction?
2. Diffusion is affected by what factors?
3. Dye tends to move faster in warmer temperatures. Why is this?

MEASURING OSMOSIS

Learning Objectives

After completing the lab, the student will be able to:

1. Describe how varying concentrations of solutes and solvents affect the rate of osmosis.
2. Describe or explain how temperature affects the rate of osmosis.

Activity 2: Pre-Assessment

1. What do you think happens to a plant when it is placed in salt water? Why might this occur?
2. Discuss the answers to question 1 with the class.

Activity 2: Measuring Osmosis

Osmosis is the diffusion of water across a selectively permeable membrane (i.e., cell membrane). Because of osmosis, water will move where water is less concentrated from an area of low solute (high water content) to an area with high solute concentration (low water content) (Figure 6.2). Dialysis tubing is used to model selectively permeable membranes because it will prohibit large molecules from crossing the membrane but will allow small molecules to cross. For example, water and glucose are small molecules that can easily cross the membrane. However, starch (a polymer of glucose), cannot cross due to its large molecular size. Additionally, cell surface area and volume can affect the rate of diffusion across a membrane. The **surface area-to-volume ratio** describes the relationship between the area outside the cell to the volume inside the cell (Figure 6.3). In this lab, we first will use dialysis tubing to model how the membrane selectively allows certain molecules to cross the membrane. Then, we will compare the diffusion rate of dialysis tubing with a small surface area-to-volume ratio to one that has a large surface area-to-volume ratio.

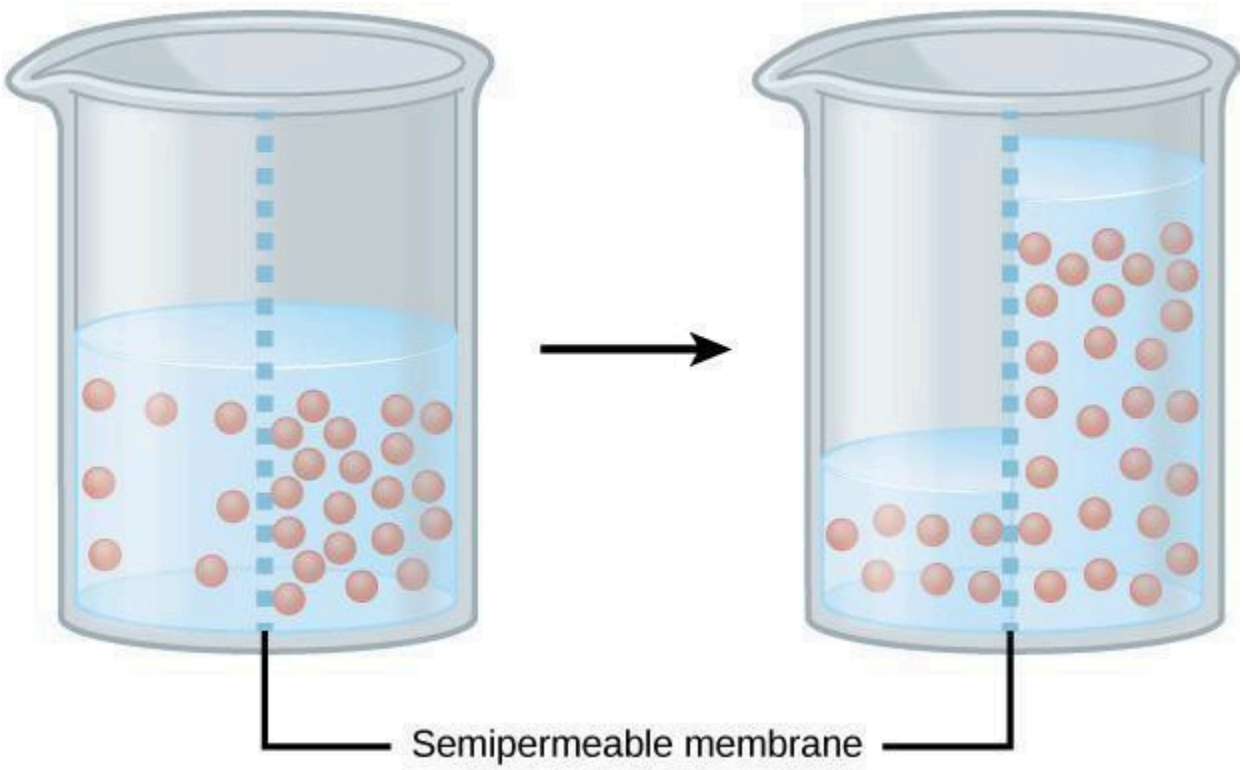
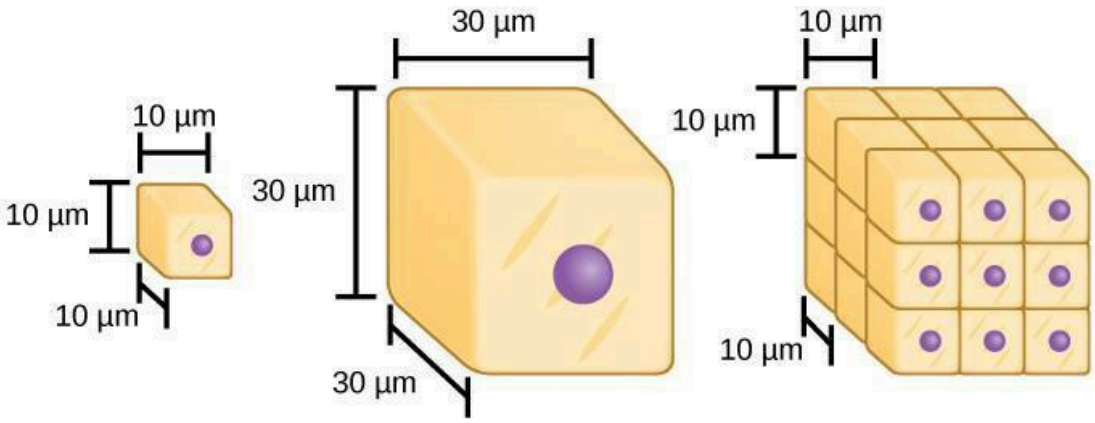


Figure 6.2: Osmosis is the diffusion of water across a semipermeable membrane. Water moves from an area of high water and low solute concentration to an area of low water and high solute concentration. Note that the solute does not move.



Surface area = height × width × number of sides × number of cubes	600 µm^2	5400 µm^2	16,200 µm^2
Volume = height × width × length × number of cubes	1000 µm^3	27,000 µm^3	27,000 µm^3
Surface area-to-volume ratio = area ÷ volume	0.6	0.2	0.6

Figure 6.3: Smaller cells have higher surface areas compared to larger cells.

Safety Precautions

- Safety goggles/glasses should be worn when chemicals or solutions are heated.
- Handle all chemicals safely.
- Inform your teacher immediately of any broken glassware, as it could cause injuries.
- Clean up any spilled water or other fluids to prevent other people from slipping.
- Dispose of all chemicals per local regulations.
- Use caution when performing the Benedict's reaction, which involves the use of a hot water bath.

For this activity, you will need the following:

- Dialysis tubing, two pieces, about 15 cm each long and narrow (pre-soaked)
- Dialysis tubing, one piece, short and wide (pre-soaked)
- Six Dialysis tubing clamps (optional)
- Three 500 mL beaker
- Water
- 5 percent Glucose solution, 10 mL

- Starch suspension, 10 mL
- Iodine
- Benedict's reagent
- Graduated cylinder
- Test tube
- Hot water bath

For this activity, you will work in *groups of four*.

Structured Inquiry

Step 1: Use a graduated cylinder to measure 250 mL of room temperature water in a beaker. Set aside. Obtain 1 piece of long and narrow dialysis tubing. Seal 1 end of the tube by tying a secure knot or attaching a dialysis tubing clamp to the end of the tubing. Put 10 mL of starch suspension and 10 mL of glucose solution in the tube. Then tie or clamp the other end of the tube. Place the sealed tube into the beaker (Figure 6.4). Develop and implement the use of an appropriate controlled experiment to go alongside your experiment. Allow both to sit for 20–30 minutes.

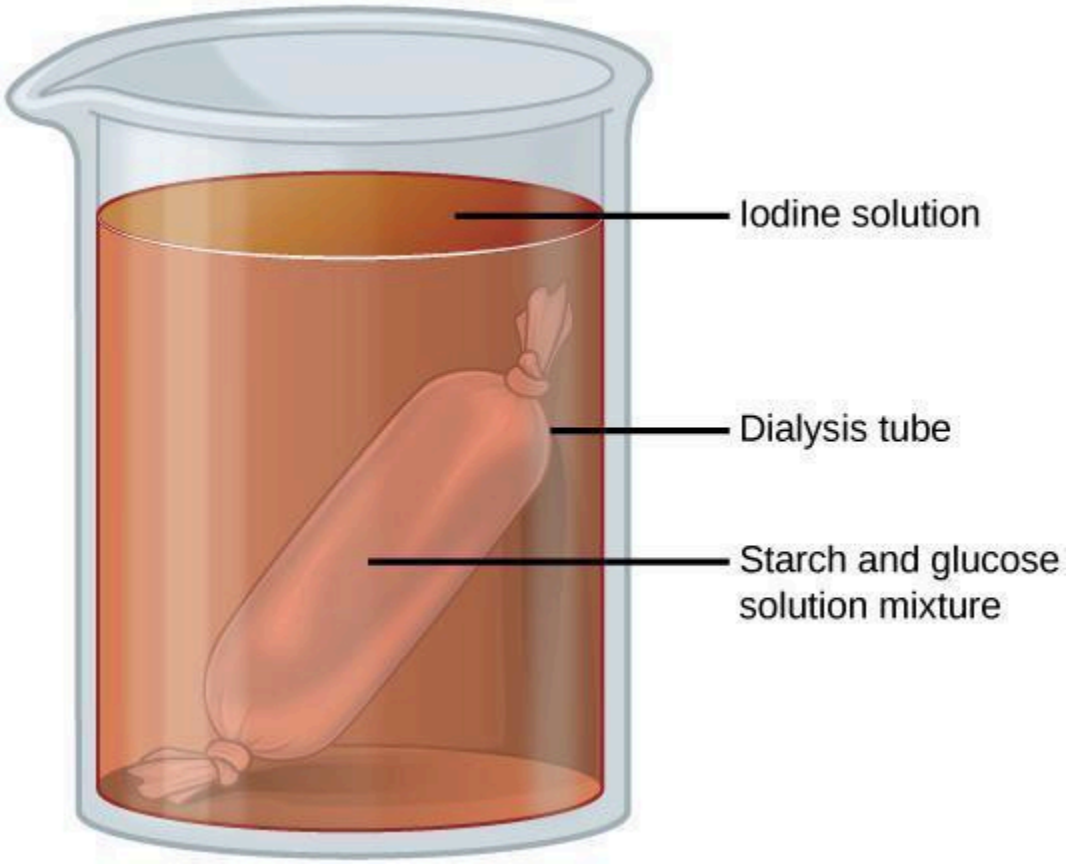


Figure 6.4: Your dialysis tube should be placed into the beaker and allowed to sit for the time specified.

Step 2: Hypothesize/Predict: Consider the molecular sizes of glucose and starch. What do you think will happen with the starch and the glucose inside of the dialysis tube? Predict whether starch and/or glucose will remain inside of the tube or if they will move out.

Step 3: Student-led planning: Briefly remove the dialysis bag from the beaker and set it aside. Pour 2 mL of the water from the beaker into a test tube. Put 10 drops of Benedict's reagent into the tube (note the initial color of the solution) and place into the hot water bath for 5 minutes. Note whether there is a color change. Place the bag back into the beaker. Put 20 drops of iodine into the water in the beaker. Allow this to sit for about 10 minutes. Determine whether starch is present in the bag and/or the beaker. Describe in your notebook what you observe. Create a data table for your observations that shows whether starch and glucose were inside and/or outside of the dialysis tubing.

Step 4: Critical Analysis: Are the predictions you made in step 2 supported by your data? Why or why not? What methods could you use to improve your results? Discuss with your group and then write your answers in your notebook.

Guided Inquiry

Step 1: Hypothesize/Predict: Predict what would happen to the rate of osmosis if the surface area-to-volume ratio was large (as it would be with small cells compared to larger ones). Record your prediction.

Step 2: Student-led planning: Determine how the rate of osmosis of water using a dialysis bag with starch solution is affected if surface area-to-volume ratio is altered. Use dialysis tubing of 2 different lengths and widths to determine this. Record weights of each bag at different time intervals. Discuss with your group the time points at which you will check the bags for differences in weight over a 20-minute period. Record your data for each. Discuss with your group how best to set up these experiments.

Step 3: Critical Analysis: Create a graph that shows how osmosis rate is affected over time because of alterations in surface area-to-volume ratios. Are the predictions you made in Step 2 supported by your data? Why or why not? What methods could you use to improve your results? Discuss with your group and then write your answers in your notebook.

Assessments

1. Would a large protein have difficulty crossing the membrane of a dialysis tube? Why or why not?
2. Why do smaller cells have more efficient diffusion compared to larger ones?

OSMOSIS IN LIVING CELLS

Learning Objectives

After completing the lab, the student will be able to:

1. Describe or explain hypertonic, hypotonic, and isotonic solutions.
2. Differentiate between the osmosis mode of action in animal and plant cells.

Activity 3: Pre-Assessment

1. Saline solutions are given to patients in an IV. Why might the salt concentration in the solution need to be the same as that in the blood cells?
2. Discuss the answer to question 1 with your group.

Activity 3: Osmosis in Living Cells[1]

A cell lacking a cell wall is affected greatly by the tonicity of the environment. In a **hypertonic** solution where the concentration of dissolved solute is high, water will be drawn out of the cell. In a **hypotonic** solution where the concentration of dissolved solute is lower than the interior of the cell, the cell will be under great **osmotic pressure** from the environmental water moving in and can rupture (Figure 6.5).

1. Activity 3: Osmosis in Living Cells includes text and images adapted from Osmosis and Diffusion in Biology OER, a site sponsored by the Ursula Schwerin Library to select and curate resources for use in General Biology 1 and originally authored and curated by Jeremy Seto, Department of Biological Sciences – New York City College of Technology. It is licensed CC-BY-NC-SA

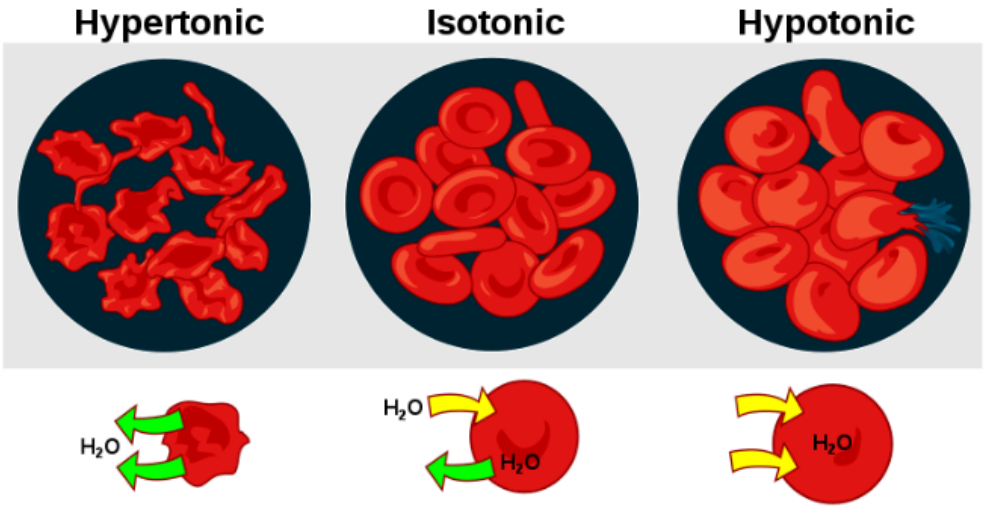


Figure 6.5 Tonicity of an animal cell's environment. (CUNY Lab: Osmosis)

Plants have rigid cell walls composed of cellulose. These cell walls permit for maintenance of cellular integrity when the external environment is **hypotonic** (less dissolved substances). In this situation, the water moves into the cell. Without the cell wall, the cell would burst open from the excessive water pressure entering the cell. This state of swelling is referred to as turgid, resulting from **turgor pressure** (Figure 6.6).

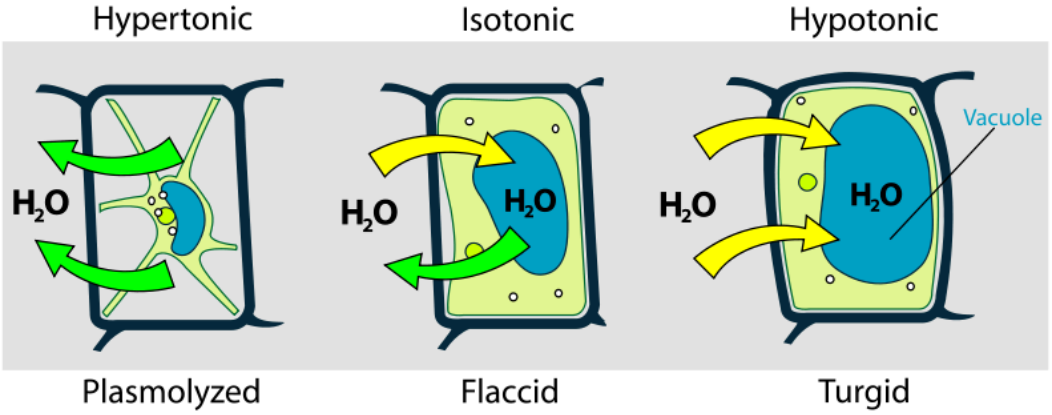


Figure 6.6 Cell walls of a plant retain the shape of the cell despite the state of external tonicity. (CUNY Lab: Osmosis)

The inside and the outside of a cell are mainly composed of water with dissolved solutes. The differences in solute concentration direct the movement of water across the plasma membrane of the cell. This difference between the two solutions (i.e., the cytoplasm and the extracellular fluid) is known as **tonicity**. Solutions are said to be **isotonic** if they both have equal concentrations of solute. The extracellular fluid is **hypertonic** to the intracellular fluid if it has a higher solute concentration. On the other hand, a lower solute concentration in the extracellular fluid compared to the inside of the cell would mean that the outside of the cell is **hypotonic** to the inside.

We can observe tonicity in *Elodea* leaves by placing them into different solutions with various solute concentrations.

Safety Precautions

- Be careful handling glass slides; the edges may be sharp.
- Observe proper use of the microscope; avoid handling the electric cord with wet hands.
- Do not use the coarse adjustment knob of the microscope at higher magnifications.
- Inform your teacher immediately of any broken glassware, as it could cause injuries.
- Clean up any spilled water or other fluids to prevent other people from slipping.
- Handle all chemicals safely.

For this activity, you will need the following:

- Two glass slides
- *Elodea* leaves
- Two coverslips
- Distilled water
- 10 percent NaCl solution
- 30 percent NaCl solution
- Microscope
- Potato cubes
- Electronic balance
- Weigh boat
- Three 50 mL beakers

For this activity, you will work in *groups of four*.

Structured Inquiry

Step 1: Obtain 2 glass slides and 2 cover slips. On 1 slide, place an *Elodea* leaf and put 1 drop of distilled water. Make a second slide with an *Elodea* leaf but put a drop of 10% NaCl. Allow these to sit for 2–3 minutes.

Step 2: Hypothesize/Predict: Predict what will happen with the leaf that has been placed into NaCl. What do you think will happen to the leaf in water? Record your predictions.

Step 3: Student-led planning: Determine what happens to the *Elodea* leaf cells in both solutions by observing them under the microscope. Record your observations for each. Draw pictures that demonstrate what you observe.

Step 4: **Critical Analysis:** Are the predictions you made in step 2 supported by your data? Why or why not? What methods could you use to improve your results? Discuss with your group and then write your answers in your notebook.

Guided Inquiry

Step 1: Hypothesize/Predict: What happens to a cell in an isotonic solution? Hypertonic? Hypotonic? Predict what might happen to a potato in these solutions. Will it gain, lose, or maintain its weight? Write your ideas in your notebook.

Step 2: Student-led planning: Design a study that would test your hypothesis in step 1. Plan which solutions you will use and how to determine if osmosis happened in each. Show your design to a teacher for approval, then create data tables that you would need to test your hypothesis. Write your results in your notebook and create a graph or table to summarize your results.

Step 3: Critical Analysis: Discuss your results with your group. Are your results what you expected? How can you improve your experiment? Write your ideas in your notebook.

Assessments

1. Have you ever seen a slug hanging out on the steps? Oftentimes, people use salt to get rid of them. What do you think this does to the slug in terms of osmosis?
2. How can you predict whether osmosis will occur into or out of a cell?
3. Describe what is meant when we say that a cell membrane is selectively permeable. What types of molecules might have a difficult time crossing the membrane?

KEY TERMS

diffusion
passive transport process of low-molecular weight material according to its concentration gradient

hypertonic
situation in which extracellular fluid has a higher osmolarity than the fluid inside the cell, resulting in water moving out of the cell

hypotonic
situation in which extracellular fluid has a lower osmolarity than the fluid inside the cell, resulting in water moving into the cell

isotonic
situation in which the extracellular fluid has the same osmolarity as the fluid inside the cell, resulting in no net water movement into or out of the cell

osmosis
passive transport process of low-molecular weight material according to its concentration gradient

surface area-to-volume ratio
describes the relationship between the area outside the cell to the volume inside the cell

LAB 7: FACTORS AFFECTING THE ENZYMATIC ACTIVITY OF LACTASE

MEASURING THE ENZYMATIC ACTIVITY OF LACTASE

Learning Objectives

After completing the lab, the student will be able to:

1. Measure enzymatic activity of the enzyme lactase over time and represent it graphically.

Activity 1: Pre-Assessment

1. How would the concentration of an enzyme, its substrate(s), and its product(s) change over time as an enzymatic reaction takes place?
2. What molecule(s) would you measure to monitor the progress of an enzymatic reaction? How might you observe the relative concentrations of these molecules over the course of the enzymatic reaction?
3. Discuss the answers to questions 1 and 2 with the class.

Activity 1: Measuring the Enzymatic Activity of Lactase

How would we measure the activity of a specific enzyme? One easy way to measure the concentration of a chemical is by using a colored indicator. As an enzymatic reaction proceeds, the color of the solution changes and can be monitored visually.

In this lab, you will measure the enzymatic activity of lactase, an enzyme that breaks down lactose, a disaccharide found in milk and other dairy products, into the monosaccharides galactose and glucose (Figure 7.1). While human infants naturally produce lactase, most adults do not, making them lactose intolerant, or unable to produce enough lactase to digest ingested lactose. Interestingly, in the past 10,000 years, several populations of humans have developed lifelong lactase activity. These human populations are often people

who raise livestock and drink animal milk. For most individuals who are lactose intolerant, lactase may be purchased in tablet form from pharmacies.

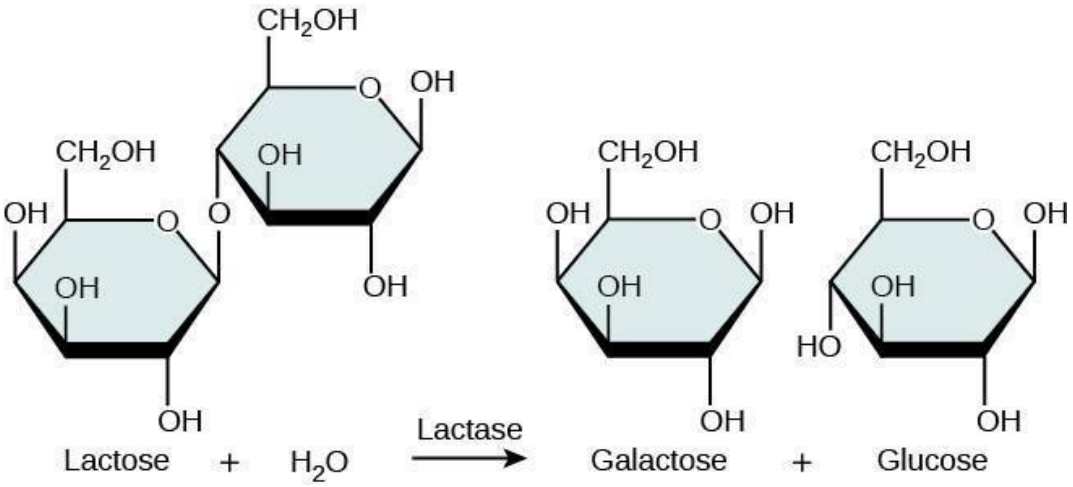


Figure 7.1: The enzyme lactase degrades the disaccharide lactose into the monosaccharides galactose (1) and glucose (2).

Glucose, one of the products of lactose degradation, can be detected visually using glucose detection strips, which can also be purchased from pharmacies. One type of glucose detection strip contains the dye toluidine blue and the enzyme glucose oxidase (Figure 7.2). Glucose oxidase within the strip converts the glucose to gluconic acid and hydrogen peroxide. Hydrogen peroxide then interacts with the toluidine blue within the strip, bringing about a color change from blue to green to yellow to brown.

Figure 7.2: Diastix glucose detection strips turn from blue to green to yellow to brown with increasing concentrations of glucose.

Safety Precautions

- Goggles should be worn at all times while in laboratory.
- No open-toe shoes worn in laboratory.
- Measure fluids carefully using graduated cylinders to avoid breakage and spillage.
- Inform your teacher immediately of any broken glassware as it could cause injuries.
- Clean up any spilled fluids to prevent other people from slipping.

For this activity, you will need the following:

- Graduated cylinder
- Beaker
- Water
- Milk

- Lactase (obtained from laboratory supply company)
- Stirring rod
- Test tubes
- Glucose (powdered)
- Balance
- Labeling pencil
- Glucose test strips
- Graph paper
- Timer

For this activity, you will work *in pairs*.

Structured Inquiry

Step 1: Make 100 mL of a 20 mg/mL glucose solution or obtain this from your instructor. Set up tubes as follows:

- Negative control: 3 mL of water
- Positive control: 3 mL of 20 mg/mL glucose solution

Insert a glucose test strip into each tube, and compare the color you observe to the color chart that comes with the test strips. What is the concentration of glucose in each tube? Are your results what you expected?

Step 2: Hypothesize/Predict: Based upon your knowledge of enzymes and lactase function, predict whether glucose will be detected in milk in the presence of lactase enzyme compared to milk lacking the lactase enzyme. Predict how the glucose concentration of milk will change over time under each of these two conditions. Write your predictions in your lab notebook.

Step 3: With your partner, prepare two tubes each containing 2 mL of milk. Additionally, make your lactase enzyme solution per your teacher's instructions.

Step 4: Student-led Planning: Your experiment should last for 15 minutes. Create a data table to record glucose concentrations in each of the two tubes of milk at 3-minute intervals. To start the experiment, add 1 mL of water to one of the tubes of milk and 1 mL of the lactase solution to the other. Then, every 3 minutes for 15 minutes, record the concentrations of glucose in each tube using the color chart that came with the test strips and record this in your data table.

Step 5: Critical Analysis: Using graph paper, graph your data of glucose concentration versus time. Which is the independent variable? Which is the dependent variable? Calculate the rates of glucose production for each tube. Are the predictions you made in step 2 supported by your data? Explain how you know in your notebook.

Guided Inquiry

Step 1: Hypothesize/Predict: How do you think increasing the amount of lactase will affect glucose formation? How do you think decreasing the amount of lactase will affect glucose formation? Formulate a hypothesis involving the effect of the lactase amount on glucose formation.

How do you think increasing the amount of milk will affect glucose formation? What would happen if you decreased the amount of milk? Formulate a hypothesis involving the effect of the amount of milk on glucose formation.

Write your hypotheses and predictions in your notebook.

Step 2: Student-led Planning: Select one of the hypotheses above to discuss with your lab partner. Design an experiment to address this hypothesis and submit it to your teacher for approval. Once your teacher approves, create a table to record your data, prepare your test tubes per your design, and record data on glucose production every 3 minutes for 15 minutes. Graph your data and calculate the rate of glucose production for each tube.

Step 3: Critical Analysis: Are the predictions you made in Step 1 supported by your data? Is there any way you could improve your experiment? Discuss your answers with your lab partner and write them in your notebook.

Assessments

1. In this experiment, assume that all the lactose provided is broken down to glucose and galactose. Based upon this assumption, if one diluted the amount of milk by half in the tube containing milk and the lactase enzyme, what would happen to the amount of glucose produced? Explain why in terms of molecular interactions of the substrate with the enzyme and enzyme function.
2. In this experiment, describe the role of each of the following:
    1. The water-only tube
    2. The tube of 20 mg/mL glucose solution

THE EFFECTS OF TEMPERATURE AND PH ON ENZYMATIC ACTIVITY

Learning Objectives

After completing the lab, the student will be able to:

1. Measure enzymatic activity of the enzyme lactase over time and represent it graphically.
2. Monitor the effects of environmental conditions on enzymatic activity.

Activity 2: Pre-Assessment

1. Which environmental conditions could alter the rate at which an enzymatic reaction takes place? Why would this occur?
2. Which environmental conditions could affect an enzyme's active site? Why would this occur?
3. Discuss the answers to questions 1 and 2 with the class.

Activity 2: The Effects of Temperature and pH on Enzymatic Activity

What types of environmental factors may affect enzymatic activity? Why? Several factors known to affect enzymatic activity are temperature, pH, and substrate concentration. In a typical chemical reaction, increasing temperature causes the substrates to become more energetic and hence more likely to bump into each other in solution. However, changes in temperature can cause an enzyme to **denature**, which changes the three-dimensional structure of the enzyme molecule. In addition, cellular enzymes each work within a certain pH range because the side chains within their active sites are optimized for efficient catalysis and are thus quite sensitive to changes in pH. Different enzymes may have different pH ranges and **pH optima**, conditions under

which they work maximally; while many enzymes work best around a neutral pH, some are adapted to an acidic pH, while others are adapted to a basic pH.

Safety Precautions

- Goggles should be worn at all times while in laboratory.
- No open-toe shoes worn in laboratory.
- Measure fluids carefully using graduated cylinders to avoid breakage and spillage.
- Be careful not to touch solutions of concentrated acids and bases directly.
- Take precautions when using a hot plate and touching hot glassware.
- Inform your teacher immediately of any broken glassware as it could cause injuries.
- Clean up any spilled fluids to prevent other people from slipping.

For this activity, you will need the following:

- Graduated cylinder
- Beaker
- Water
- Buffer solutions of pH 3.0, pH 4.0, pH 5.0, pH 6.0, pH 7.0, pH 8.0, and pH 9.0
- Milk
- Lactase (obtained from laboratory supply company)
- Test tubes
- Stirring rod
- Labeling pencil
- Hot plate
- Ice bath
- Refrigerator set to approximately 4 °C
- Incubator set to 37 °C
- Thermometers
- Glucose test strips
- Timer
- Graph paper

For this activity, you will work *in pairs*.

Structured Inquiry: Temperature

Step 1: Prepare a large beaker of boiling tap water on a hot plate. Prepare five identical test tubes, each containing 2 mL of milk. Label five test tubes accordingly with each of the following temperatures: 0°C (ice bath), 4°C (refrigerator), room temperature, 37°C, and 100°C (boiling temperature). Place one tube of milk at each of the five temperatures. Create a data table to enter your results for each of these test tubes over time. Measure the room temperature using a thermometer.

Step 2: Hypothesize/Predict: Based upon your knowledge of enzymes and the effects of temperature on their activity, rank the tubes from fastest (1) to slowest (5) glucose production predicted over time after the addition of lactase. Add your predictions to the data table you created in step 1.

Step 3: Student-led Planning: Discuss with your partner how you could use the data you collect to calculate a rate of lactase activity for each temperature.

Step 4: Make your lactase enzyme solution per your teacher's instructions. Add 1 mL of the lactase enzyme solution to each of the five tubes listed above and immediately start timing. Immediately after adding the lactase enzyme solution, determine the glucose concentration in each tube using glucose test strips and the color chart that came with the test strips. Record this in your data table.

Monitor the temperatures of each of these locations, both before and after the experiment using thermometers.

Step 5: Every 3 minutes for 15 minutes, record the concentrations of glucose in each tube using the color chart that came with the test strips and record in your data table.

Step 6: Critical Analysis: Calculate the rate of enzymatic activity for lactase at each temperature using the method you devised in step 3. Using graph paper, graph your data of rates of lactase activity versus temperature. Which is the independent variable? Which is the dependent variable? Are the predictions you made in step 2 supported by your data? Explain how you know in your notebook.

Guided Inquiry: Temperature

Step 1: Hypothesize/Predict: Based on the data already collected, predict a temperature range that includes the optimal temperature for lactase activity. How do you think you could more finely pinpoint the optimal temperature for lactase activity? Write your ideas in your notebook.

Step 2: Student-led Planning: Determine how you could change the set-up of your test tubes to determine the optimal temperature for lactase activity. Once your teacher approves, create a table to record your data, prepare your test tubes per your design, and record data on glucose production every 3 minutes for 15 minutes. Determine the rates of lactase enzyme activity under each of your chosen conditions as you did in the Structured Inquiry. Graph the rates of lactase enzyme activity versus temperature and estimate the optimal temperature.

Step 3: Critical Analysis: Are the predictions you made in step 1 supported by your data? Is there any way you can improve your experiment? Discuss your answer with your lab partner and write it in your notebook.

Structured Inquiry: pH

Step 1: Prepare three test tubes, each containing 2 mL of milk, and label the three tubes as follows: 4.0 (acidic), 7.0 (neutral), and 9.0 (basic). To the first test tube, add 1 mL of pH 4.0 buffer. To the second test tube, add 1 mL of pH 7.0 buffer. To the third test tube, add 1 mL of pH 9.0 buffer. Create a data table to enter your results for each of these test tubes over time.

Step 2: Hypothesize/Predict: Based upon your knowledge of enzymes and the effects of pH on their activity, order the tubes from highest (1) to lowest (3) glucose production predicted over time. Add your predictions to the data table you created in step 1.

Step 3: Student-led Planning: Discuss with your partner how to calculate a rate of lactase activity for each pH.

Step 4: Make your lactase enzyme solution per your teacher's instructions. Add 1 mL of the lactase enzyme solution to each of the three tubes listed above and immediately start timing. Immediately after adding the lactase enzyme solution, determine the glucose concentration in each tube using glucose test strips and the color chart that came with the test strips. Record this in your data table.

Step 5: Every 3 minutes for 15 minutes, record the concentrations of glucose in each tube using the color chart that came with the test strips and record in your data table.

Step 6: Critical Analysis: Calculate the rate of enzymatic activity for lactase at each pH. Using graph paper, graph your data of rates of lactase activity versus pH. Which is the independent variable? Which is the dependent variable? Are the predictions you made in step 2 supported by your data? Explain how you know in your notebook.

Guided Inquiry: pH

Step 1: Hypothesize/Predict: Based on the data already collected, predict a pH range that includes the optimal pH for lactase activity. How do you think you could more finely pinpoint the optimal pH for lactase activity? Write your ideas in your notebook.

Step 2: Student-led Planning: Determine how you would change the set-up of your test tubes to determine the optimal pH for lactase activity. Once your teacher approves, create a table to record your data, prepare your test tubes per your design, and record data on glucose production every 3 minutes for 15 minutes. Determine the rates of lactase enzyme activity under each of your chosen conditions. Graph the rates of lactase enzyme activity versus pH and estimate the optimal pH.

Step 3: Critical Analysis: Are the predictions you made in step 1 supported by your data? Is there any way you could improve your experiment? Discuss your answer with your lab partner and write it in your notebook.

Assessments

1. If an enzyme has an optimal activity at 25°C, what do you think will happen to the enzyme's activity if the temperature is raised to 37°C? Why?
2. If an enzyme has a largely acidic active site, what do you think will happen to the enzyme's activity if the pH is made basic? Why?

SUBSTRATE SPECIFICITY OF THE ENZYME LACTASE

Learning Objectives

After completing the lab, the student will be able to:

1. Measure enzymatic activity of the enzyme lactase over time and represent it graphically.
2. Determine the specificity of the enzyme lactase.

Activity 3: Pre-Assessment

1. How could we determine experimentally the specificity of an enzyme to its substrate?
2. Discuss the answer to question 1 with the class.

Activity 3: Substrate Specificity of the Enzyme Lactase

An enzyme's active site contains side chains of the amino acids that can only bind to certain molecules. For example, if the active site is largely positively charged, then negatively charged substrates will be attracted. Additionally, the three-dimensional shape of the active site within the enzyme is key in determining which substrates will fit into the active site (Figure 7.3). What does this suggest about how specific enzymes are to their substrates?

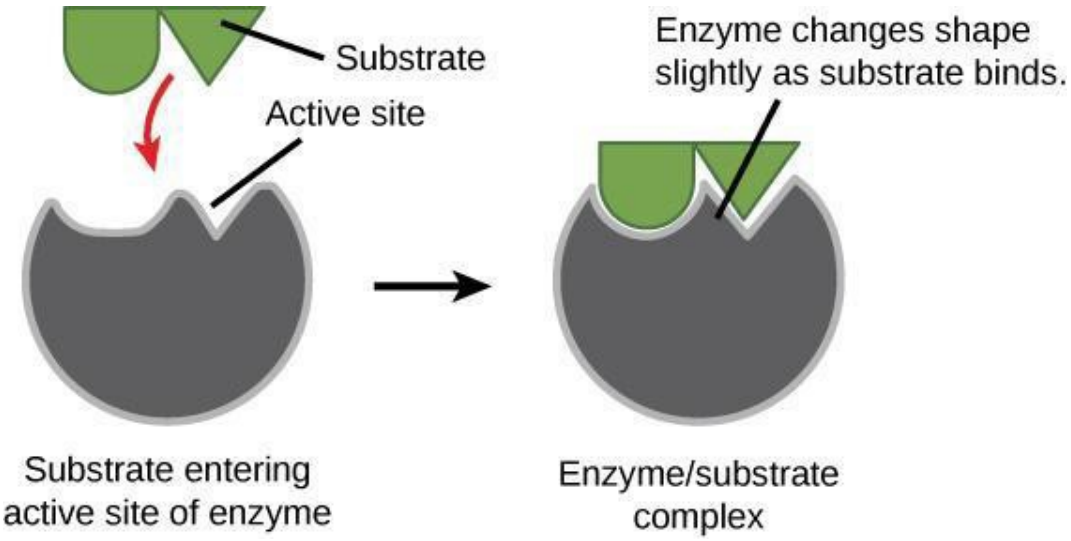


Figure 7.3: The three-dimensional shape of the active site is also important in determining which substrates will bind to the active site.

Safety Precautions

- Goggles should be worn at all times while in laboratory.
- No open-toe shoes worn in laboratory.
- Measure fluids carefully using graduated cylinders to avoid breakage and spillage.
- Inform your teacher immediately of any broken glassware as it could cause injuries.
- Clean up any spilled fluids to prevent other people from slipping.

For this activity, you will need the following:

- Graduated cylinder
- Beaker
- Water
- 20 mg/mL lactose solution
- 20 mg/mL sucrose solution
- Various foods for testing for presence of lactose, including both dairy products and those marketed as lactose-free
- Lactase (obtained from laboratory supply company)
- Test tubes
- Stirring rod
- Timer
- Labeling pencil
- Glucose test strips
- Graph paper

For this activity, you will work *in pairs*.

Structured Inquiry

Step 1: Prepare two test tubes, one with 2 mL of 20 mg/mL lactose and the other with 2 mL of 20 mg/mL sucrose. Label one tube as lactose-containing and the other as sucrose-containing. Create a data table to enter your results for each of these test tubes over time.

Step 2: Hypothesize/Predict: Based on your knowledge of enzymes and their specificity, predict whether you will observe enzyme activity for each of the two tubes indicated above. Add your predictions to the data table you created in Step 1.

Step 3: Student-led Planning: Discuss with your partner how to calculate a rate of lactase activity for each substrate.

Step 4: Make your lactase enzyme solution per your teacher's instructions. Add 1 mL of the lactase enzyme solution to each of the two tubes listed above and immediately start timing. Immediately after adding the lactase enzyme solution, determine the glucose concentration in each tube using glucose test strips and the color chart that came with the test strips. Record this in your data table.

Step 5: Every 3 minutes for 15 minutes, record the concentrations of glucose in each tube using the color chart that came with the test strips and record in your data table.

Step 6: Critical Analysis: Calculate the rate of enzymatic activity for lactase in each substrate. Using graph paper, make a bar graph showing the rates of lactase activity in each of the two substrates. Are the predictions you made in step 2 supported by your data? Explain how you know in your notebook.

Guided Inquiry

Step 1: Hypothesize/Predict: Based upon what you have learned, how could we use this assay for lactase activity to determine the relative amounts of lactose in various food products, including typical dairy products as well as those that are advertised as lactose-free? Write your ideas in your notebook.

Step 2: Student-led Planning: With your lab partner, choose two food products to test—one that you suspect contains lactose and one that is supposed to be lactose-free. Determine how you would change the setup of your test tubes above to assay for lactase activity in food products. Once your teacher approves your experimental design, create a table to record your data, prepare your test tubes per your design, and record data on glucose production every 3 minutes for 15 minutes. Determine the rates of lactase enzyme activity for each food product. Make a bar graph showing the lactase activity for each of the two foods chosen.

Step 3: Critical Analysis: Are the predictions you made in Step 1 supported by your data? Is there any way you could improve your experiment? Discuss your answers with your lab partner and write them in your notebook.

Assessments

1. How can substrate specificity of an enzyme be used to determine the presence of its substrate in a sample? Explain in terms of the active site.
2. What might you conclude if you observed glucose in a lactose-free food at the start of the experiment?
3. How could enzyme specificity be used to determine the concentration of a substrate in a food product?

KEY TERMS

denature
: to breakdown or unfold a molecular conformation

pH optima
: conditions under which enzymes work maximally

LAB 8: CELLULAR RESPIRATION

CELLULAR RESPIRATION

Learning Objectives

After completing the lab, the student will be able to:

1. Determine the site of respiration in the cell.

Activity 1: Pre-Assessment:

1. What function do mitochondria fulfill in the cell? What kind of staining would allow visualization of mitochondrial activity?
2. Which plant tissue would you choose to stain mitochondria? Explain your choice.
3. Discuss the answers to questions 1 and 2 with the class.

Activity 1: Staining Mitochondria with Janus Green B

You will investigate the site of oxidative phosphorylation and the effect of environmental conditions on the mitochondria. **Janus Green B** is a stain that appears blue-green when oxidized (that is, when it loses electrons) and is colorless or light pink when reduced (when it gains electrons).

Safety Precautions

- Use the single-edge razor blade with caution. Do not leave blades exposed on the bench. When you are finished using the razor blade, dispose of it as instructed by your teacher.
- Dispose of coverslips in a broken glass container.
- Be careful handling glass slides; the edges may be sharp.
- If using cheek cells, dispose of flat toothpicks and slides in a jar containing 10 percent bleach.

- Handle dyes with care and be careful not to ingest.
- Inform your teacher immediately of any broken glassware, as it could cause injuries.
- Clean up any spilled fluids to prevent other people from slipping.
- Wash your hands with soap and water after completion of the activity.

For this activity, you will need the following:

- Onion; alternatively slices of celery branch or cheek epithelial cells may be used
- Toothpicks and 10 percent bleach container if using epithelial cells
- Forceps
- Water and dropper
- 0.001 percent solution of Janus Green B
- Absorbent paper such as a paper towel or filter paper
- Microscope
- Slides and coverslips
- Mounting fluid to be chosen by students (7 percent sucrose, 0.9 percent salt, vinegar, ammonia)

For this activity, you will work *in pairs*.

Structured Inquiry

Step 1: Preparation and observation of wet mount:

- Slice a layer from an onion with the single-edge razor blade and grab the edge of the layer with the forceps peeling back a thin transparent layer of epidermal tissue. The thickness of the layer is one or a few cells which will allow you to visualize clearly the inside of the cells.
- Add a drop of water and place a cover slip over the onion slice; do not remove air bubbles if they form.

Step 2: Hypothesize/Predict: Janus Green B is an indicator of the redox state. Knowing that it appears blue/green in its oxidized state, and loses its color when it is reduced, allows you to predict which organelle will show a progressive change in color because it is the active site of oxidation-reduction. Discuss with your lab partner how you would expect Janus Green B color to reflect active respiration. Which experimental conditions would you choose to investigate with your current setup? Record your prediction in your notebook.

Step 3: Student-Led Planning: Decide which mounting solution(s) you will choose to observe changes in respiration in the epidermal layer. View the sample first under low magnification to focus on the cells. Proceed to the highest magnification available to you (highest dry objective or oil immersion) and observe internal structures.

Stain with Janus Green B by using the wicking method as shown in Figure 8.1. Place a piece of filter paper or tissue on one side of the cover slip. Add one or two drops of Janus Green on the opposite site of the coverslip close to the edge. The stain solution will flow in by capillary action.

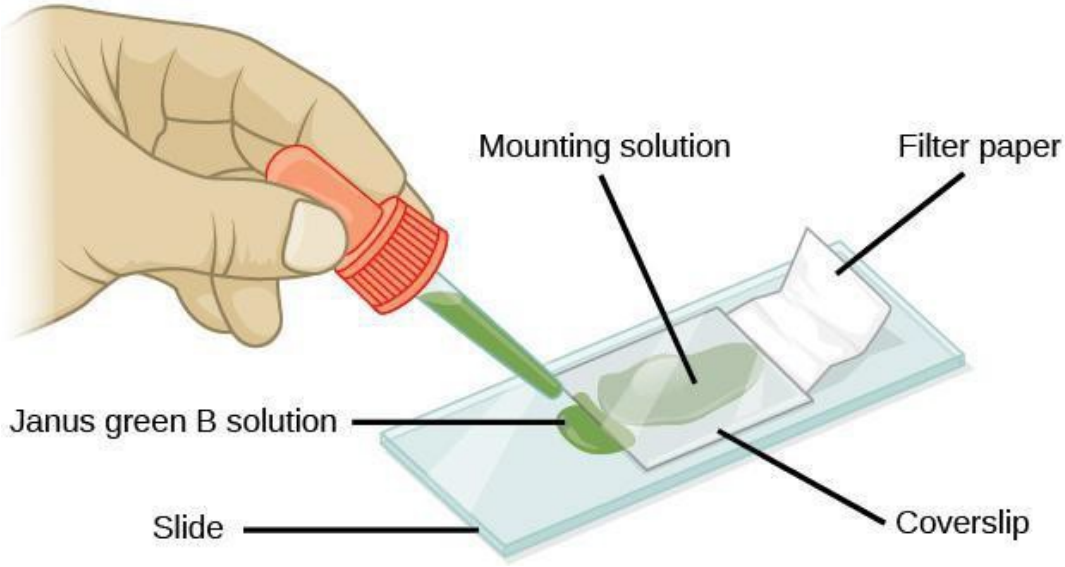


Figure 8.1 Staining a wet mount by capillary action.

Monitor the changes in stain appearance for five to 10 minutes, taking turns observing the slide. Record your observation in your lab notebook.

Step 4: Critical Analysis: Draw and label all the structures that you can identify. Do not forget to add a title and the final magnification to all the drawings. Draw only what you observe. Do not copy from existing micrographs from published or online work. Compare the effect of different mounting solutions to distilled water. Did you observe a change in the color of mitochondria? Did it change over time? If there was an effect, how can you explain your observations? Can you think of other experiments that would support your conclusions?

Guided Inquiry

Step 1: Hypothesize/Predict: Janus Green B is a vital stain and an indicator of redox state. Knowing that it appears blue/green in its oxidized state and loses its color when it is reduced, allows you to predict which organelle will show a progressive change in color because it is the active site of oxidation-reduction and which organelle will appear colorless. What environmental conditions would be essential to observe a blue color of stain?

Step 2: Student-led planning: Prepare a wet mount of tissue under the microscope. Stain with Janus Green B, using the wicking method described earlier, while observing under the microscope. Which organelles can you distinguish? Are there any changes with time? Record your observations in your notebook. Repeat

your experiment with a different mounting medium, staining with Janus Green B according to the wicking method. Record your observations in your notebook.

Step 3: Critical analysis: How did the various mounting fluids you used influence the response of the mitochondria to Janus Green B? How can you explain the effect that you observed? Compare the effect of different mounting solutions to distilled water. Was there an effect? If there was an effect, how can you explain your observations? Can you think of other experiments that would support your conclusions? Write your ideas in your notebook.

Assessments

1. What do you predict would be observed if the epidermal layer of an onion is incubated in a solution of rotenone, an inhibitor of respiration?
2. A student carefully mounts a specimen of onion epidermal layer, pushing out all the air bubbles. She is very disappointed that she does not observe a change in the color of Janus Green B. Can you explain her observation?
3. Cyanide is a known metabolic poison that acts mainly by blocking cytochrome oxidase, an enzyme embedded in the inner membrane of mitochondria, and preventing the reduction of oxygen. If cyanide were added to an onion layer stained with Janus Green B, what you would observe and why?

MEASUREMENT OF RESPIRATION AND EFFECT OF TEMPERATURE

Learning Objectives

After completing the lab, the student will be able to:

1. Measure the consumption of oxygen during respiration.
2. Measure the effect of environmental conditions on respiration in pea seeds.

Activity 2: Pre-Assessment

1. Students stain corn seeds over a period of several days after the seeds are soaked with water to promote germination with iodine. Iodine stains starch blue. The students observe that the amount of starch decreases during germination. Can you explain this observation? Which metabolic process uses up starch?
2. What kind of biological catalysts are involved in the reactions of respiration? If the rate of a chemical reaction doubles with the temperature, would you expect that rates of respiration to increase continuously with temperature?
3. Discuss the answers to questions 1 and 2 with the class.

Activity 2: Measurement of Respiration and Effect of Temperature on Respiration Rate

Imagine that you plan to monitor respiration in a whole organism, such as a small invertebrate or a seedling. You may decide to follow the disappearance of the reactants, either glucose or oxygen. Your second choice is to measure the formation of the products, either water or carbon dioxide. In this laboratory, you will design experiments to assess the effect of environmental conditions on the process of cellular respiration.

In respiration, oxygen is consumed and CO2 is released. In this experiment, we will measure the disappearance of oxygen. A **respirometer** consists of an enclosed chamber in which the studied organism is placed and a graduated pipette with which we measure changes in the gas volumes. The CO2 gas that forms will be removed by adding Ca(OH)2, which reacts with carbon dioxide to form the insoluble salt CaCO3, calcium carbonate.

While measuring the changes in the amount of gas produced, you will consider the ideal gas law equation which can be stated as

$$PV = nRT$$

P represents the atmospheric pressure in mmHg, *V* is the volume of the gas in liters, *n* is the number of moles of gas, *R* is the ideal gas constant, and *T* is the temperature in degrees Kelvin. In the respirometer, pressure remains constant as the gas produced displaces water in the tube. We will set up the respirometers in a water bath to minimize fluctuations in temperature.

In this experiment, you will use pea seeds. In a seed, like the yellow peas shown in Figure 8.2, a tough coat protects the plant embryo. Nutrients in the form of starch and lipids surround the embryo and support its **germination**, or growth from seed, until the appearance of photosynthetic structures. Seeds are normally **dormant**, that is metabolically inactive, until the environmental conditions helpful for growth are available. In order to bring the seeds to an active state, (out of dormancy), the seeds you will use were soaked in water via a process called imbibition, for 6 to 8 days.

Figure 8.2: Dried yellow peas are the seeds of a variety of the plant Pisum sativum and are considered a staple food. They contain yellow cotyledons and a large reserve of starch.

Cellular respiration involves three major sequential stages: glycolysis, the citric acid cycle, and oxidative phosphorylation. Oxygen serves as a terminal electron acceptor. Glycolysis takes place in the cytoplasm whereas mitochondria are the site of the citric acid cycle and the electron transport chain.

All the steps of respiration are mediated by **enzymes**, biological catalysts—mainly proteins—that lower the **activation energy**, the energy required to be available in a system before a chemical reaction can take place. Enzymes are not used up by the reactions they catalyzed. The process of respiration responds to the same environmental factors that affect the activity of enzymes. In this activity, you will measure the effect of temperature on respiration rates.

Safety Precautions

- Handle test tubes or glass containers with care; insert the plug by holding the container in a paper towel.
- Use plastic pipettes rather than glass pipettes.
- Wear goggles or safety glasses.
- Wear gloves when working with KOH or lime [$Ca(OH)_2$], which are corrosive chemical compounds.
- Use care while handling hot water. Wear mitts and do not leave boiling water or a hot plate unattended.
- Protect your clothes with an apron.
- Inform your teacher immediately of any broken glassware as it could cause injuries.
- Clean up any spilled fluids to prevent other people from slipping.
- Wash your hands with soap and water after completion of the activity.

For this activity, you will need the following:

- Dried yellow peas
- Glass beads
- Balance and weigh boats
- Paper towels to imbibe seeds
- KOH or lime water
- Food coloring
- Absorbent and non-absorbent cotton
- Drilled rubber stoppers that fit the opening of the test tubes or bottles
- 1-ml plastic pipettes
- Top loading balance
- Thermometers
- Water baths
- Weights such as clamps or hex keys
- Wide glass test tubes or bottles

- Stirring rod
- Ice
- Hot plate to boil water
- Mitts

For this activity, you will work *in pairs*.

Structured Inquiry

Step 1: Obtain 25-30 germinating peas, dry peas, and glass beads to start your experiment. Place the germinating peas in a weigh boat and measure their weight. Record the weight in your notebook and then repeat for the dried peas and glass beads.

Step 2: In this activity, you will indirectly measure the rate of respiration of the peas by monitoring the decrease in gas when the peas are placed in the respirometer chamber. What gas will decrease in the chamber as the peas undergo respiration? Hypothesize how much the gas levels will likely change for the germinating seeds, dry seeds, and glass beads. Record your hypotheses and predictions in your notebook.

Step 3: Student-Led Planning: Which of your treatments serve as a control? Is this a positive or negative control? How will this control reveal whether or not the experiment is functioning properly? Write your answers in your notebook.

Step 4: Assemble a respirometer using Figure 8.3 as a guide and following the steps below.

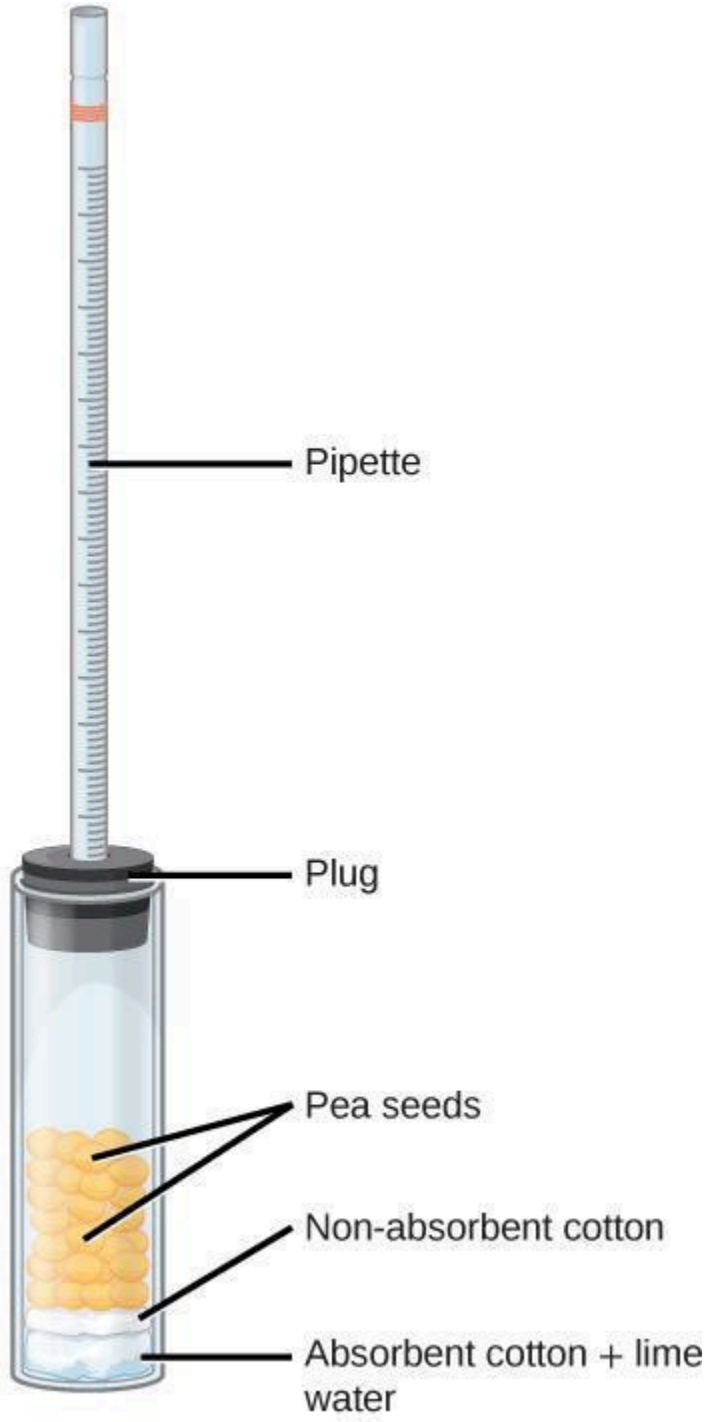


Figure 8.3: Assembled respirometer.

1. In a wide test tube (or bottle), drop a pad of absorbent cotton. Pack down the cotton with a stirring rod. Add lime water Ca(OH)2, being careful not to oversaturate the pad or drip the lime water on the side of the tube.
2. Insert a thin layer of non-absorbent cotton, pushing down with the glass rod. The cotton protects the seeds from lime water; however, if it is too thick, it will interfere with the diffusion of CO2.

3. Plug the test tube with a bored rubber stopper. Add a drop of colored water in a 1-ml graduated pipette and insert the pipette in the hole of the stopper. Adjust the position of the drop by inserting a syringe in the stopper until you can easily read the position of the dye. (The syringe is not shown in Figure 8.3.) Rub some petroleum jelly where the pipette comes into contact with the rubber stopper. The respirometer must be water tight to yield reliable results. It is also possible to wrap the openings with stretchable plastic film.
4. You may want to test for leaks by immersing the respirometer with the plug and pipette *before* filling it with reagents and cotton.

Step 5: Assemble the respirometer containing the control sample in the same manner.

Step 6: Immerse the respirometers with the experimental sample and the control in the water bath. Lining the water bath with a white paper towel will make it easier to read the markings on the pipettes. Make sure that the pipettes are resting across a piece of ribbon or string that spans the width of the water bath, as illustrated in Figure 8.4. The goal is to keep the pipettes out of the water while the test tubes remain submerged.

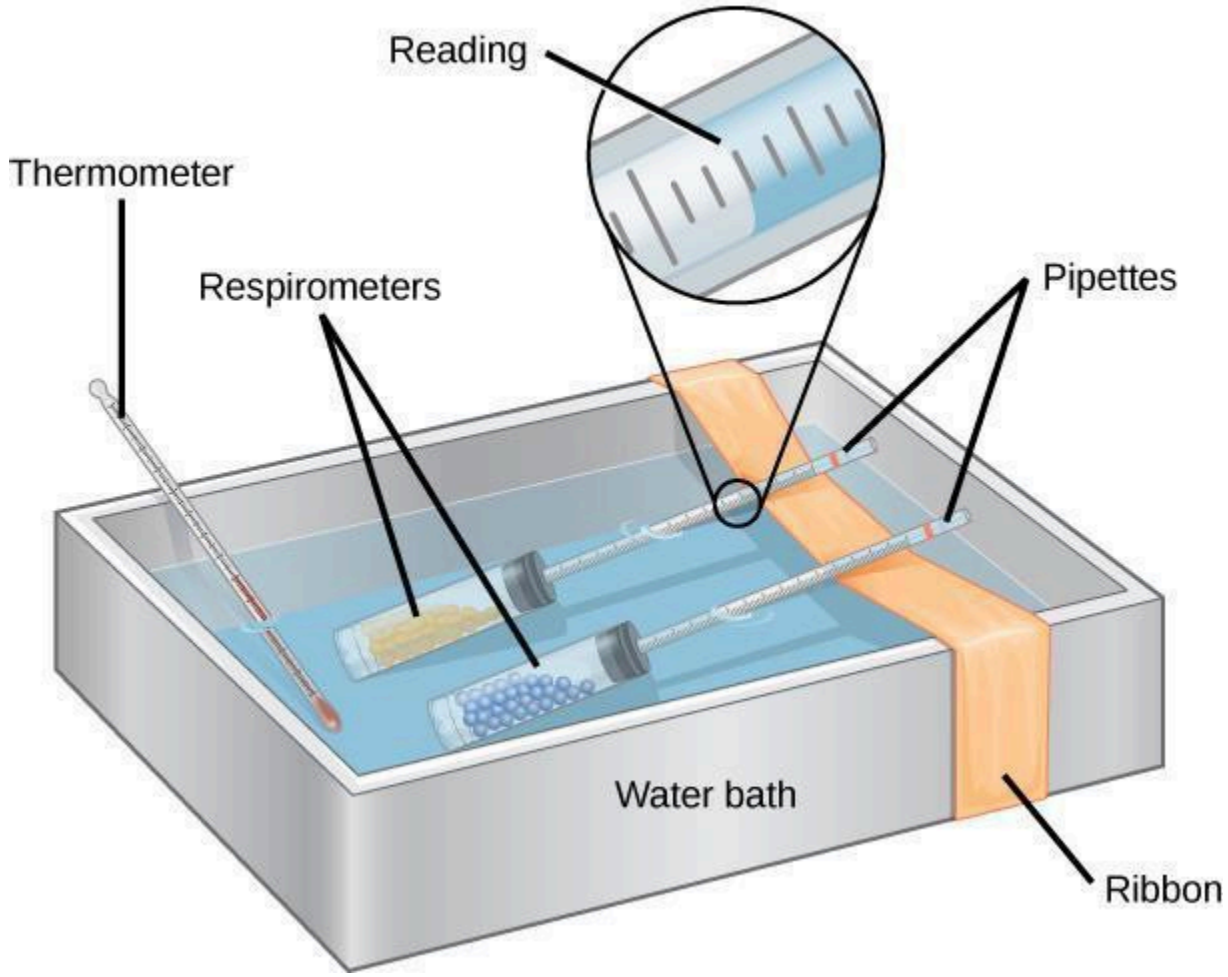


Figure 8.4: Respirometers in water bath.

Step 7: Let the respirometers equilibrate for 5–10 minutes.

Step 8: Read the starting volume on the pipette. This is time 0 min. Record the displacement of the colored bead for all samples every 2 minutes for 20 minutes and enter data in a table of measurements.

Step 9: Critical Analysis: Calculate the changes in volume where the reading at time 0 is subtracted from every subsequent reading. Subtract the rate of volume change measured in the control samples to obtain a corrected rate of respiration.

Graph the changes in volume in respirometers as a function of time and calculate the rate of change from the slopes of the line plots. Calculate the rate of change per gram of seed. This will allow you to compare values obtained from different samples. Draw a plot of changes in gas volumes from the data in your table. What measurements will you enter on the axis? What measurements will you enter in the *y*-axis? Determine the rate of respiration in your experiment. How did you use the data from your control or controls? Did volumes change during the experiment? Which gas caused the change in volume? Do the results support your hypothesis? Can you explain unexpected results? Were the respirometers water-tight at all times? How could you modify the experiment in the future? Write your answers in your notebook.

Guided Inquiry

Step 1: Repeat the steps to set up the respirometers described in the Activity 2 Structured Inquiry. Use three water baths at the following temperatures: 10°C, room temperature (see Structured Inquiry), and 50°C.

Step 2: Hypothesize/Predict: Discuss with your partner what kind of influence temperature might have on metabolic processes. How would the respiration rate measured at 10°C compare to the rate measured at room temperature? Will the rate of respiration be higher at 30°C than room temperature? Do you predict that the rate of respiration will be higher at 50°C than at room temperature or 30°C? Enter your hypotheses in your notebook.

Step 3: Student-Led Planning: You will now measure the rate of respiration at three different temperatures. Discuss with your partner if you need to run the experiment at room temperature again. Decide which control you will set up for this experiment. Make a note of all the steps you will perform, as you did in Activity 2, and create tables for your observations in your lab notebook. You will take readings of the colored water bubble at 2-minute intervals for 20 minutes. Have your teacher approve your experimental procedure before proceeding.

Step 4: Once approved, carry out your experimental procedure, closely monitoring the temperature as you take measurements.

Step 5: Critical Analysis: Graph the changes in gas volumes from the data in your table for all three temperatures for the experimental and control set-up, as you did for the Structured Inquiry. Determine the rate of respiration for each temperature. Because the gas law shows that differences in temperature affect volumes, you must correct for any changes in volume that are a consequence of temperature variations rather than respiration. To do this, subtract changes in volumes measured in the respirometer containing glass beads from

the changes in volume measured in the tubes containing germinating seeds held at the same temperature. Do the results support your hypothesis? Explain whether your results support or refute your hypothesis. How could you modify the experiment in the future? Write your ideas in your notebook.

Assessments

1. Students record changes in gas released from respirometers containing germinating seeds and dry seeds. They set up their tubes in air rather than in a water bath. A thermometer probe is inserted in each respirometer. The tube that contains germinating seeds shows an increase in temperature. No such increase is recorded in a respirometer that contains dry seeds. What is the reason for the difference in temperature?
2. The ideal gas law shows that volume depends on temperature as well as pressure. Why do you set your respirometers in a water bath?
3. A classmate insists that there are no mitochondria in leaves because chloroplasts produce ATP through photosynthesis. How would you experimentally disprove this claim?

KEY TERMS

activation energy
: the energy required to be available in a system before a chemical reaction can take place

dormant
: metabolically inactive

enzymes
: biological catalysts, many of which are proteins

germination
: growth from seed

respirometer
: an enclosed chamber in which the studied organism is placed. We measure changes in the gas volumes in the chamber with a graduated pipette

LAB 9: FERMENTATION IN YEAST

COMPARING RATES OF FERMENTATION IN YEAST

Learning Objectives

After completing the lab, the student will be able to:

1. Describe how different carbon compounds affect the rate of fermentation.

Activity 1: Pre-Assessment

1. What is the difference between real sugar and a sugar substitute? Could either substance be used during cellular respiration?
2. How could you tell that an organism, such as yeast, has switched from aerobic respiration to fermentation?
3. Discuss the answers to questions 1 and 2 with the class.

Activity 1: Comparing Rates of Fermentation in Yeast

Yeast carry out **fermentation** as a means to access the chemical energy from their food, which, in this case, will be sugars such as glucose. In this activity, you will be comparing fermentation between these food sources and a control group of water. Yeast can exist in a state of **dormancy**, where they are alive, but their physical activity has temporarily stopped to minimize energy use. This adaptation allows these organisms to survive extended periods of drought and other harsh environmental conditions. As you add water to the dry yeast, you will be activating the cells out of dormancy, and they will resume physical activity and begin eating. Once the yeast absorbs the food molecule, it will first break the molecule down in a process called **glycolysis**. The word glycolysis literally translates into the breaking apart of glucose and this is the first step of the metabolism of sugars (Figure 9.1).

Shortly after mixing the activated yeast with the sugar molecules, fermentation will begin, and you will be able to observe gas bubbles being produced. This gas is carbon dioxide, one of the products of fermentation.

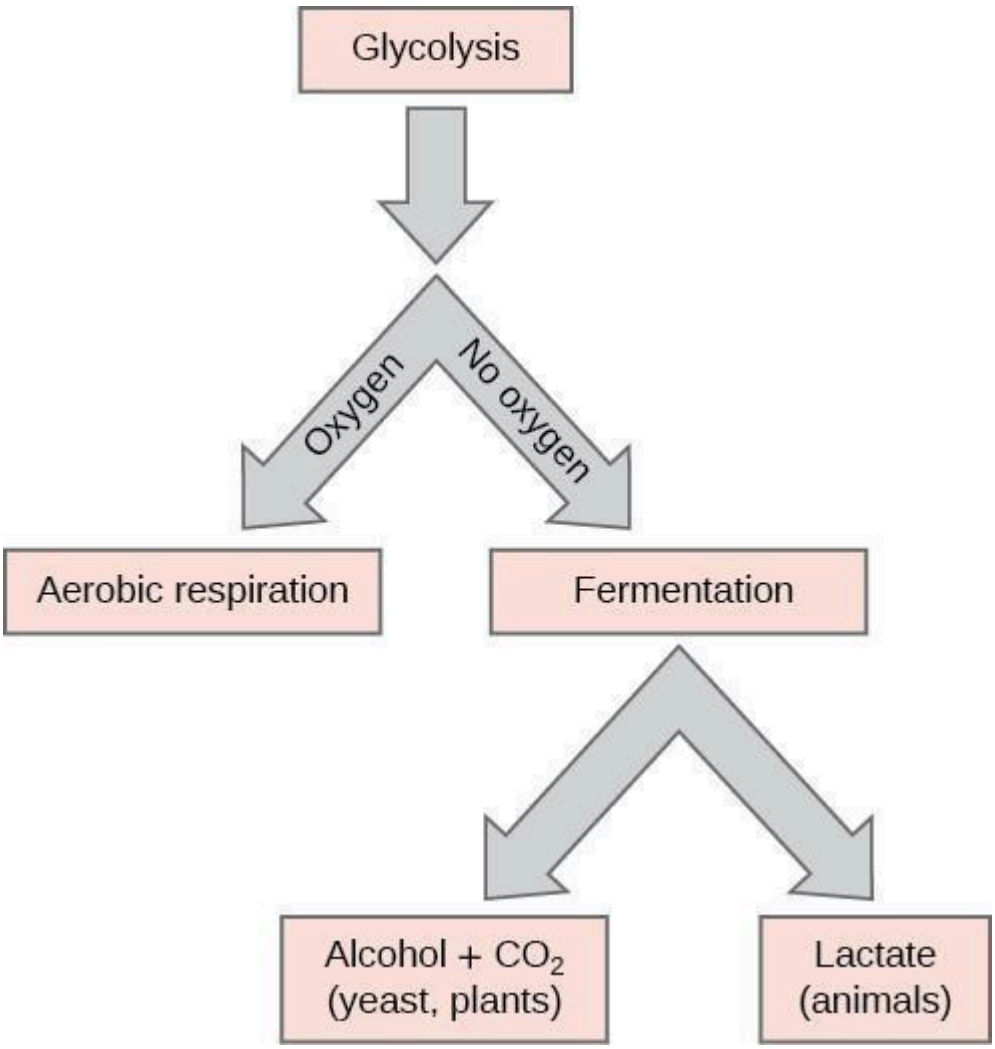


Figure 9.1: Glycolysis is the first step of both aerobic respiration and fermentation. Aerobic respiration only produces carbon dioxide as a toxic byproduct, but fermentation also produces alcohol or lactate.

Safety Precautions

- Use care when using glassware.
- Be careful when inverting the graduated cylinder into the Petri dish.
- Inform your teacher immediately of any broken or cracked glassware, as it could cause injuries.
- Clean up any spilled liquids to prevent people from slipping.

For this activity, you will need the following:

- Graduated cylinder (50 ml)
- Petri dishes
- Beaker/container for yeast solution (150–200 ml)

- One package of dry yeast
- Warm water
- Room temperature water
- Glucose
- A sugar substitute
- Pipette or droppers
- Paraffin wax paper
- Paper towels

For this activity, you will work *in pairs*.

Structured Inquiry

Step 1: Add the yeast packet to the beaker and add 100 ml of warm water to activate it. Swirl slightly until water and yeast are mixed. Allow 10 minutes for activation. During this time, create a data table to measure the volume in milliliters of the carbon dioxide gas bubbles as a function of time. Include space for two trials for each variable of water, glucose, and the sugar substitute, as well as predictions of the amount of respiration that will occur for each sugar.

Step 2: Hypothesize/Predict: Based on what you know about the differences between water, glucose, and the sugar substitute, predict how much carbon dioxide concentrations will differ in the presence of 1) glucose, 2) the sugar substitute, and 3) water. Add your predictions to the data table created in Step 1.

Step 3: Student-Led Planning: You will now start with the first trial of measuring carbon dioxide gas bubbles produced from fermentation. Choose a time interval to take down measurements. After the first trial, adjust the time intervals if necessary for taking down measurements. These should be in your data table. Determine and record the quantity of glucose and sugar substitute that you will use, as you should perform more than one trial of each test.

Step 4: Pour 15 ml of yeast solution into the graduated cylinder. Then add in the water and fill up the rest of the cylinder. For the other trials, glucose or sugar substitute will be added instead of water. As soon as you add the water, cover the cylinder with paraffin wax paper and invert it, to avoid spilling the fluid. One person can hold the graduated cylinder in place displacing it carefully and completely onto the Petri dish, as shown in Figure 9.2. As fermentation takes place, bubbles will rise into the cylinder, and the volume of the bubbles can be recorded. To do this, you will read the measurement markings at either end of the bubbles to get at total volume reading.

Step 5: Student-Led Planning: Discuss with your partner how the first trial worked and whether any adjustments need to be made to the set-up to ensure more accurate results. If the original trial did not go well due to measurement issues, repeat after adjusting the procedure. Then use the corrected trial as the first one. All subsequent trials will then use the same exact procedure.

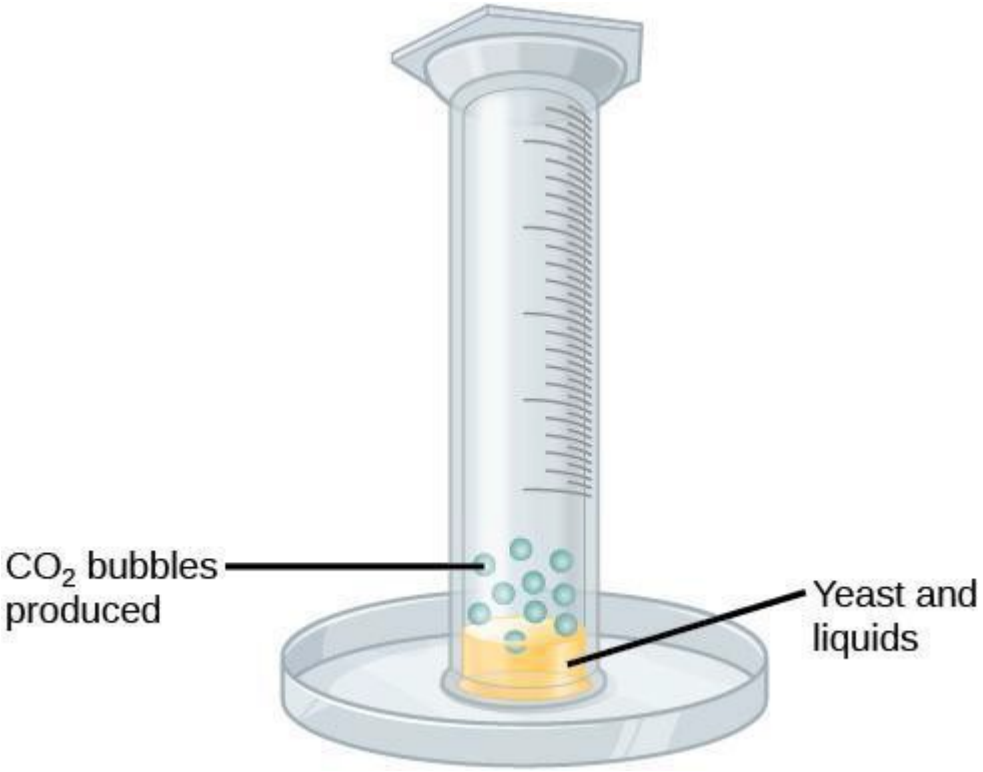


Figure 9.2: As fermentation takes place, carbon dioxide bubbles will rise into the cylinder. Volume measurements can be taken at specific time increments.

Step 6: Critical Analysis: Record the amount of carbon dioxide gas produced in your data table. Are the predictions made in step 2 supported by the data? Why or why not? What is the control in this experiment? Is it a positive or negative control and why? What changes to the quantities of liquids, time period, or set up can you make to improve your results? Discuss with your partner and write your answers in your notebook.

Guided Inquiry

Step 1: Hypothesize/Predict: How do you think the chemical makeup of the compound added to the yeast solution affects the rate of fermentation? How different do you think the results will be with the glucose and sugar substitute trials? Write your hypotheses in your notebook.

Step 2: Student-Led Planning: Make any adjustments to your first trial set up and re-do if necessary. Then, carry out the second trial for water and two additional trials each using glucose and sugar substitute. Record your measurements in the data table.

Step 3: Critical Analysis: Which compound led to the highest rate of fermentation? Was there a significant difference between the glucose and sugar substitute trials? Discuss your answers with your partner and write them in your notebook.

Assessments

1. What does data from the experiment show about the energy needs of yeast?
2. What do you think would happen to the activated yeast if no carbon-based molecules were provided?
3. What environmental reasons would cause yeast to go dormant?

DEPENDENCE OF FERMENTATION ON TEMPERATURE

Learning Objectives

After completing the lab, the student will be able to:

1. Describe how different carbon compounds affect the rate of fermentation.
2. Explain how temperature affects the rate of fermentation.

Activity 2: Pre-Assessment

1. How does temperature affect the rate of chemical reactions? Explain your answer.
2. What would likely influence the optimum temperature of cellular respiration for an organism? Do you think there is a temperature that cellular respiration would cease and what would determine this upper temperature limit for cellular respiration?
3. Discuss the answers to questions 1 and 2 with the class.

Activity 2: Dependence of Fermentation on Temperature

All cellular processes, like **fermentation**, consist of an interconnected series of chemical reactions. However, temperature can influence the rate of chemical reactions by affecting how quickly the reactants move, and therefore, how often they collide with each other. If the temperature gets too high, enzymes or other cellular **proteins** involved with cellular respiration can break apart, or **denature**, rendering them inactive. Therefore, it is very important for cells and organisms to regulate their internal temperature to ensure that cellular respiration and other chemical reactions can continue at the proper rate.

Safety Precautions

- Use protection when handling hot glass and materials.
- Do not mix very hot water with very cold water in glass containers.
- Clean up any spilled liquids to prevent slipping.

For this activity, you will need the following:

- Graduated cylinders
- Water
- Ice
- Thermometer
- Beakers
- Sugar substitute solution
- Yeast solution (one packet of yeast with 100 ml of water; can use from Activity 1)
- Hot plate
- Droppers or pipettes
- Medium-size, un-inflated balloons
- Cloth measuring tape or pieces of string and a ruler
- Balloons

For this activity, you will work *in pairs*.

Structured Inquiry

Step 1: Hypothesize/Predict: Unlike Activity 1, you will measure the volume of carbon dioxide gas by placing balloons over the graduated cylinders after the reactants are added. Use 10 ml of each reactant. As the balloons fill with gas, you can measure their circumference to find the volume. How do you think temperature will affect the rate of fermentation? How can this be tested? Write your hypotheses in your notebook.

Step 2: Student-Led Planning: In this lab, you will measure the changes in the circumference of a balloon. Decide, with your partner, how you will take this measurement using a practice balloon that you inflate with your mouth. Make sure that your measurement is repeatable regardless of the size of the balloon. Create a data table for your measurements of balloon circumference versus solution temperature. You should record the volume of the gas in the balloon every 2 minutes for 10-15 minutes per trial.

Step 3: Prepare the first beaker with 100 ml of water at room temperature, approximately 21 °C. Add cold or hot water to get the water as close to 21 °C as possible. Once the temperature is stable, place the graduated

cylinder containing the yeast and sugar substitute directly into the water beaker. Be sure to record the actual temperature of your room temperature solution in your notebook.

Step 4: Repeat Step 3 for the cold and hot water beakers one at a time. For the cold water treatment, fill the beaker with cold water and then add ice. Wait until the temperature stabilizes before beginning the timer. For the hot water beaker, bring the water to a boil using a hot plate. Then, turn down the hot plate to maintain the water at a low boil. Record the stabilized temperatures for both treatments before immersing the graduated cylinders containing the yeast into the beakers.

Step 5: Critical Analysis: Does this set up seem to capture the carbon dioxide gas effectively? Should you adjust the quantities of any of the liquids and/or the time intervals? Discuss with your partner and then write your answers in your notebook.

Guided Inquiry

Step 1: Hypothesize/Predict: How do you think the more extreme temperatures, such as (~ 0–4 °C) and (55–60 °C) will affect the rate of fermentation? Are there any new safety concerns to consider with such changes to the temperature?

Step 2: Student-Led Planning: Pick two additional temperatures to test within the range (~ 0–4 °C) and (55–60 °C). Use the water, ice, hot plate, and thermometer to make these baths before adding the graduated cylinders containing the yeast and sugar substitute solutions. Then perform your trials as in the structured inquiry, measuring the volume of gas in the balloon. Create appropriate data tables before beginning your trials.

Step 3: Critical Analysis: How did temperature affect the rate of fermentation for this species of yeast? Is it likely any of the proteins involved in the reaction denatured and at what temperature did this occur? Graph the circumference of the balloon versus the temperature of the reaction. Based on your graph, what is the best estimate of the optimal temperature for fermentation in yeast? Discuss your answers with your partner and write them in your notebook.

Assessments

1. What likely caused the differences in reaction rate at the three temperatures examined in Activity 2?
2. How could this experiment better measure the effect of temperature changes in the natural habitat of the yeast?
3. What other biotic and abiotic factors would likely affect the rate of cellular respiration in yeast? Explain your answers.
4. What patterns can you identify in the data you collected between the yeast and the abiotic factor of temperature?

KEY TERMS

aerobic respiration
: the process of cellular respiration that occurs in the presence of oxygen

anaerobic respiration
: the process of cellular respiration in which some other molecule other than oxygen is the terminal electron receptor. In this process, cells break down sugars in the absence of oxygen

carbohydrates
: Biological macromolecule compounds composed of carbon, hydrogen, and oxygen. Also called carbs and/or sugars. A nutrient required by living organisms for the production of energy in the form of ATP

dormancy
: state of continuing normal biological function with physical function slowed or suspended in what is considered deep sleep or inactivity

denature
: to breakdown or unfold a molecular conformation

enzymes
: form of proteins that act as biological catalysts

fermentation
: anaerobic respiration that utilizes enzymes to break down organic substrates and produce energy, often in the form of heat

glycolysis
: the breaking down of the glucose molecule (carbohydrates) into pyruvic acid, producing ATP

proteins
: biological macromolecules composed of one or more amino acids

yeast
: eukaryotic, single-cell microorganisms classified in the fungus kingdom

LAB 10: PLANT PIGMENTS

EXTRACTING PIGMENTS FROM PLANT MATERIAL

Learning Objectives

After completing the lab, the student will be able to:

1. Extract pigments from plant material.
2. Separate pigments by paper chromatography.

Activity 1: Pre-Assessment

1. Think about what colors you have seen in leaves of plants. What determines the color of a leaf? What color or colors do you expect to see if you extract pigments from leaves and separate them chemically?
2. Other structures in plants besides leaves are brightly colored. Fruit, flowers, and some roots display colors. Are these colors associated with photosynthesis? What are the purposes of those colors?
3. **Discuss the answers to questions 1 and 2 with the class.**

Activity 1: Extracting Pigments from Plant Material

The chloroplast, as illustrated in Figure 10.1, is the photosynthetic organelle in the plant cell. The chloroplast is surrounded by a double membrane. Within the inner membrane is a fluid compartment called the stroma. The **thylakoids** form a complex network of membranes that appear as stacked disks called grana (singular, granum). The photosystems, and photosynthetic pigments they contain, are embedded in the thylakoid membrane. Carbon fixation takes place in the stroma. What chemical properties would you expect photosynthetic pigments to display and how can these chemical properties be used to extract the pigments from tissues? How do the solubility properties enable scientists to separate individual pigments for analysis?

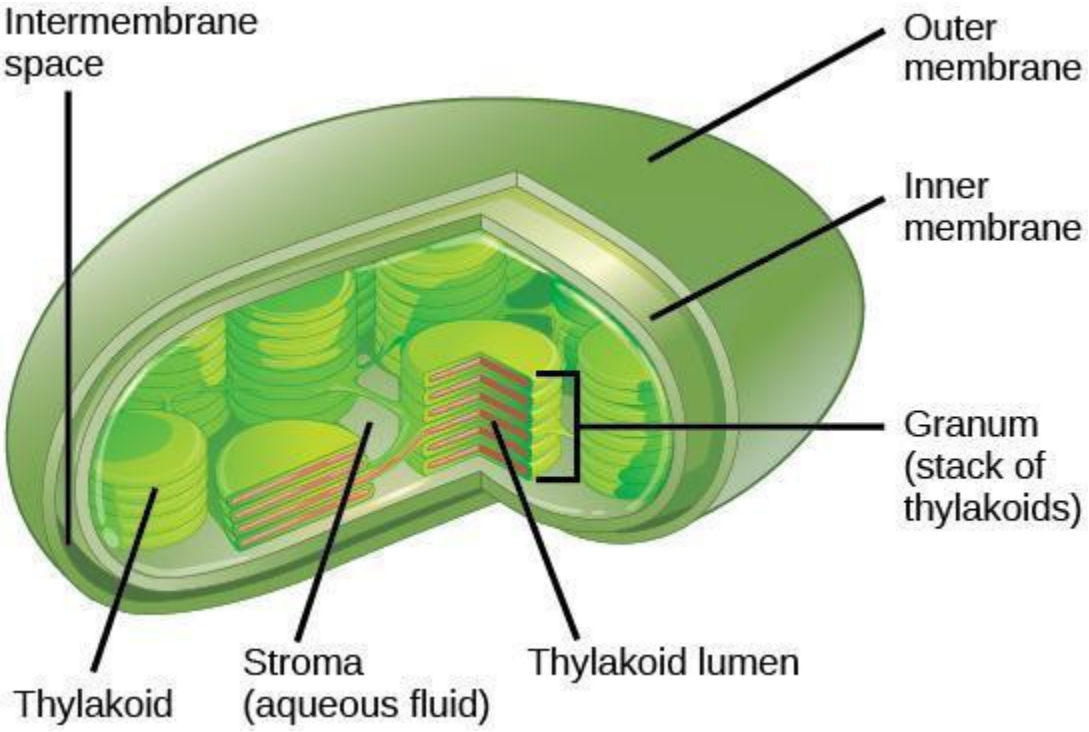


Figure 10.1: A section through a chloroplast shows the arrangement of the thylakoid membranes in stacks and single membranes. The thylakoids are pockets enclosed by membranes.

Safety Precautions

- Wash your hands after completion of this activity.

For this activity, you will need the following:

- Plant material: intact leaves of spinach and *Coleus* (one leaf of each per pair of students)
- Filter or chromatography paper
- Ruler (one per group)
- Coins
- Pencils
- Colored pencils
- Forceps
- Scissors

In this activity, you will work *in pairs*.

Structured Inquiry

Step 1: Hypothesize/Predict: In this activity, you will extract pigments from spinach leaves. Discuss and predict what colored pigments you will observe after extraction. Write your hypotheses in your notebook.
Step 2: Student-led Planning: Assemble all the material needed for extracting pigments from spinach leaves. Read the procedure in step 3 and Figure 10.2 to extract pigments from plant material. Throughout the procedure, you will need to avoid touching the paper with your fingers, because oils from your skin can interfere with the chromatography. Handle the paper by the edges. Devise a method for handling the paper with your partner. Use a few scraps of paper to practice extracting pigments from loose leaves. If you smear pigments all over the piece of paper while you apply pigment, the stains will also separate by chromatography and interfere with the interpretation of the results. Throw any smeared paper away and start over.
As pigments separate along the paper, the color intensity of each pigment decreases and makes it more difficult to identify each color. What approach will you use to obtain a dark line of concentrated pigment on the paper?

Step 3: Complete the steps below to extract pigments from plant material, as illustrated in Figure 10.2. Use the method you devised in step 2 to avoid touching the paper with your fingers.

1. Measure the height and the diameter (D) of the container with your partner to determine the size of the piece of chromatography paper you need for the experiment. Subtract 1 cm from the height of the container. The width of the chromatography paper should be about 1 cm shorter than the circumference of the container. Record your calculations and show them to your instructor before you cut the paper.
2. With a pencil, draw a line across the entire width of filter/chromatography paper about 1.5 cm from the bottom. This line is called the **origin** or start of the separation. It is the place where the sample is applied.
3. Select 1 large spinach leaf and carefully blot it dry with paper towels. Note in your lab notebook why it is important to blot dry the leaf.
4. Position the leaf on top of the pencil line leaving a space on each end. The leaf should reach the edge of the paper.
5. Align a plastic ruler over the leaf with the edges of the pencil line that was left uncovered by the leaf.
6. Roll a coin pressing down firmly along the ruler edge without crushing the rest of the leaf and smearing pigment on the surface of the filter paper. You may find it helpful to lay the filter paper on top of the paper towels taking care not to cover the line where the pigments are deposited.
7. Let the pigment line dry for 3 to 5 minutes. Move to a different section of the leaf and roll the coin again over the same line. Keep the band of pigments as narrow as possible. The pigments will diffuse during separation and the bands will smear if they are laid down too thick.

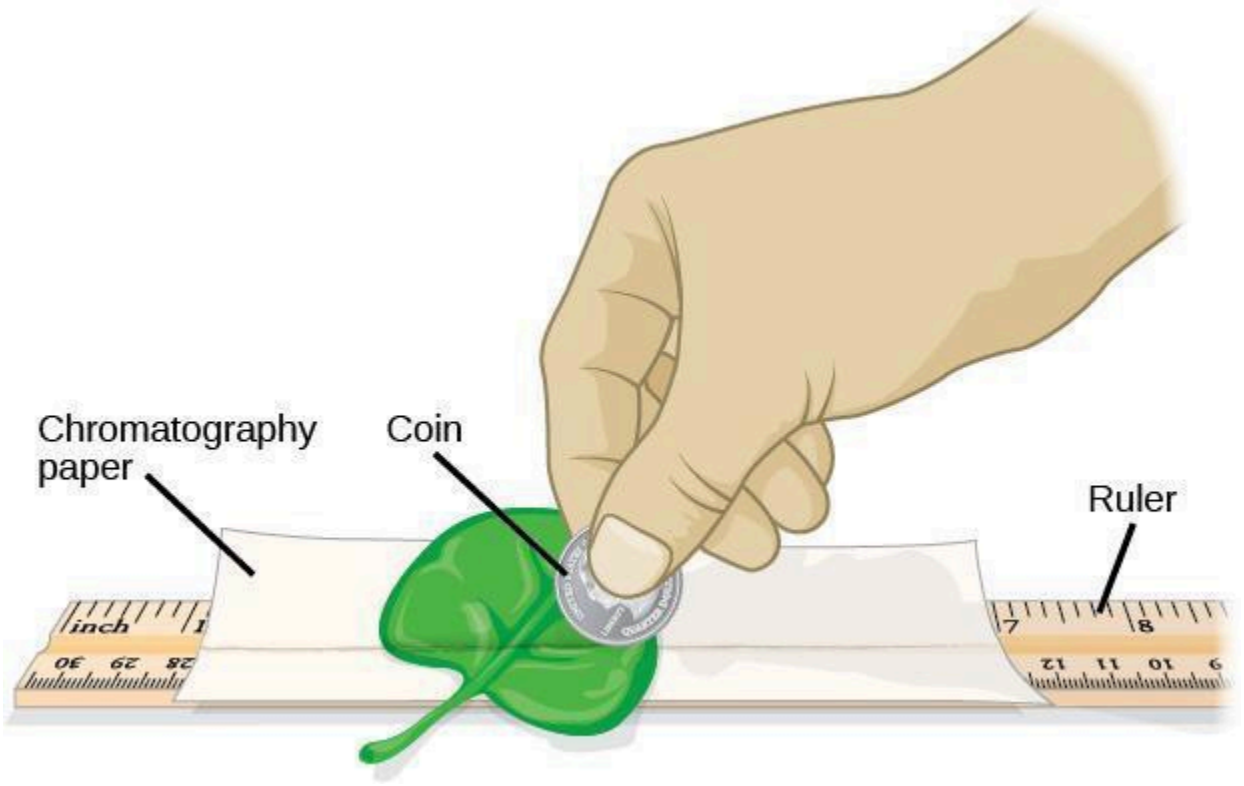


Figure 10.2: Extract pigments by rolling a coin along a ruler. Make sure that the leaf does not cover the entire paper and align the ruler with the starting line to avoid edge effects.

Step 4: Critical Analysis: Record the results from your experiment. Try to identify the pigment or pigments you extracted. Can you state conclusively what types of pigments are present in your extract on the line? How could you determine which pigments are present in the extract without performing a chemical separation? Write your answers in your lab notebook. You may refer to Figure 10.2 while discussing the answer.

Guided Inquiry

Step 1: Hypothesize/Predict: If pigments are colored molecules, can you decide which pigments are present by looking at biological material? Do spinach leaves contain other pigments besides the green chlorophylls? Do *Coleus* leaves contain additional pigments? Do you see a red pigment on the line for *Coleus*? Write your hypotheses in your notebook.

Step 2: Student-led Planning: Decide with your lab partner how you will test your hypotheses on the kinds of pigments you expect to find in your extracts of spinach and *Coleus* leaves. One lab partner should use spinach and the other one should use *Coleus.* Decide with your lab partner which plant material you will use to test your hypothesis. Extract pigments from leaves by the coin method described in step 2 of the Structured Inquiry. Record all the steps you perform and draw the colors seen on the line in your lab notebook.

Step 3: Critical Analysis: Observe the pigment lines on the paper. Record in your lab notebook the color or colors you detected. Note the differences, if any, between the spinach and *Coleus* samples.

Assessments

1. Plants in the rainforest that live under the canopy of tall trees must adapt to low light conditions. Can you predict how the pigments of plants that receive low light levels will differ from the pigments of plants that receive high light levels? What would happen to the forest if the tall trees were removed for timber?
2. You analyze a pigment extract from a mutant plant and discover that the plant defective in its carotenoid biosynthetic pathway. What would happen to that plant if it is grown in bright sunlight? Where would you expect it to thrive and how would such a plant compensate for the loss of carotenoids?
3. The Rhodophyta are algae that can grow deeper in the ocean than other plants. Based on the information shown in Figure 10.3, what color of the visible spectrum would you expect to be absorbed by an accessory pigment found in Rhodophyta? Why do you think plants have reddish colors?

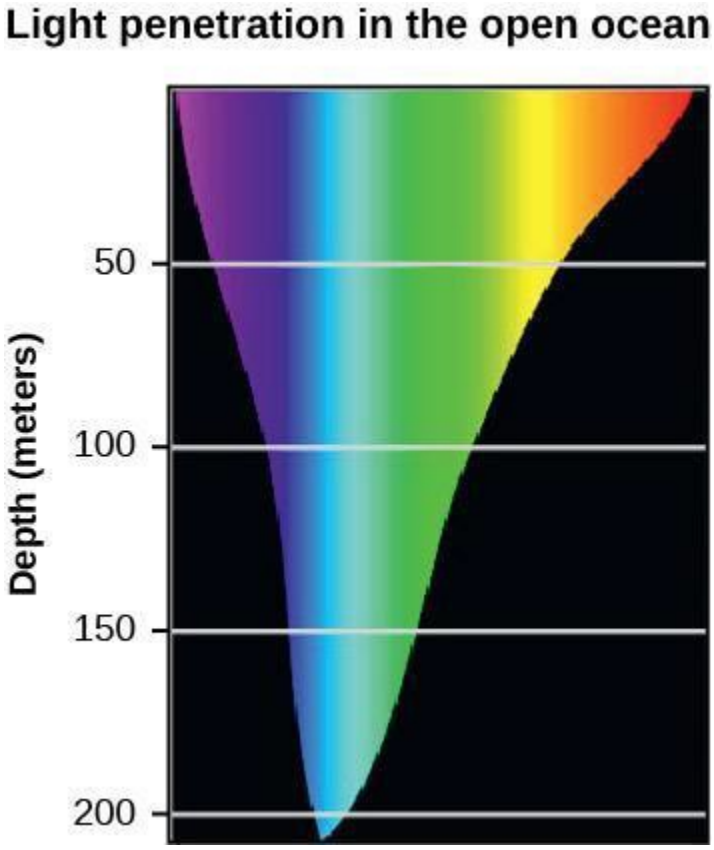


Figure 10.3: This graph shows the light penetration in the open ocean as a spectrum of color from violet to red. The depth of the ocean is measured in meters, and the peak light penetration occurs in blue light, which progresses past 200 m.

PAPER CHROMATOGRAPHY OF PLANT PIGMENTS

Learning Objectives

After completing the lab, the student will be able to:

1. Extract pigments from plant material.
2. Separate pigments by paper chromatography.
3. Measure R_f (retention factor) values for pigments.

Activity 2: Pre-Assessment

1. The leaves of some plants change color in fall. Green foliage appears to turn to hues of yellow and brown. Does the yellow color appear because carotenoids replace the green chlorophylls? Explain your reasoning.
2. Examine the molecular structures of photosynthetic pigments in Figure 10.1. Photosynthetic pigments are hydrophobic molecules located in thylakoid membranes. Will these pigments dissolve in water?

Activity 2: Paper Chromatography of Plant Pigments

Paper **chromatography** is an analytical method that separates compounds based on their solubility in a solvent.

The solvent is used to separate a mixture of molecules that have been applied to filter paper. The paper, made of cellulose, represents the **stationary** or immobile phase. The separation mixture moves up the paper by capillary action. It is called the **mobile** phase. The results of chromatography are recorded in a chromatogram. Here, the chromatogram is the piece of filter paper with the separated pigment that you will examine at the end of your experiment (see Figure 10.4).

We separate the compounds based on how quickly they move across the paper. Compounds that are soluble in the solvent mixture will be more concentrated in the mobile phase and move faster up the paper. Polar compounds will bind to the cellulose in the paper and trail behind the solvent front. As a result, the different compounds will separate according to their solubility in the mixture of organic solvents we use for chromatography.

One or more interactive elements has been excluded from this version of the text. You can view them online here: https://louis.pressbooks.pub/generalbiology1lab/?p=505#oembed-1

This video demonstrates the principles and examples of chromatography. You will experiment with only paper chromatography in this lab; however, you will see that you are already familiar with some uses of thin layer chromatography.

Safety Precautions

- Work under a hood or in a well-ventilated space and avoid breathing solvents.
- Do not have any open flames when working with flammable solvents.
- Wear aprons and eye protection.
- Do not pour any organic solvent down the drain.
- Dispose of solvents per local regulations.
- Use forceps to handle chromatography paper that has been immersed in solvent and wash your hands after completing this activity.

For this activity, you will need the following:

- Plant material: intact leaves of spinach and *Coleus* (one leaf of each plant per pair of students)
- Filter or chromatography paper
- Ruler (one per group)
- Coins
- Pencils
- Colored pencils
- Beakers (400 mL) (Mason jars are an acceptable substitute)
- Aluminum foil
- Scissors
- Forceps

- Freshly prepared solvent mixture
  - Petroleum ether: acetone: water in a 3:1:1 proportion
  - If no hood or well-ventilated place is available, the mixture can be substituted with 95 percent isopropyl alcohol. Note that, if isopropyl alcohol is used, the pigment bands will smear. You may not be able to separate and identify the chlorophylls or carotene from xanthophyll.

For this activity, you will work *in pairs*.

Structured Inquiry

Step 1: Hypothesize/Predict: Discuss with your lab partner what color pigments will likely be present in the spinach leaves. Write your predictions in your lab notebook and draw a diagram of how you think the pigments will separate out on the chromatography paper.

Step 2: Student-led Planning: Read step 3 below. Discuss with your lab partner the setup of the experiment. Then agree upon the dimensions of the filter/chromatography paper that you will use. To allow good separation, the paper should not touch the walls of the container. The paper must fit inside the container while being long enough for maximum separation. Write all your calculations in your lab notebook.

Step 3: Follow the steps below to set up your filter paper and perform the chromatography experiment.

1. Prepare the chromatogram by cutting a piece of filter paper. Transfer pigments from spinach leaves as in Activity 1. A heavy application line will yield stronger colors when the pigments separate, making it easier to read results. Allow the pigments to dry between applications. Wet extracts diffuse on the paper and yield blurry lines.
2. Form a cylinder with the filter paper without overlapping the edges (to avoid edge effects). The sample should face the outside of the cylinder. Secure the top and bottom of the cylinder with staples.
3. Pour enough separation mixture to provide a mobile phase while staying below the origin line on the chromatogram. The exact volume is not critical if the origin, the start line where you applied the solvent, is above the solvent. See Figure 10.4.

Figure 10.4: Chromatography can be set up in a container such as a Mason jar. The start line of the sample, the origin, is shown above the solvent

1. Label the beaker with a piece of tape with your initials and your partner's initials.
2. Lower the paper into the container with the band from the extraction in the lower section. The paper must touch the solvent, but not reach the band of pigment you applied. Why must the band be above the solvent line? Write your answer in your notebook.
3. Cover the container tightly with a piece of aluminum foil.
4. Track the rising of the solvent front. Can you see a separation of colors on the paper?
5. When the solvent front is within 1 cm of the upper edge of the paper, remove the cylinder from the beaker using forceps. Trace the solvent front with a pencil before it evaporates and disappears! Draw the colored bands seen on your chromatography paper in your lab notebook immediately. The colors will fade upon drying. If no colored pencils are available, record the colors of the lines.
6. Let the paper dry in a well-ventilated area before making measurements because the wet paper is fragile and may break when handled. This is also a precaution to avoid breathing fumes from the chromatogram.
7. Discard solvent mixture per your instructor's directions. Do not pour down the drain.

Step 4: Critical Analysis: Open the dried cylinder by removing the staples. Measure the distance from the first pencil line to the solvent front, as shown in Figure 10.5. This is the distance traveled by the solvent front.

Measure the distance from the pencil line to the middle point of each color band and the original pencil line. Record your results in your notebook in a table modeled after Table 10.1. The retention factor (R_f) is the ratio of the distance traveled by a colored band to the distance traveled by the solvent front. Calculate $\mathbf{R_f}$ values for each pigment using the following equation:

$$R_f = \text{Distance traveled by colored band} / \text{Distance traveled by solvent front}$$

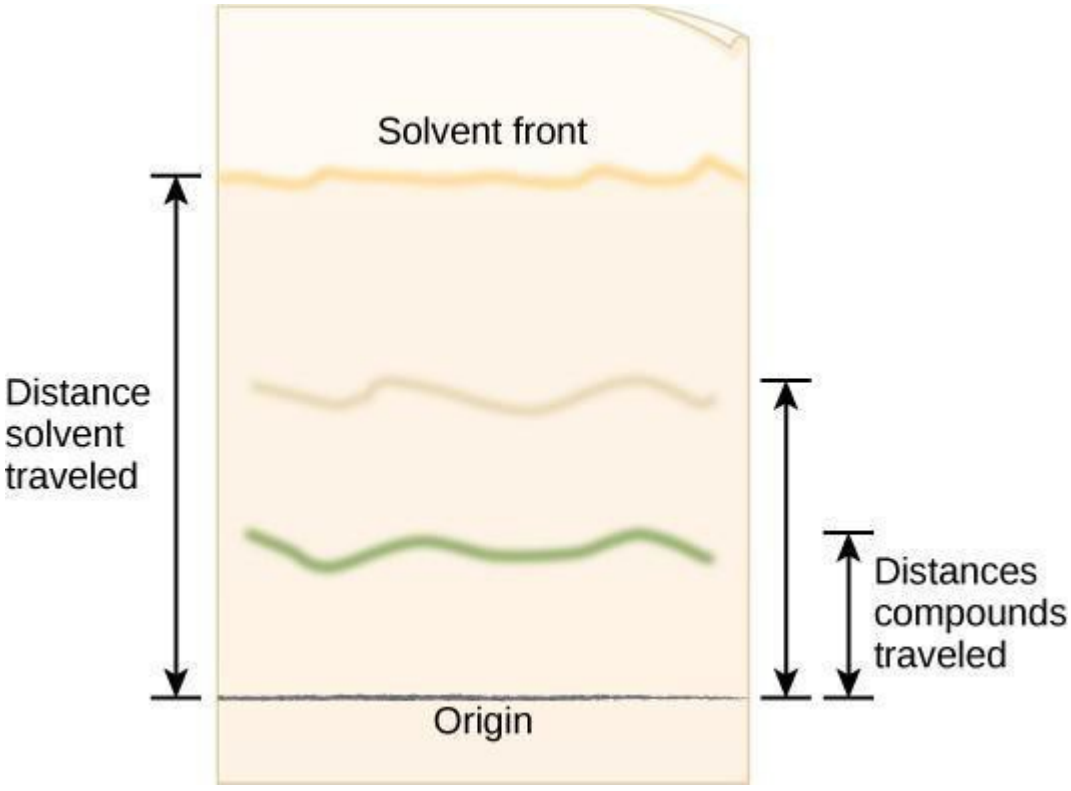


Figure 10.5: Chromatogram shows the distance traveled by the solvent front and the compounds separated by chromatography.

Step 5: After determining the color of the band, tentatively identify each band. Did your results support your hypothesis about the color of each band? Discuss which aspects of the experiments may have yielded inconclusive results. How could you improve the experiment?

Guided Inquiry

Step 1: Hypothesize/Predict: What type of pigments are present in *Coleus* leaves and where are the different colors located? Can you make a hypothesis based on the coloration of the variegated leaves? Write your hypothesis down in your lab notebook. Would there be a difference if you performed chromatography on pigment composition from different colored regions of the leaves?

Step 2: Student-led Planning: Cut the chromatography/filter paper to the dimensions needed. Apply pigments from different parts of the *Coleus* leaves following the procedure described under Activity 1, keeping in mind that a darker line will yield stronger colors when the pigments are separated, which will make it easier

to read the results. Allow the pigments to dry between applications. Wet extracts diffuse on the paper and yield blurry lines.

Step 3: When the solvent front reaches 1 cm from the top of the filter paper, stop the procedure. Draw the pigment bands you see on the filter paper in your lab notebook. Clearly indicate the color you observed for each band.

Step 4: Let the cylinder dry and measure the distance the front traveled from the origin and the distances traveled by each of the pigments. If the bands broadened during separation, take measurements to the middle of each band.

Step 5: Critical Analysis: Calculate R_f for each of the bands and record them in a table in your notebook. Compare the R_f you obtained with those of other groups. Are the R_f values similar? What may have altered R_f values?

Assessments

1. Carotenoids and chlorophylls are hydrophobic molecules that dissolve in organic solvents. Where would you find these molecules in the cell? What would happen if you ran the chromatography in this lab with water as the solvent?
2. All chlorophyll molecules contain a complexed magnesium ion. Your houseplant is developing yellow leaves. What may cause this, and how can you restore your plant's health?
3. Seeds that grow under dim light are said to be etiolated, which describes their pale and spindly appearance. They soon waste away after exhausting their food reserves. Can you explain this observation?

KEY TERMS

chromatography
: analytical method that separates compounds based on their solubility in a solvent

mobile
: the solvent moving by capillary action

stationary
: substance that remains fixed; the paper in paper chromatography

thylakoids
: disc-shaped, membrane-bound structure inside a chloroplast where the light-dependent reactions of photosynthesis take place; stacks of thylakoids are called grana

LAB 11: THE LIGHT REACTION OF PHOTOSYNTHESIS

INFLUENCE OF THE LIGHT REACTION ON THE CONCENTRATION OF CO2

Learning Objectives

After completing the lab, the student will be able to:

1. Measure the dependence of CO_2 fixation on the light-dependent reaction.
2. Explain the chemical principles of pH indicators and the bicarbonate-CO_2 equilibrium.

Activity 1: Pre-Assessment

1. **Answer the following questions in your notebook:** Which gas is released by plants during photosynthesis? Which gas is absorbed during photosynthesis? How does the overall chemical equation for photosynthesis compare to the overall chemical equation for respiration?
2. **Answer the following questions in your notebook**: What chemical reactions take place when CO_2 is released in water? What is the connection between an increase in CO_2 in the atmosphere and the acidification of oceans? Why are plants essential in mitigating the effects of climate change?
3. Discuss the answers to questions 1 and 2 with the class.

Activity 1: Influence of the Light Reaction on the Concentration of CO_2

In this experiment, you will predict and test the effect of environmental conditions on the process of photosynthesis. Gaseous carbon dioxide dissolves in water per the reaction

$$CO_2 + H_2O \leftrightarrow H_2CO_3 \leftrightarrow H+ + HCO_3-$$

Carbon dioxide and water combine to generate the unstable carbonic acid molecule that separates into hydrogen and bicarbonate ions effectively lowering the pH of the solution. The reaction is reversible. When

CO_2 molecules are consumed by photosynthesis, the reaction shifts to the left. Hydrogen ions and bicarbonate ions combine to replace CO_2 with the net effect of increasing the pH of the solution.

A **pH indicator** is a chemical compound that changes color based on pH level. In this experiment, you will use phenol red, which appears yellow at pH below 7, orange at neutral pH, and bright red or pink at basic pH values (see Figure 11.1). How can the presence of phenol red allow measurement of the consumption of CO_2 by a plant submerged in water? Discuss the method with your partner and write your answer in your notebook.

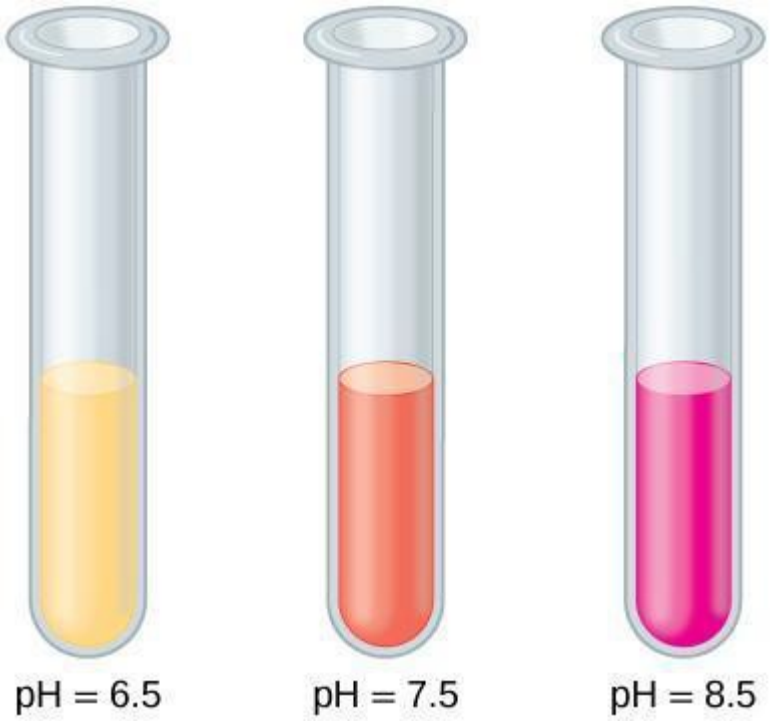


Figure 11.1: The pH indicator phenol red appears yellow at an acidic pH, pinkish orange at a neutral pH, and bright red or pink at a basic pH.

Safety Precautions

- Be careful when blowing into the straw inside the reaction vessels. Do not suck up the solution.
- Do not touch incandescent lamps, because they can be very hot.
- Wear aprons and eye protection.

For this activity, you will need the following:

- Plant material: *Elodea* or other aquatic plant
- Flasks
- Transfer pipettes
- Solution of phenol red
- New drinking straws

- Test tubes and test tube racks
- Lamp with LED bulb
- Beakers of water
- Hot plate
- Ice
- Aluminum foil
- Acetate colored filter (at a minimum, use blue, red, and green filters)
- Thermometer
- Meter stick

For this activity, you will work *in pairs*.

Structured Inquiry

Step 1: Hypothesize/Predict: In this experiment, you will measure CO_2 uptake in *Elodea* exposed to light compared to a control *Elodea* in a control tube where light is blocked. Make a hypothesis with your partner about the color changes you expect to see in the pH indicator when the test tubes are exposed to light. Write down your hypothesis in your notebook. Discuss what you predict will happen to the test tubes kept in the dark. Decide what you will do if you observe no change in the test tubes exposed to light within the time allocated.

Step 2: Student-led planning: Set up the materials described in Step 3 below. If you have access to a camera, you may take pictures of your set-up. Otherwise, draw the set-up in your lab notebook indicating the colors in words or by using colored crayons. What is the control treatment in this setup? Is it a positive control or a negative control? Explain your reasoning in your notebook.

Step 3: Monitor the photosynthetic response by following CO_2 uptake, as outlined in the following steps.

1. Prepare test tubes for the experiment. First, label all tubes with a piece of tape and a marker to identify the sample. The first tube will contain the sprig of *Elodea* exposed to light. This is the experimental sample that will be labeled *Light*. The second tube will contain a sprig of *Elodea* and will be wrapped in aluminum foil. Label this tube *Dark Control* because it will serve as a control. The third tube will be labeled *Light Control* and will contain water and indicator without plant material.
2. Prepare a dilute solution of phenol red (mixed with tap water) in sufficient quantity for the test tubes. For example, if you set up test tubes that will contain 25 ml each, prepare 120 ml of solution to make it easier to aliquot equal amounts of solution. If the color is close to red or pink, it means that the tap water is basic. In this case, it may be more practical to use distilled water; since it may be difficult to blow enough CO_2 to adjust the pH. The reason for using distilled water is that freshly distilled water has a pH of 7.0. As CO_2 from the atmosphere dissolves in the distilled water, the pH drops below 7.0.

3. If the starting phenol solution is a light shade of pink, take a clean drinking straw and gently blow bubbles into the solution until it turns bright yellow. What does the yellow color indicate? Write your answer in your notebook.
4. Fill test tubes with water as indicated by your teacher (see Step 2). Then add the pH indicator solution.
5. Cut leafy sprigs of *Elodea* with the scissors without crushing the material. To produce comparable results, the sprigs should be similar in length and number of leaves.
6. Place the sprigs in the test tubes you prepared for the experiment. Top with water to make sure that the sprigs are fully immersed.
7. Record the color of each solution in your lab notebook in a table like Table 11.1. Remember to modify the table to accommodate all your data.
8. Wrap a piece of aluminum foil around the tubes if you are measuring photosynthesis in the *Dark Control* tube. Make sure that there are no light leaks.
9. Place a lamp about 0.5 m from your experimental set-up. Place a container of water between the lamp and the test tube to absorb the heat radiating from the bulb if you use an incandescent bulb, as shown in Figure 11.2. LED lamps radiate less heat making it possible to bypass the use of a heat sink.
10. Start your watch and record the color of the tap water with phenol indicator solution every 30 minutes. To compare CO_2 production in the presence and absence of light, record the end-point color of the test tubes wrapped in aluminum foil after two hours of incubation.

Table 11.1: Change in Phenol Red Color

Time (min)	Tube with *Elodea* exposed to light	Tube without *Elodea* exposed to light	Tube with *Elodea* in the dark	Tube without *Elodea* in the dark
0				
30				
60				
90				
120				

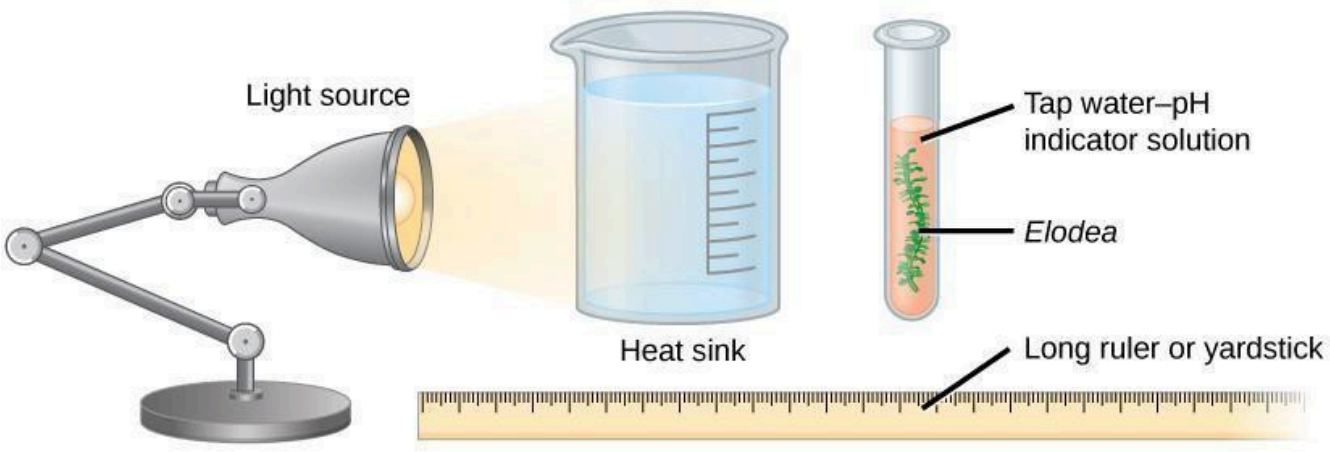


Figure 11.2: Experimental set-up to measure release of CO_2 during photosynthesis.

Step 4: Critical Analysis: Record the colors you observe in the test tubes over time in the table you prepared in your lab notebook. What happened to the *Dark Control* test tubes compared to the *Light Control* tubes and what does this tell you about the validity of your experiment? Do the experimental results support your hypothesis?

Guided Inquiry

Step 1: Hypothesize/Predict: With your partner, review the process of photosynthesis and decide on an environmental condition you want to test. You may choose to investigate the influence of temperature, light intensity, or light color. Can you dim the room lights to minimize the effect of white light when experimenting with the effect of colored light? Decide with your lab partner how you will test the environmental condition and what you predict will happen to the pH in the solution. Include all necessary controls in your hypothesis. You should also devise a positive and negative control for your experiment. Check with your instructor on the availability of supplies to carry out your experiment.

Step 2: Student-led planning: Set up your experiment following the guidelines described under step 2 of structured inquiry. You may need to shut off the room lights and cover the windows so there is only one light source. When measuring the effect of light intensity, use the equation $I=1/D^2$ where I is light intensity and D is distance. In every case, ensure that you measure only one variable in your set up. The temperature can be kept constant by placing the tube in a water bath. Prepare a table like Table 11.1 in your lab notebook to enter your data.

Step 3: Critical analysis: Record your measurements in your lab notebook. Analyze the results and compare them to your hypothesis. Did the environmental variable affect pH changes as you predicted? What do the changes in pH tell you about the changes in CO_2 production? What other factors may have contributed to the results? If you measured the effect of temperature, explain which reactions in photosynthesis would be dependent on temperature? How would you modify the experiment if you were to repeat it? Discuss your results with groups that tested different environmental variables.

Assessments

1. In an experiment, the water was first boiled, which drove out all of the existing gases. The water was then added to the test tube containing *Elodea*, which was then exposed immediately to light. The photosynthetic activity of the *Elodea* was then monitored but no photosynthetic activity was detected. How can you correct the situation by modifying the composition of the *Elodea* environment?
2. Scientists report that the levels of carbon dioxide are rising in the atmosphere and driving climate change. Another observation is that acidification of oceans is also a consequence of climate change. Can you explain what link may exist between the two phenomena and how it affects the marine ecosystem?
3. Farmers report that planting corn plants too closely can stunt growth even when the plants are heavily fertilized and receive plenty of light. Furthermore, the effect seems to be reduced in recent years. What is the growth limiting factor under these conditions? What may be an explanation? How do plants respond to decreased availability of the limiting factor?

DEPENDENCE OF PHOTOSYNTHESIS ON SELECTED WAVELENGTH IN THE LIGHT SPECTRUM

Learning Objectives

After completing the lab, the student will be able to:

1. Analyze the dependence of O_2 production on the color of light spectrum and other environmental variables.

Activity 2: Pre-Assessment

1. **Answer the following questions in your notebook**: *Spirogyra* are thin, thread-like green algae that cluster up in rafts and float on the surface of ponds buoyed by bubbles of gas. What gas buoys the network of algae? What happens when there is no light?
2. **Answer the following questions in your notebook:** If chlorophyll absorbs maximally in the blue and red regions of the visible spectrum and carotenoids extend absorbance in the blue-green region, which color of irradiation will be the most effective: red, green, or white?
3. **Discuss the answers to questions 1 and 2 with the class.**

Activity 2: Dependence of Photosynthesis on Selected Wavelength in the Light Spectrum

Several environmental factors influence the **light-dependent reaction** of photosynthesis. In this activity, you will investigate the rate of the light-dependent reaction by measuring indirectly the production of oxygen. Leaf disks are suspended in a solution of bicarbonate. A small drop of dishwashing soap in the solution breaks the

surface tension of the leaf disks and allows the bicarbonate to penetrate inside the leaf. The bicarbonate in the solution provides a source of CO_2 for photosynthesis. The inner spaces in a leaf are normally filled with air. When a bicarbonate solution is forced into those air spaces, the leaf disks become heavier and sink to the bottom of the solution.

As photosynthesis proceeds, the oxygen produced by **Photosystem II (PSII)** pushes liquid out of the air spaces and eventually bubbles out of the disks, which float to the surface. By monitoring the time it takes the disks to float, the rate of photosynthesis can be estimated under various conditions. To compare results of experiments, you will use the estimated time for 50 percent of the disks to float to the surface, labeled as ET_{50}. This is a median value that allows you to discount outliers, such as a floating disk that never sank in the first place or a disk stuck to the bottom of the cup. Because ET_{50} values decrease when photosynthetic rates go up, you may choose to plot $1/ET_{50}$ as a function of your independent variable.

Safety Precautions

- Wear goggles and an apron
- Use caution while working with the lamp, as light bulbs become very hot
- Use caution while handling glass

For this activity, you will need the following:

- Plant material: intact leaves of spinach and *Coleus*, and light-colored leaves such as iceberg lettuce, celery leaves, or cabbage (one leaf per pair of students). Avoid fuzzy leaves that trap air on their surface and do not sink easily.
- 0.2 M sodium bicarbonate solution (0.4 M, 0.6 M, and 0.8 M should be available)
- Two to three plastic 10 mL syringes
- Clear plastic cups
- Dilute dishwashing soap solution
- Single-hole punch
- Lamps
- Acetate colored filters (at a minimum, use blue, red, and green filters)
- Aluminum foil

For this activity, you will work *in pairs*.

Structured Inquiry

Step 1: Hypothesize/Predict: Discuss with your partner how different the wavelengths of light would affect

the rate of photosynthesis. Write in your lab notebook the justification for your predictions. Include the controls you will need to run a meaningful experiment.

Step 2: Student-led planning: In this guided inquiry, you will measure the ET50 in white light, in the dark, and in different regions of the visible spectrum. Design your experiment so you change only one variable at a time. Can you devise a set-up to minimize stray white light when you test the effect of colored light? Some experimentalists build a cardboard box with an opening that can be covered by a filter. In this case, run all your experiments under the same conditions using a transparent filter for white light and aluminum foil to block the light. Create a table for your data.

Step 3: Set up of experiment:

1. Using a single-hole punch, punch 10-15 disks from an intact leaf being careful not to damage the leaf in the process.
2. Transfer the disks to the barrel of a 10 mL syringe and replace the plunger.
3. Pull about 4–5 mL suspension solution in the syringe. Tapping gently on the barrel until all disks float freely
4. Push the plunger to expel excess air.
5. Create a vacuum in the syringe by holding your index finger on the tip of the syringe where the needle would be inserted and slowly draw the plunger down. You will feel the vacuum pulling on your finger. Do not draw the barrel out of the syringe. The solution should be flowing into the airspaces of the leaf disks. Wait two to three minutes and release the vacuum. The disks should sink after you repeat this procedure two to three times. See Figure 11.3.
6. Tap the syringe gently to re-suspend the disks and empty the contents of the syringe into a clear cup. Cover the disks with additional solution if needed to submerge them.
7. Continue with your experimental set-up. Illuminate the clear cups with the lamp and record the number of floating disks every minute for 20 minutes. For the dark control, check the disks after 20 minutes.

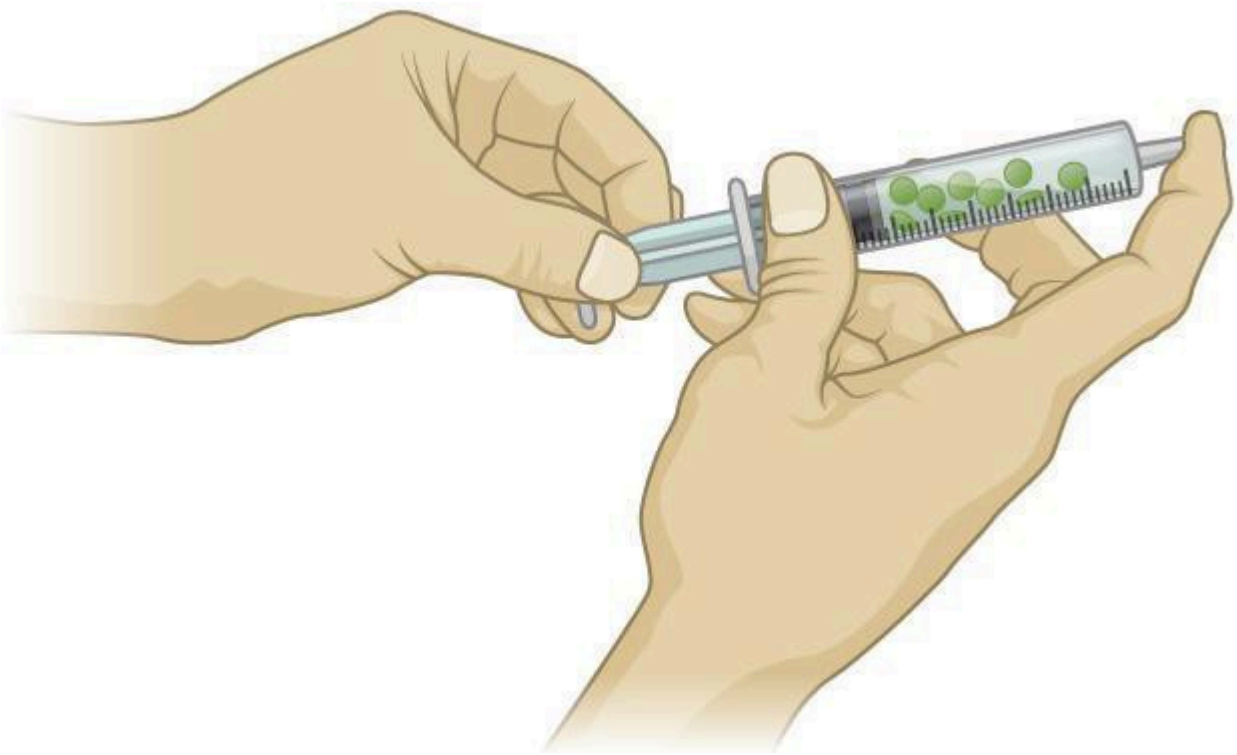


Figure 11.3: Pulling the barrel of the syringe and capping the tip with a finger creates a vacuum inside the syringe.

Step 4: Critical analysis: Plot the number of floating disks for each condition as a function of time and determine the ET_{50} for each condition. Compare the estimated photosynthetic rates as $1/ET_{50}$ at the wavelengths you tested. Do the results confirm your hypothesis? Plot your results in a graph.

Guided Inquiry

Step 1: Hypothesize/Predict: Discuss with your partner which conditions would impact the rate of photosynthesis in the disk. Conditions include the following:

1. **The effect of varying CO_2 concentration**. This is achieved by using different concentrations of a sodium bicarbonate buffer that releases CO_2.
2. **Plant material differences,** such as comparing the deep green leaves of spinach to the pale leaves of cabbage or celery
3. **Light intensity differences**, such as by changing the distance between the light source and the cup

Recall that $I=1/D^2$, where I is the intensity of light and D is the distance between the light source and the target. You can use different areas of a variegated leaf from *Coleus*.

Choose a single variable you will study in your experiment. Write down in your notebook the justification for your experiment and your prediction. How will it improve your understanding of photosynthesis? Include

the controls you will need to run a meaningful experiment. Once you have determined a condition, discuss the feasibility of the experiment with your instructor and be prepared to modify your experiment. After choosing a condition, design your experiment carefully to limit the effect of a single variable.

Step 2: Student-led planning: Sketch your set-up in detail and ask your teacher for approval. Modify your set-up per the feedback from your teacher if need be. Now you are ready to assemble all the materials that you will need to perform the experiment. Follow the instructions described in Step 3 under the structured inquiry. Count the number of disks that float over time to estimate the ET_{50}. Enter all data in a table like Table 11_02, which you will adapt to your experimental conditions.

Step 3: Critical analysis: Plot the number of floating disks for each experimental condition as a function of time and determine the ET_{50} for each condition. Plot the estimated photosynthetic rates using $1/ET_{50}$ as a function of your independent variable, either bicarbonate concentration or light intensity. Compare the $1/ET_{50}$ values for darkly pigmented leaves and pale leaves. In each case, discuss the validity of the data and how you would improve on the experiment. How reliable is it to use bicarbonate as a source of CO_2? Can you compare light intensities without actually measuring light flux? Is it a fair comparison to use the response of disks from different types of plants? What else could have been at play?

Assessments

1. In an experiment, water is labeled with the radioactive O^{18} isotope. The labeled water is then supplied to the leaf disks. In a second experiment, CO_2 labeled with the isotope O is supplied to the disks. In which of the two experiments will you detect O^{18} in the product of photosynthesis? Why is this so?

1. DCMU (3-(3, 4-dichlorophenyl)-1, 1-dimethyl urea) is an herbicide that specifically inhibits transfer of electrons from PSII. What would you predict would happen to the production of oxygen in an *Elodea* twig submerged in a solution treated with DCMU? How would an animal such as a water snail be affected by the presence of DCMU in the same environment? You can assume that DCMU doesn't affect the snail directly.
2. What would happen to gas bubbles in *Elodea* if a test tube is exposed to light for a prolonged period without aeration? What would happen if water snails were added to the test tube? Illustrate your answer with a diagram showing gas the interaction between the *Elodea* and the water snail.

Extension Activities

1. Measure the ET_{50} of disks from plants maintained overnight in the light and in the dark. Do plants maintained in the dark perform photosynthesis faster, slower, or at the same rate? Write your prediction with an explanation and discuss your results.

2. Compare ET_{50} at different temperatures, such as 10°C, room temperature, and 50°C. Which steps of photosynthesis may be temperature dependent and how do they affect the evolution of O_2?

KEY TERMS

light-dependent reaction
: first stage of photosynthesis where certain wavelengths of the visible light are absorbed to form two energy-carrying molecules (ATP and NADPH)

pH indicator
: a chemical compound that changes color based on pH level

photosystem
: group of proteins, chlorophyll, and other pigments that are used in the light-dependent reactions of photosynthesis to absorb light energy and convert it into chemical energy

photosystem II
: integral protein and pigment complex in thylakoid membranes that transports electrons from water to the electron transport chain; oxygen is a product of PSII

LAB 12: MITOSIS AND MEIOSIS

STAGES OF MITOSIS IN THE BLASTULA OF A WHITEFISH

Learning Objectives

After completing the lab, the student will be able to:

1. Observe the stages of mitosis in whitefish blastula cells.
2. Identify and describe the stages of mitosis in whitefish blastula cells.

Activity 1: Pre-Assessment

1. List three reasons why organisms need to produce new cells.
2. What cellular structures must be replicated to ensure that new cells are functional after cell division?
3. Why are karyokinesis and cytokinesis distinct steps in cell division?
4. Discuss the answers to the questions with a partner (think, pair, share) and then the class.

Activity 1: Observe the Stages of Mitosis in the Blastula of a Whitefish

A fundamental property of somatic (nonreproductive or body) cells of multicellular organisms is mitosis, which basically provides new cells for growth and regeneration or replacement of dying and dead cells of the living body. A simple example in humans is our continuous shedding of skin cells and their replacement by new skin cells. Mitosis is also vital for development. Many single-celled organisms depend on mitosis as their sole or primary way of asexual reproduction. This asexual reproduction is distinct from multicellular organisms which undergo meiosis to produce reproductive cells (sperms or eggs). Cell division involves the chromosomes and genes of the dividing cells, which are duplicated and passed on to the new cells or daughter cells and are the reason why, for example, all these new skin cells are genetically identical. Mitosis is a controlled process,

and loss of control can lead to cancerous cells. Certain DNA sequences on the ends of the chromosomes called telomeres become shorter during every mitotic cycle of somatic cells, a regulatory mechanism that contributes to the number of mitotic cycles. If the telomeres fail to shorten, cells may become immortalized as they continue to divide indefinitely, typical of cancer cells.

As explained, all living organisms have the need to stay alive and reproduce. Mitosis, therefore, addresses the need for cell growth, maintenance, and repair. Mitosis is part of the cell cycle (see Figure 13.1). The cell cycle refers to the series of events that describe the metabolic processes of growth and replication of cells. The bulk of the cell cycle is spent in the "living phase," known as interphase. Interphase is further broken down into 3 distinct phases: G1 (Gap 1), S (Synthesis), and G2 (Gap 2). G1 is the phase of growth when the cell is accumulating resources to live and grow. After attaining a certain size and having amassed enough raw materials, a checkpoint is reached where the cell uses biochemical markers to decide if the next phase should be entered. S phase is when metabolism is shifted towards the replication (or synthesis) of the genetic material. During S phase, the amount of DNA in the nucleus is doubled and copied exactly in preparation to divide. The chromosomes at the end of G1 consist of a single chromatid. At the end of S phase, each chromosome consists of two identical sister chromatids joined at the centromere. When the DNA synthesis is complete, the cell continues on to the second growth phase called G2. Another checkpoint takes place at the end of G2 to ensure the fidelity of the replicated DNA and to re-establish the success of the cell's capacity to divide in the environment. If conditions are favorable, the cell continues on to mitosis. During interphase, the cell's DNA is replicated so that there are two copies of each chromosome, called the **sister chromatids.** In eukaryotes, all chromosomes must be duplicated prior to mitosis and cytokinesis to ensure each new daughter cell has the full complement of genetic information. Other structures, such as organelles, are replicated during the G1 portion of the cell cycle (See Figure 13.1). The checkpoints are prone to DNA damage, which can cause a disease like cancer (see Figure 13.2).

During **prophase**, the chromosomes coil up, and sister chromatids become visible under a microscope. The nuclear membrane surrounding the chromosomes also disappears. In **metaphase**, the sister chromatids align in the center of the cell, attached to **spindle fibers.** During **anaphase**, the sister chromatids separate and move to opposite poles of the cell. In **telophase**, the chromosomes arrive at the poles and begin to decondense while the nucleus reforms. Figure 13.3 makes it look like the phases are very distinct. The phases, however, transition without stopping. Notice also other important structures, such as the spindle fibers and **kinetochores** on the **centromeres** of each chromosome. Each pair of sister chromatids has a protein structure, called a kinetochore, which becomes attached to spindle fibers. The spindle fibers pull the sister chromatids apart, toward opposite ends of the cell.

Cancer cells are not healthy cells. Healthy cells die through programmed cell death or apoptosis after a number of generations of cell divisions. Cells become mature or differentiate, which enables them to carry out their function in the body. Cancer is the uncontrolled growth and mitotic division of cells which can be caused by mutations in genes. Some chemotherapy drugs, including taxanes such as Taxol from the Yew tree

and alkaloids from the Vinca plant, interfere with mitosis by binding to microtubules and preventing spindle fibers from separating sister chromatids, thus leading to cell death.

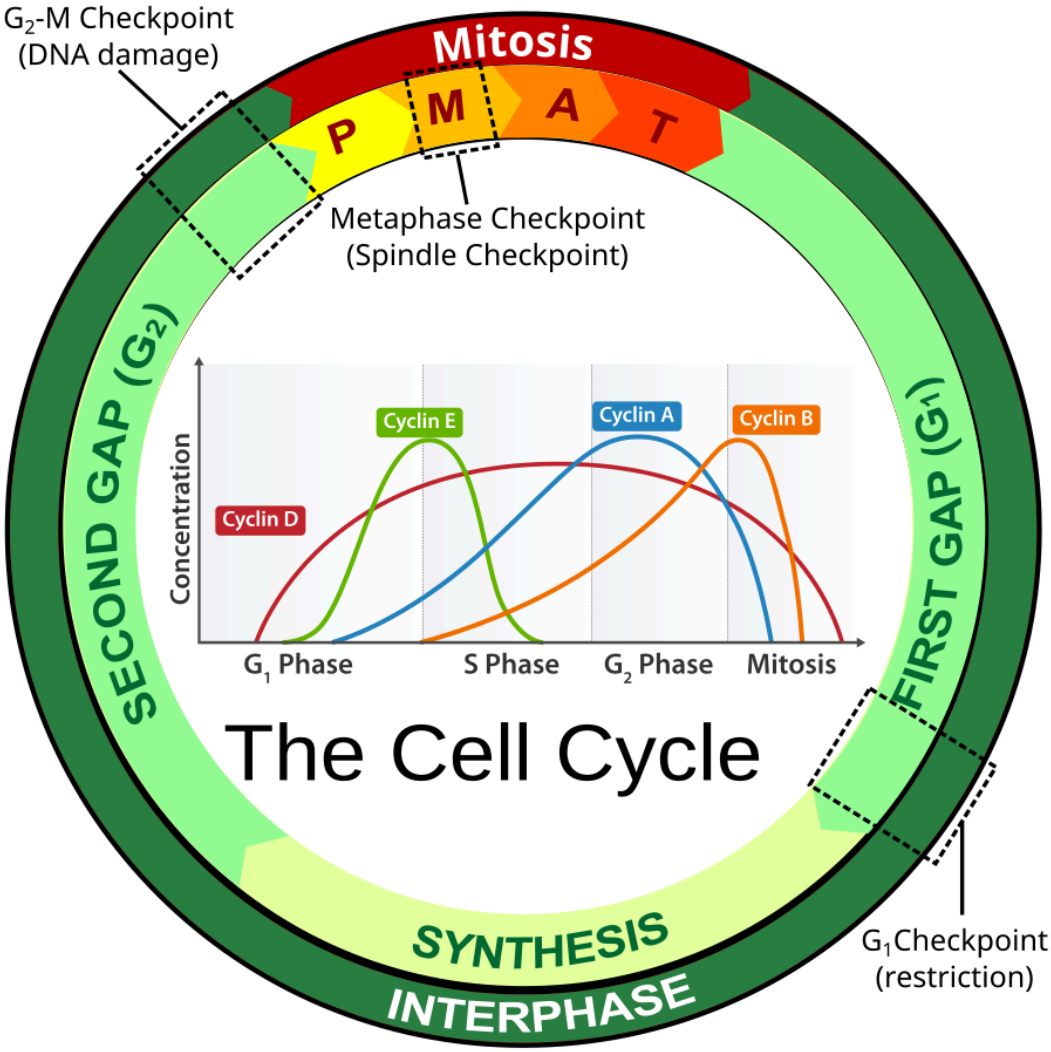
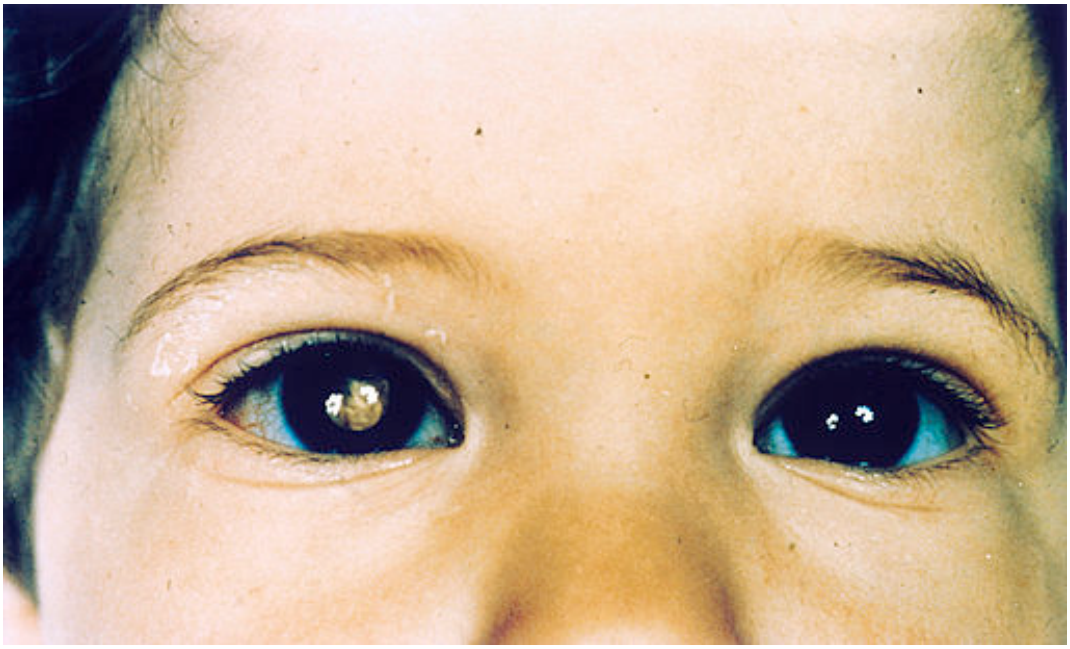


Figure 13.1: Eukaryotic cell cycle is governed by expression of cyclin proteins along with their activity. Credit: Jeremy Seto (CC-BY-SA)



https://commons.wikimedia.org/wiki/File:Retinoblastoma.jpg Figure 13.2: The checkpoints of the Cell Cycle are prone to DNA damage which can cause a disease like cancer. Exposure of tumor in the right eye by white light reflection.

Defective Cell Cycle Checkpoints: A white light shone on a child's eye should yield a clear view of the retina. In the above image, the right eye shows a white light reflecting and indicates a retinoblastoma (Figure 13.2). This cancer is caused by a defect in the Rb gene, a tumor suppressor gene. This defect permits the continuation of the cell cycle despite damage to DNA. Retinoblastoma is the most common primary childhood cancer which often stems from a genetic background.

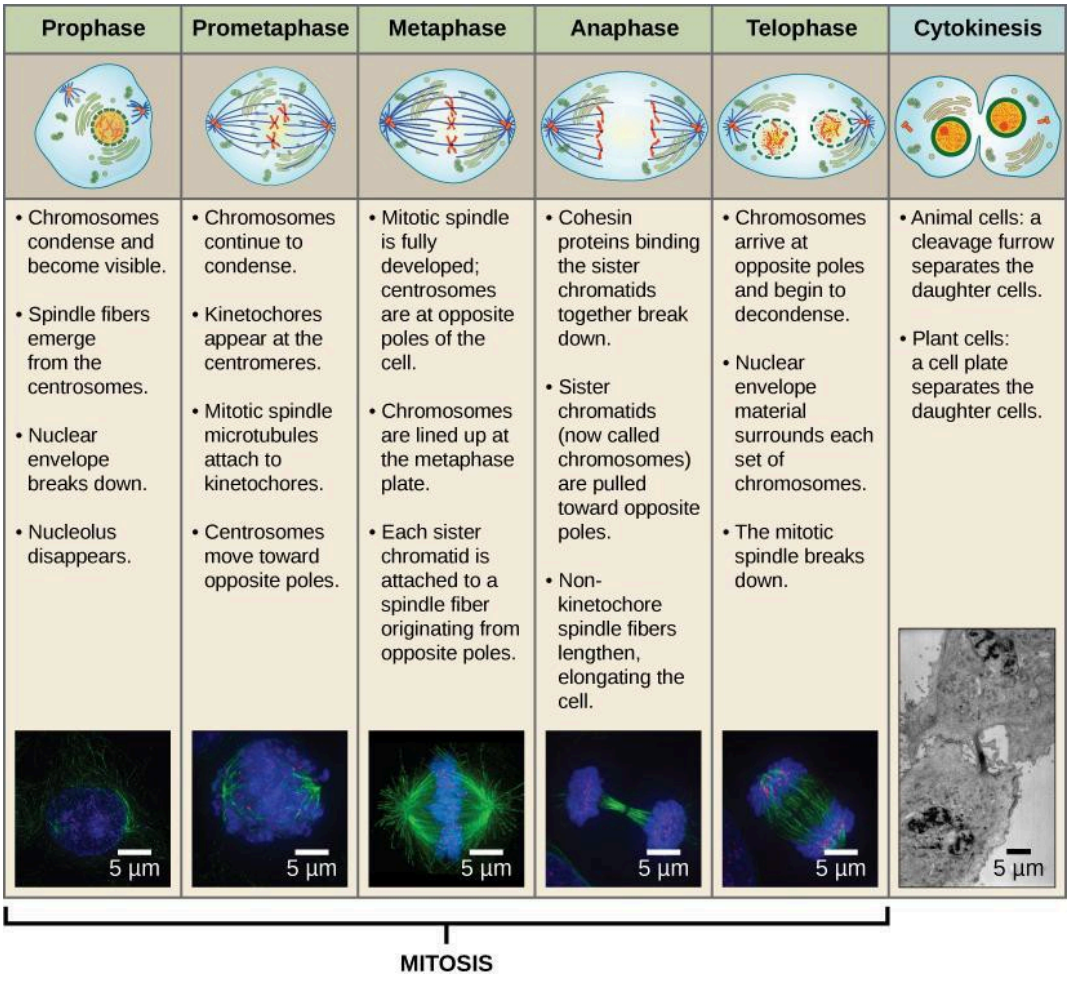


Figure 13.3: Mitosis is divided into five stages: prophase, prometaphase, metaphase, anaphase, and telophase.

Safety Precautions

- Be careful handling glass slides, as the edges may be sharp.
- Observe proper use of the microscope; avoid handling the electric cord with wet hands.
- Do not use the coarse adjustment knob of the microscope at higher magnifications.
- There is a separate marked disposal for sharp objects like broken glass. If you cannot locate it, inform your Instructor/Lab Technician immediately of any broken glassware, as it could cause serious injuries.

For this activity, you will need the following:

- Prepared slide of whitefish blastula cells, or use online images and resources

One or more interactive elements has been excluded from this version of the text. You can view them online here: https://louis.pressbooks.pub/generalbiology1lab/?p=385#oembed-1

For this activity, you will work *in pairs*.

Structured Inquiry

Step 1: Hypothesize/Predict: Using Figure 13.1, predict the percent of time that a cell would spend in each phase. Based on this prediction, how much time do you think cells will spend in interphase as opposed to mitosis? Write your prediction in your notebook.

Step 2: Student-Led Planning: Look at Figure 13.3. In your notebook, make a table defining the characteristics of the stages of mitosis, as well as interphase, that you can use to identify each stage under the microscope. Note: There are four major phases of mitosis, plus prometaphase, which is a transition phase between prophase and metaphase.

Step 3: Using the prepared slide, record the number of cells in each phase of the cell cycle. Use the method shown in Figure 13.4 to count the cells. Record your count in a data table like that shown in Table 13.1. Share your data with the class to create a group total count.

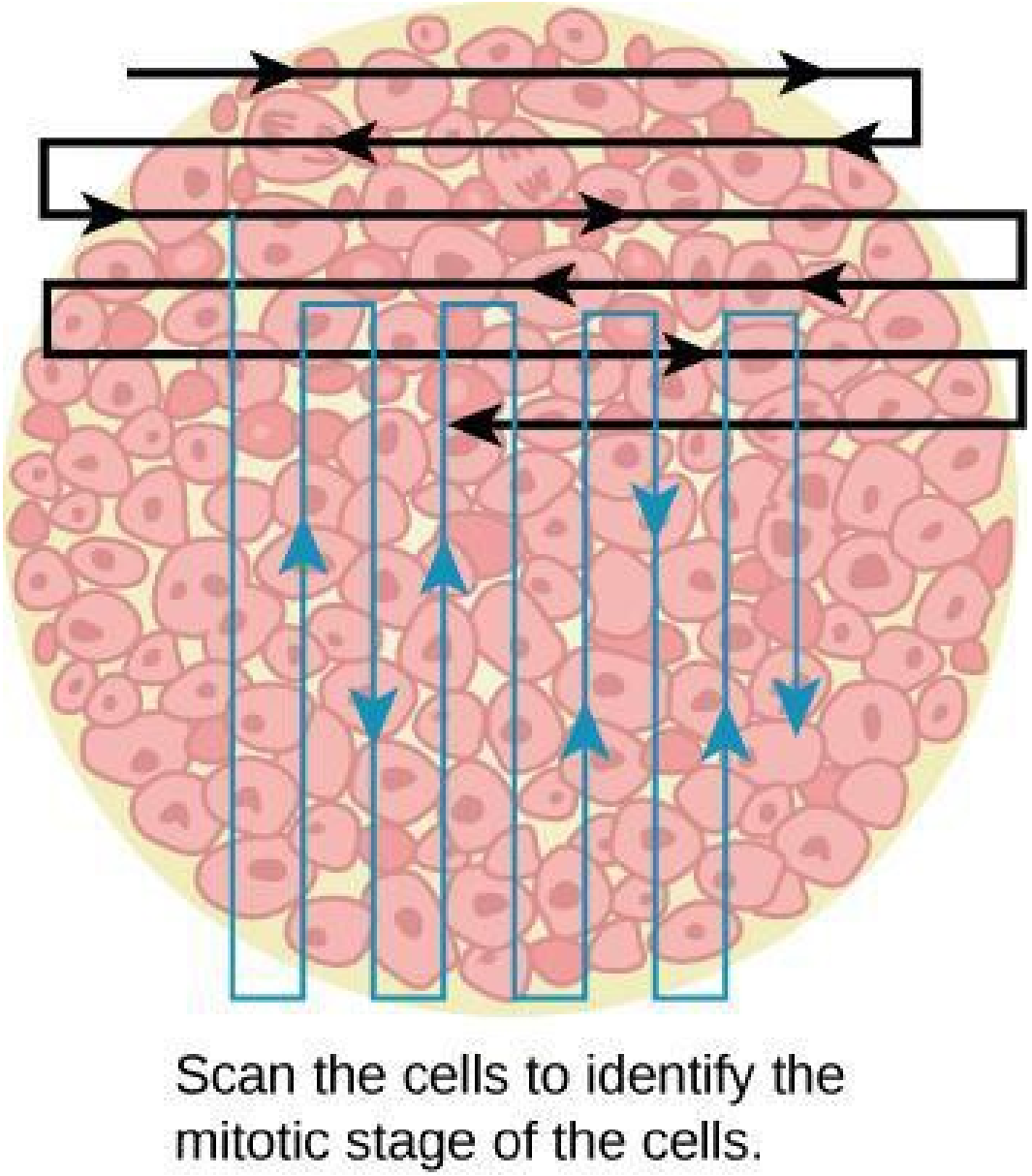


Figure 13.4: Scan and count cells by using either a horizontal (black line) or vertical (blue line) back-and-forth scanning technique with your eyes.

Table 13.1: Results of Cell Stage Identification

Phase or Stage	Individual Totals	Class or Group Totals	Percent
Interphase			
Prophase			
Metaphase			
Anaphase			
Telophase			
Cytokinesis			
Totals			100%

Step 4: In your data table, calculate the percentage of cells in each phase.

Step 5: Critical Analysis: Are the predictions you made supported by your data (observations and calculations)? Do your results match the diagram as presented in Figure 13.1? Why or why not?

Assessments

1. Explain why interphase could be the longest phase and mitosis and cytokinesis are generally much shorter phases of the cell cycle.
2. Explain the importance of spindle fibers in mitosis and why antimitotic drugs that block spindle fiber formation are used to treat cancer.

STAGES OF MITOSIS IN ONION ROOT CELLS

Learning Objectives

After completing the lab, the student will be able to:

- Observe and describe the stages of mitosis in onion root tips.

Activity 2: Pre-Assessment

1. In a plant, where would you likely find cells that are actively undergoing mitosis? Where would you likely find cells that aren't actively undergoing mitosis? Explain your answers.
2. Think about how a plant grows. In which parts of the plant would many cells likely be undergoing mitosis?
3. Discuss the answers to the questions with a partner (think, pair, share) and then the class.

Activity 2: Observe the Stages of Mitosis in Onion Root Cells

Plant cells also use mitosis for growth, maintenance, and repair. The plant's cell wall, as well as the nuclear material, makes observing mitosis much easier. Figure 13.5 shows some of the similarities and differences between plant and animal mitosis. For example, during cytokinesis, an animal cell pinches apart into two daughter cells, while a plant cell develops a new cell wall, called a **cell plate**, between the new daughter cells.

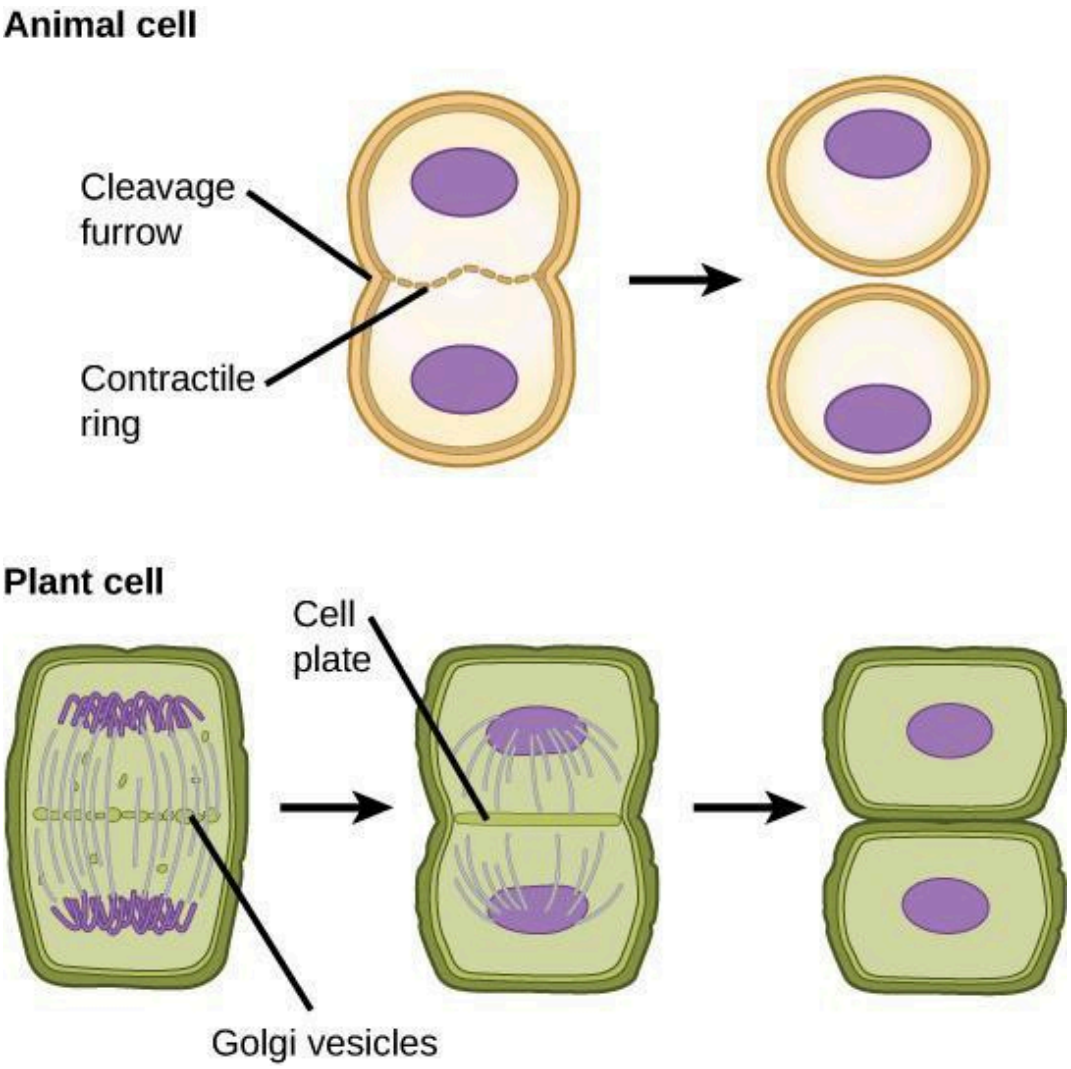


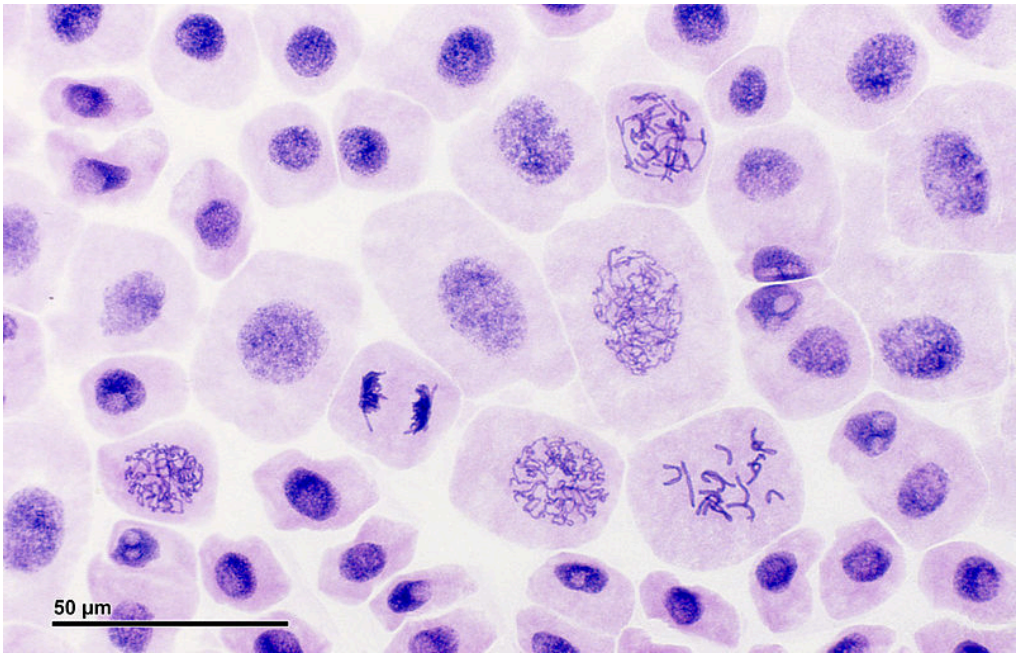
Figure 13.5: Cytokinesis differences between plant and animal cells

Safety Precautions

- Be careful handling glass slides, as the edges may be sharp.
- Observe proper use of the microscope; avoid handling the electric cord with wet hands.
- Do not use the coarse adjustment knob of the microscope at higher magnifications.
- Inform your teacher immediately of any broken glassware, as it could cause injuries.

For this activity, you will need the following:

- Prepared microscope slides of stained onion root tips or online images and resources



https://commons.wikimedia.org/wiki/File:Mitosis_(261_15)_Pressed;_root_meristem_of_onion.jpg Stained actively dividing cells of onion root tip

This video provides a demo of materials including onion or garlic bulbs, germination of root, preparation of root tip, staining, and observing under the microscope:

One or more interactive elements has been excluded from this version of the text. You can view them online here: https://louis.pressbooks.pub/generalbiology1lab/?p=390#oembed-1

For this activity, you will work *in pairs.*

Structured Inquiry

Step 1: Hypothesize/Predict: In your notebook, hypothesize what regions of the onion root tip might differ in the number of cells undergoing mitosis. What is your reasoning behind this hypothesis? Record your answers in your notebook.

Step 2: Student-Led Planning: In your notebook, make a drawing of each phase of mitosis, as well as interphase, in a plant cell. Label, in each drawing, the defining features that you will look for when identifying each stage under the microscope.

Step 3: Critical Analysis: Are the predictions you made in Step 1 supported by your observations? Why do you think cells undergoing mitosis in the onion root cell are distributed the way they are? What differences between plant and animal cells are visible under the microscope slide?

Assessments

1. Describe some similarities and some differences between animal and plant mitosis.
2. Based on your observations, explain how onion roots grow at the cellular level.

MEIOSIS AND FERTILIZATION

Learning Objectives

After completing the lab, the student will be able to:

1. Read a karyotype.
2. Determine karyotype abnormalities and identify an associated disorder or syndrome.

Activity 3: Pre-Assessment

1. Looking at Figures 13.3 and 13.6, compare the outcomes of mitosis versus meiosis.
2. After meiosis, some daughter cells may not contain the correct number of chromosomes. What failure in the meiosis process could cause this to occur?
3. Discuss the answers to questions with a partner (think, pair, share) and then the class.

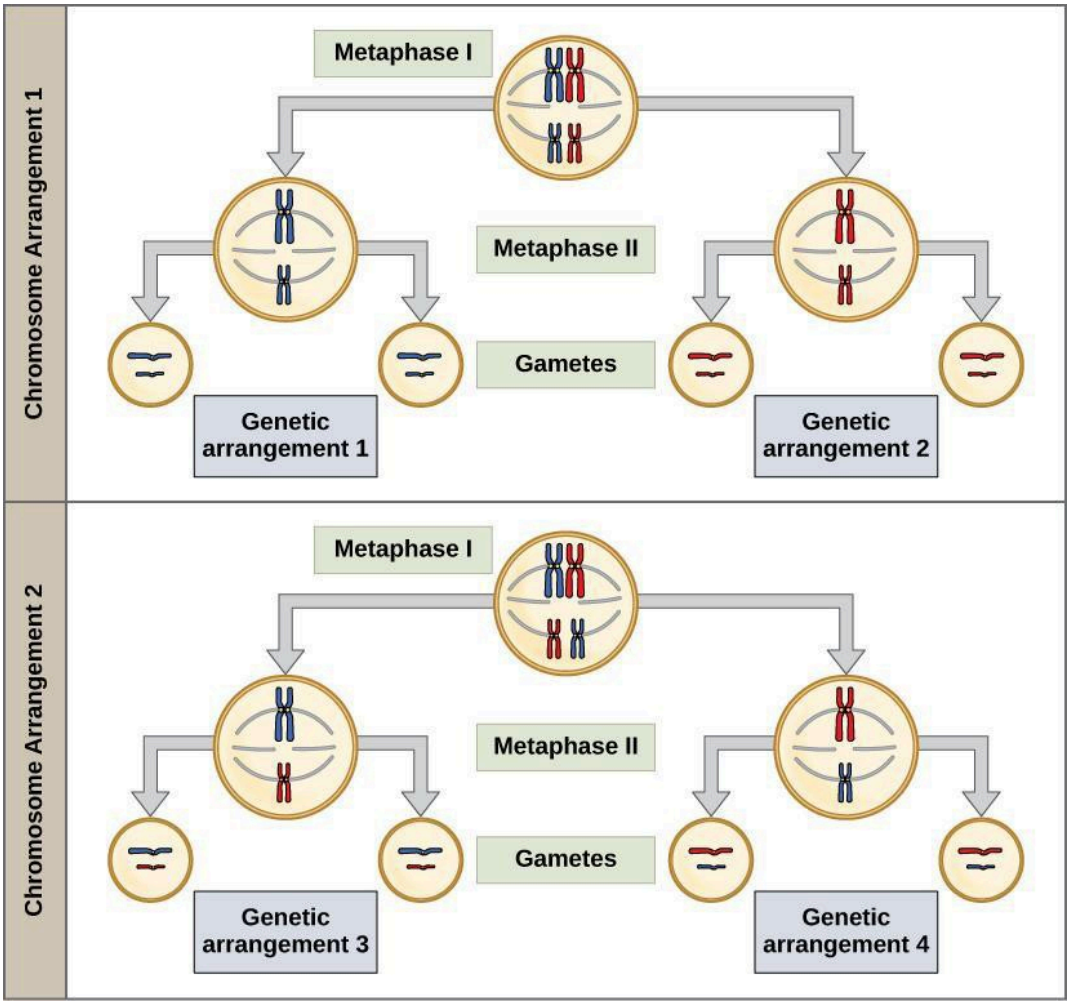
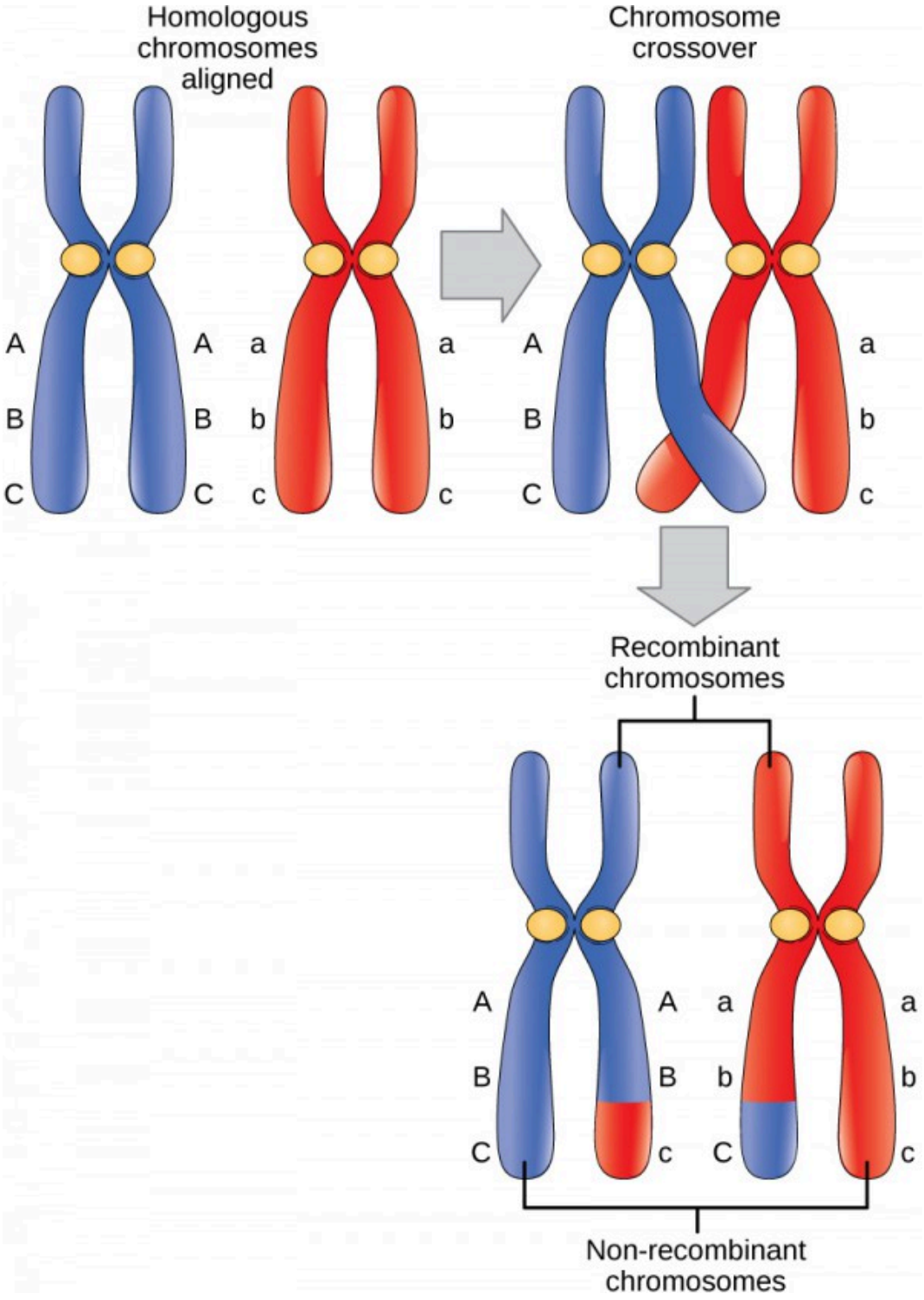


Figure 13.6: Overview of meiosis typically in sexually reproducing organisms that reduces the number of chromosomes to produce gametes or sex cells (egg in female and sperm in male)

Activity 3: Meiosis and Fertilization

Meiosis is the process in sexually reproducing eukaryotes that forms sex cells or gametes, which include sperm and eggs (ova). To avoid doubling the number of chromosomes in each generation, **reduction division** (halving the number of chromosomes) in gamete production is necessary. Chromosomes are typically diploid (2N) or occur as double sets (homologous pairs) in each nucleus. Each homolog of a pair has the same sites or loci for the same genes. You might recognize that you have one set of chromosomes from your mother and the remaining set from your father. Meiosis reduces the number of chromosomes to a haploid (1N) or single set. Meiosis uses very similar mechanisms to mitosis. There are, however, several significant differences. The source cells for meiosis are found in the reproductive organs of animals (gonads: ovaries; oogenesis, where the eggs are produced and testes; spermatogenesis, where the sperms are produced) and plants (flowers: ovaries and anthers). Major differences between mitosis and meiosis include the association of **homologous chromosomes** (sister chromatids of the same chromosomes) attached as a **tetrad** group of four, as well as crossover of chromosome regions during prophase I of meiosis I. Meiosis shuffles the genetic material so that

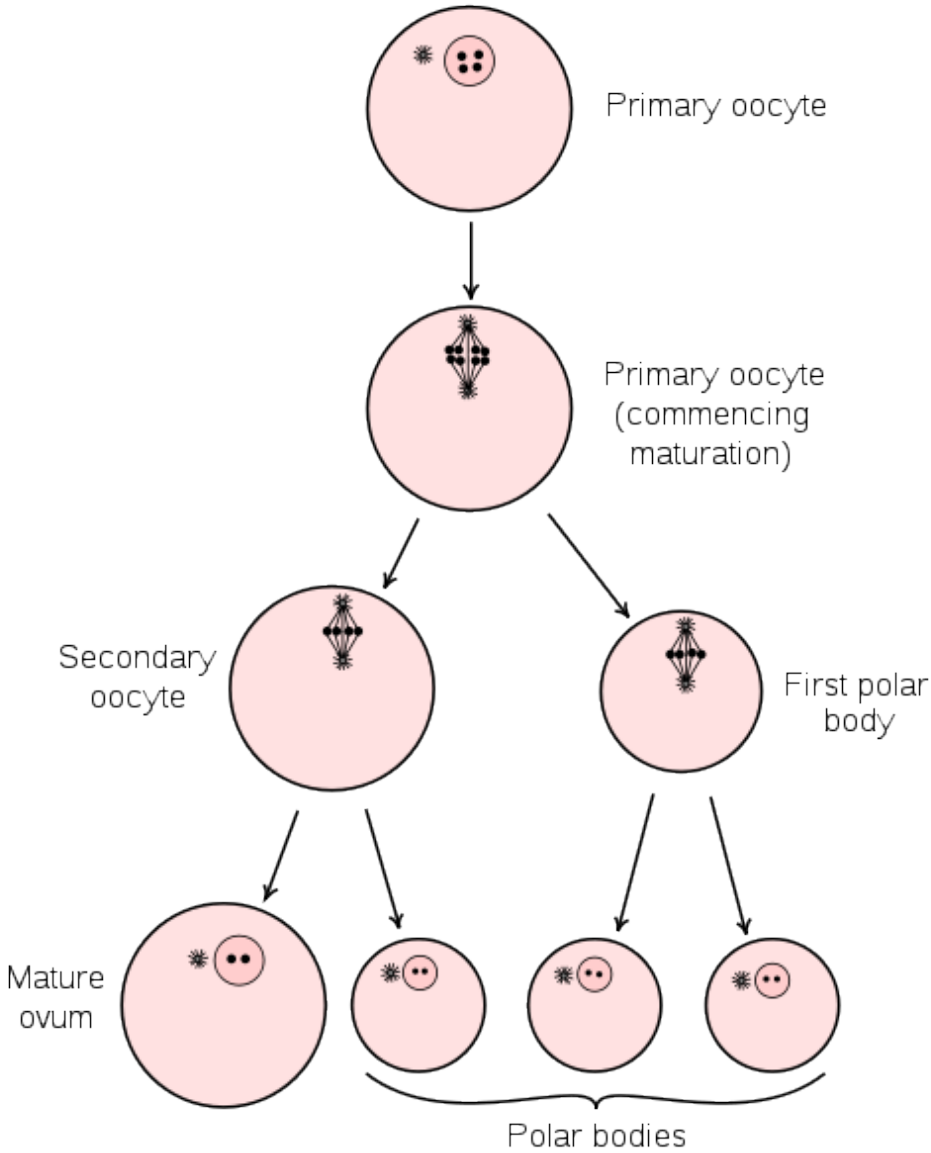
each resulting cell carries a new and unique set of genes in a process of independent assortment. Crossover provides one source of genetic variation in offspring.



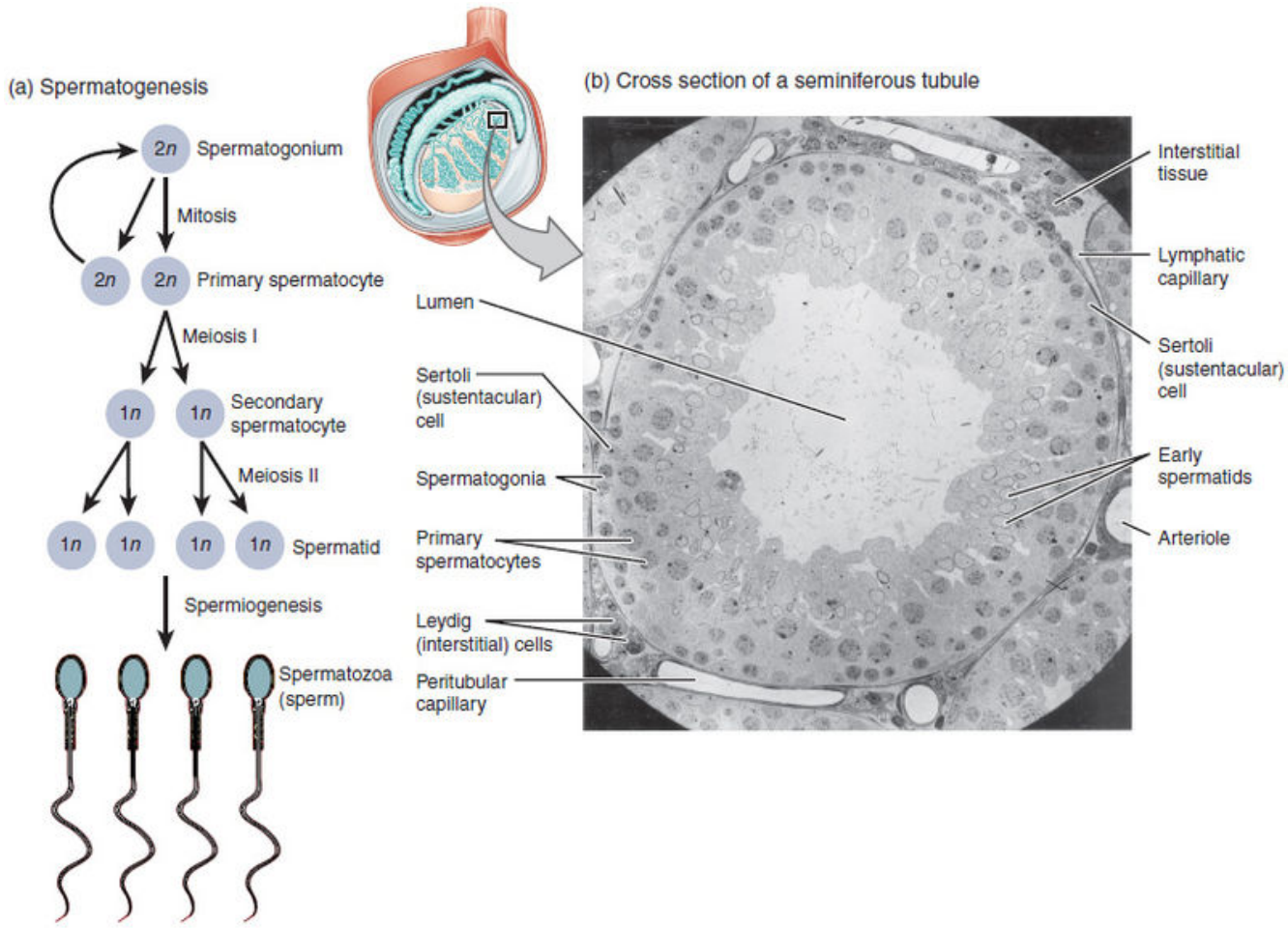
In this illustration (above) of the effects of crossing over, the blue chromosome came from the individual's father and the red chromosome came from the individual's mother. Crossover occurs between non-sister chromatids of homologous chromosomes. The result is an exchange of genetic material between homologous chromosomes. The chromosomes that have a mixture of maternal and paternal sequence are called recombinant and the chromosomes that are completely paternal or maternal are called non-recombinant. Image from Chapter 17: Meiosis in Introductory Biology: Evolutionary and Ecological Perspectives by Various Authors, licensed under a Creative Commons Attribution 4.0 International License, except where otherwise noted.

This video demonstrates the sperm producing cells of a male crane fly, a species with only 8 chromosomes per cell during meiotic division.

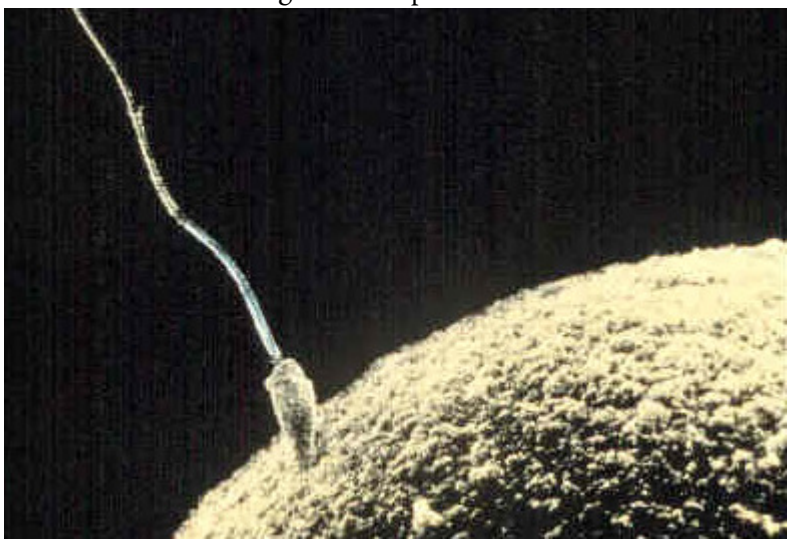
One or more interactive elements has been excluded from this version of the text. You can view them online here: https://louis.pressbooks.pub/generalbiology1lab/?p=394#oembed-1



Oogenesis: In the human female genitalia reproductive structures, a growth process in which the primary egg cell (or ovum) matures into an ovum (1N)



Spermatogenesis: In this process, haploid spermatozoa (1N) develop from germ cells in the seminiferous tubules of the testis male genitalia reproductive structures



Fertilization: The union or fusion of the 2 gametes, ovum (1N) haploid and spermatozoa (1N) haploid in which the fertilized egg or zygote (2N) diploid has the double chromosome set restored. The size of the egg is emphasized by the sperm in contact

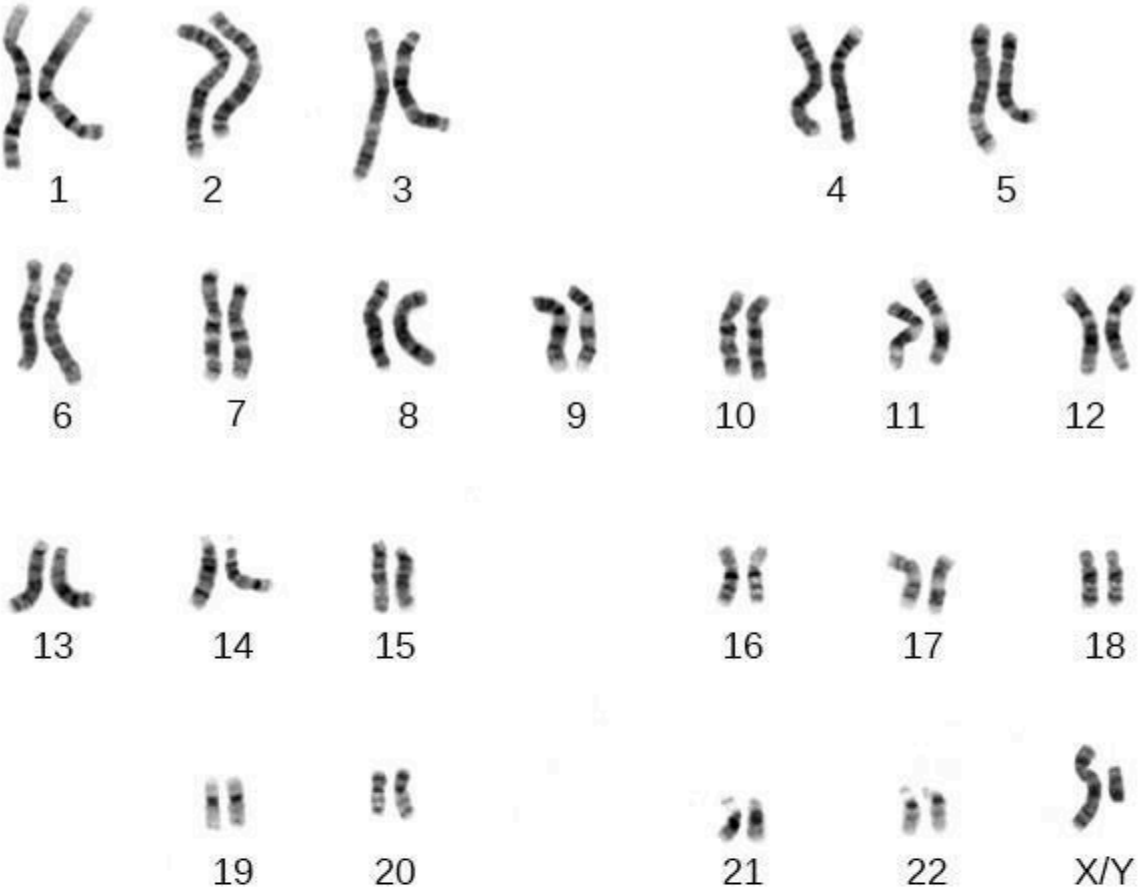
Humans have 23 different chromosomes. As stated, we have a **diploid** (2N) set of chromosomes: a full set

of 23 chromosomes received from the sperm and another set of 23 from the ovum (egg) during fertilization. See Figure 13.7. During metaphase I of meiosis I, homologous chromosomes are separated. The chromosome number is reduced from diploid (2N) to haploid (1N) by the end of meiosis I, with each cell retaining duplicate sister chromatids. Meiosis II results in four haploid (1N) cells in sperm, each with only one set of each of the 23 chromosomes. In female humans, meiosis results in uneven division of the cytoplasm and the formation of two **polar bodies** (nuclei only) that are not involved in **fertilization**. One note about human fertilization: meiosis II is completed only after the sperm has entered the ovum.

There are several malfunctions that can occur during meiosis. **Nondisjunction** occurs when the sister chromatids in tetrads separate unevenly. Nondisjunction results in one cell getting an extra chromosome while another cell is missing a chromosome. Nondisjunction is associated with certain human genetic disorders. For example, Down syndrome is usually caused by nondisjunction that results in three copies of chromosome 21. **Translocations** can occur when chromosomes exchange genetic information with nonhomologous chromosomes. For a more detailed list, see this web page: https://www.genome.gov/11508982/chromosome-abnormalities-fact-sheet/

Karyotypes (chromosome spreads) are made by stopping cells in mitosis with a chemical and then dyeing with Giemsa stain. A picture is taken through a microscope and then digitally enlarged to see the chromosomal banding, or G-bands. Dark and light banding patterns help identify chromosomes and alterations to normal chromosomes. The chromosome spreads can be seen in Figure 13.7. Human females have two X chromosomes, and males have one X and one Y.

Human Male



Human Female

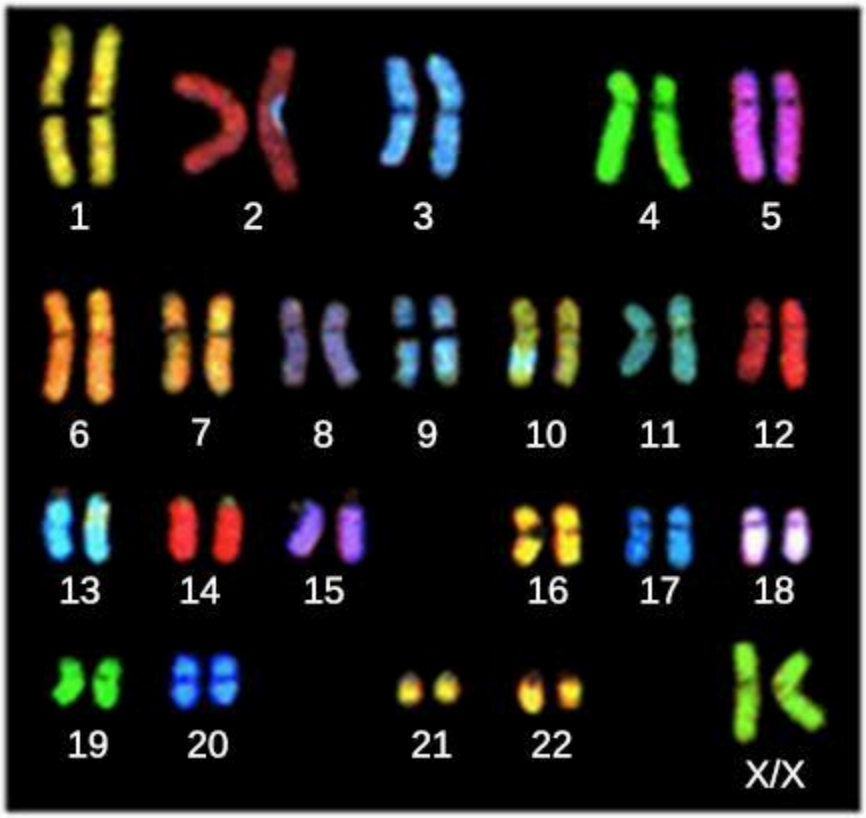


Figure 13.7 Sorted human male and female chromosomes

Safety Precautions

None

For this activity, you will need the following:

- Images of karyotypes

For this activity, you will work *in pairs*.

Structured Inquiry

Step 1: Hypothesize/Predict: A karyotype shows the number and appearance of chromosomes in the nucleus of a cell. Predict what you would look for in an abnormal karyotype. Record your predictions in your notebook.

Step 2: Examine the three karyotypes 1, 2, and 3 shown in Figure 13.8, Figure 13.9, and Figure 13.10 respectively (below). Compare these karyotypes to the normal karyotypes shown in Figure 13.7 (above). Can you tell if the individual is female, male, or indeterminate (does not have a normal distribution of sex chromosomes)? Record any abnormalities in your notebook, and research the meaning of the changes in chromosome number or appearance.

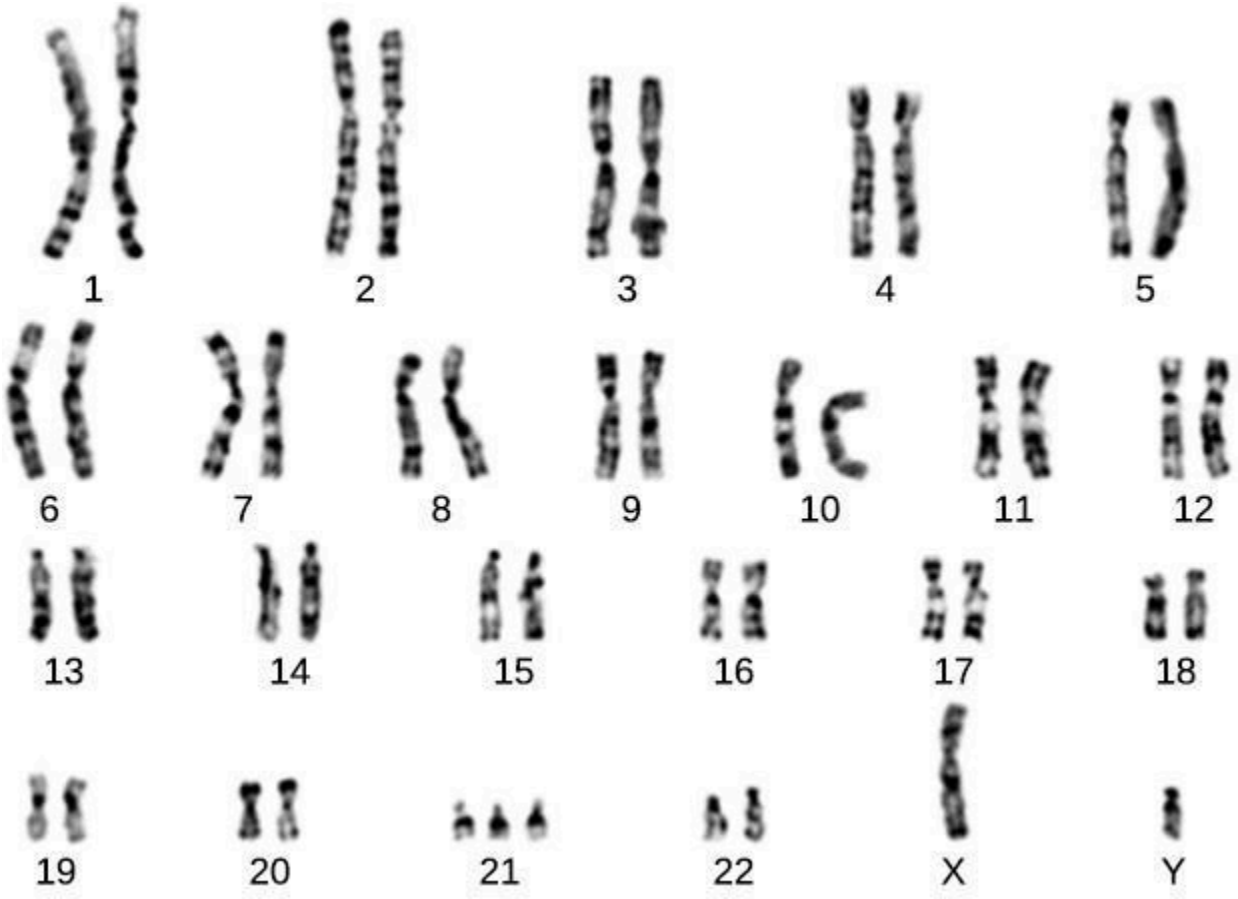


Figure 13.8: Karyotype 1

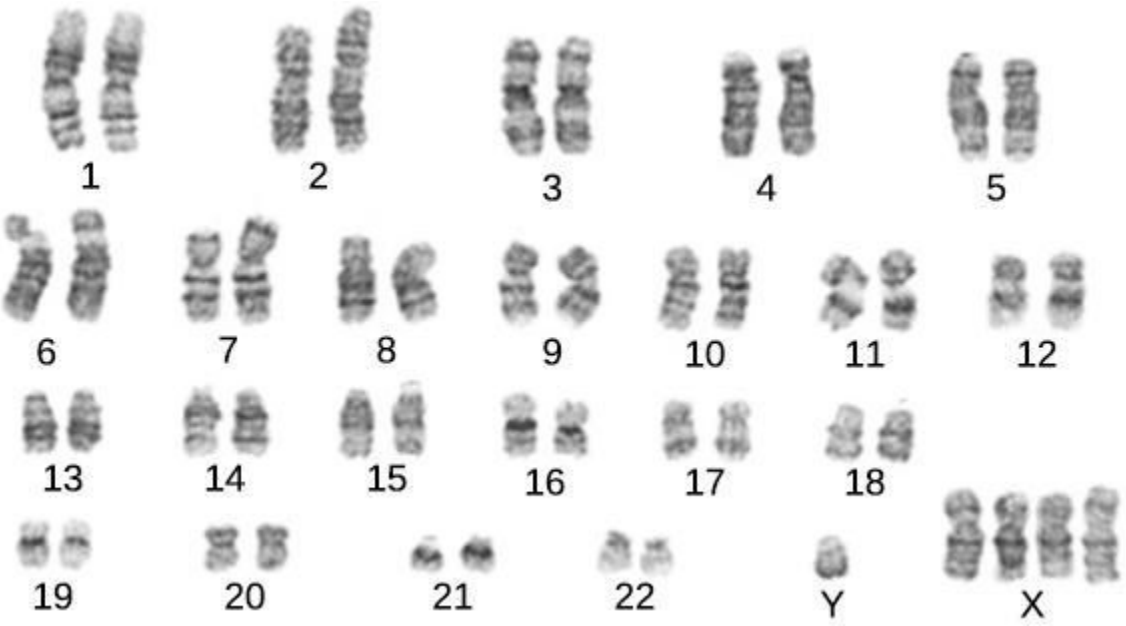


Figure 13.9: Karyotype 2

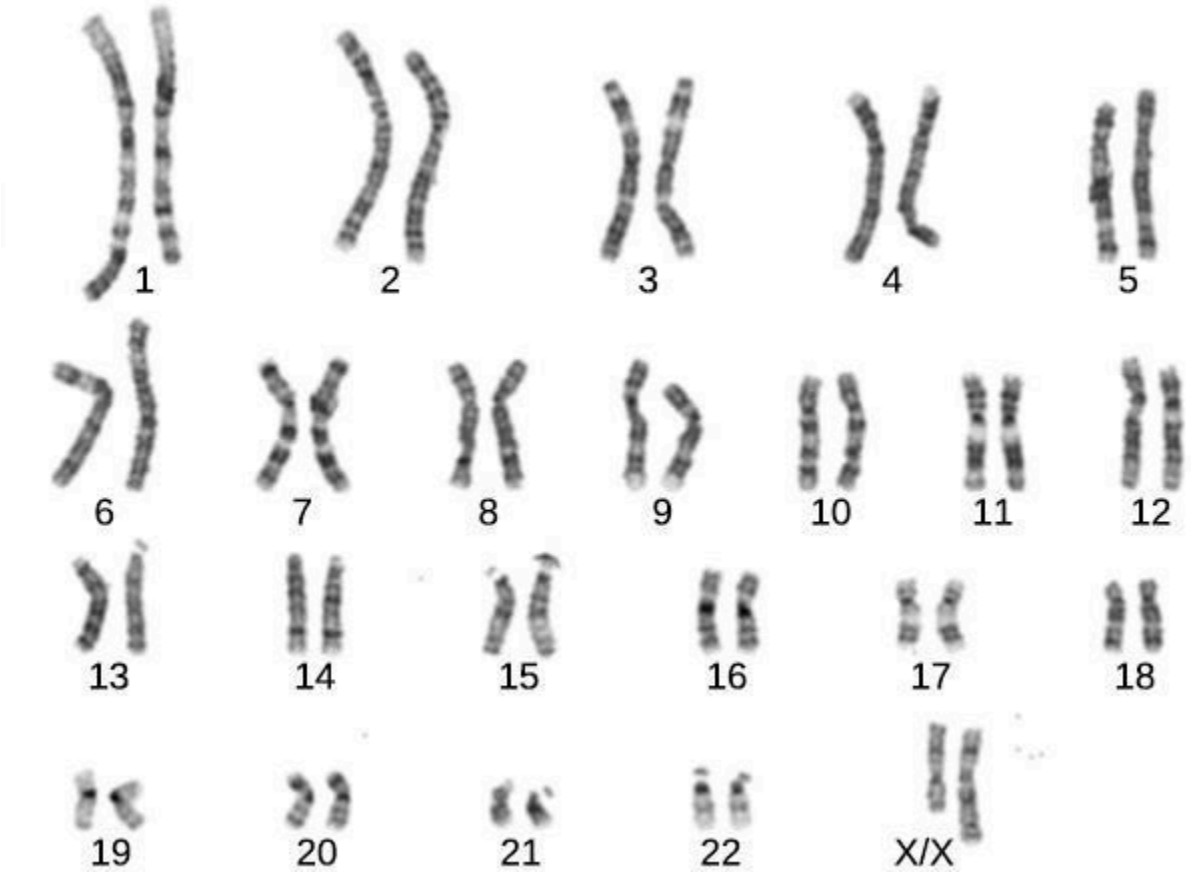
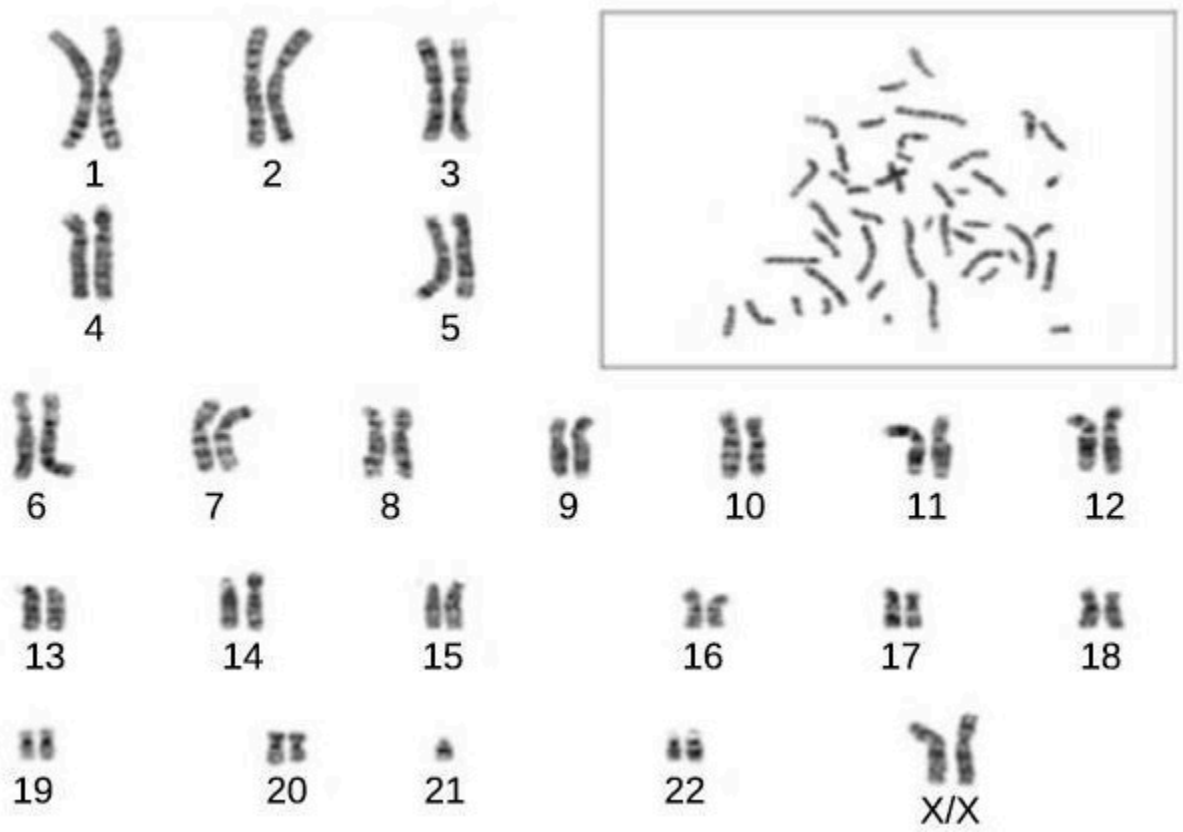


Figure 13.10: Karyotype 3

Step 3: Critical Analysis: What are the implications of nondisjunction in a karyotype? Using Karyotype 1 (Figure 13.8) as an example, explain the implications of the nondisjunction malfunction during meiosis for the chromosome count of the daughter cells.

Assessments

1. This Robertsonian translocation involves a fusion of the long arms of two non-homologous chromosomes during meiosis. A patient possesses a Robertsonian translocation among chromosome 14 onto chromosome 13. No genetic material is missing from either chromosome. Explain why it is possible that there is no abnormality in the person but could be in the patient's offspring.



Chromosomes

2. Crossing over of chromosomes during prophase I is common. How does this process increase the genetic diversity of the daughter cells?

KEY TERMS

anaphase
: stage of mitosis during which sister chromatids are separated from each other

cell plate
: structure formed during plant cell cytokinesis by Golgi vesicles, forming a temporary structure (phragmoplast) and fusing at the metaphase plate; ultimately leads to the formation of cell walls that separate the two daughter cells

centromere
: region at which sister chromatids are bound together; a constricted area in condensed chromosomes

diploid
: cell with two sets of chromosomes

fertilization
: union of two haploid cells from two individual organisms

homologous chromosomes
: sister chromatids of the same chromosomes

karyotypes
: a cell's chromosomes

kinetochores
: protein structure associated with the centromere of each sister chromatid that attracts and binds spindle microtubules during prometaphase

metaphase
: stage of mitosis where the chromosomes are at their most compact

nondisjunction
: failure of synapsed homologs to completely separate and migrate to separate poles during the meiosis's first cell division

prophase
: stage of mitosis during which chromosomes condense and the mitotic spindle begins to form

reduction division
: halving the number of chromosomes

sister chromatids
: identical copies of the same chromosome

spindle fiber
: filaments formed during mitosis and meiosis

telophase
: stage of mitosis during which chromosomes arrive at opposite poles, decondense, and are surrounded by a new nuclear envelope

tetrad
: two duplicated homologous chromosomes (four chromatids) bound together by chiasmata during prophase I

translocation
: process by which one chromosome segment dissociates and reattaches to a different, nonhomologous chromosome

LAB 13: MENDELIAN GENETICS

SINGLE TRAIT INHERITANCE

Learning Objectives

After completing the lab, the student will be able to:

1. Explain the basic principles of inheritance, the definition of genotype and phenotype, and the assumptions of Mendelian inheritance.
2. Handle, identify, and cross Mendelian traits in the fruit fly Drosophila melanogaster and predict offspring genotypes using a Punnett square.

Activity 1: Pre-Assessment

1. How would you know if a trait follows a Mendelian inheritance pattern, assuming you know and can track the genotypes and phenotypes of an organism as it produces offspring?
2. What is a Punnett square, and what does it show? Using a Punnett square, predict the offspring of a cross between two heterozygous parents for gene A (Aa × Aa).
3. Discuss the answers to questions 1–2 with the class.

Activity 1: Single Trait Inheritance

Based on the principles set forth by Mendel, we can predict what genotypes and phenotypes offspring will have based on the genotypes and phenotypes of their parents. One efficient way to do this involves using a Punnett square. A Punnett square is a grid where all the alleles of one parent are provided as the column headers while all of the alleles from the second parent are provided as the row headers (Figure 14.1). When the alleles from the two parents are combined in the grid, the internal squares predict the genotypes of their offspring. In addition, multiple Punnett squares can predict offspring genotypes across several generations. The first filial generation

(F1) is the offspring that results from crossing the original, parental generation. The second filial generation (F2) is the offspring that results from crossing F1 individuals.

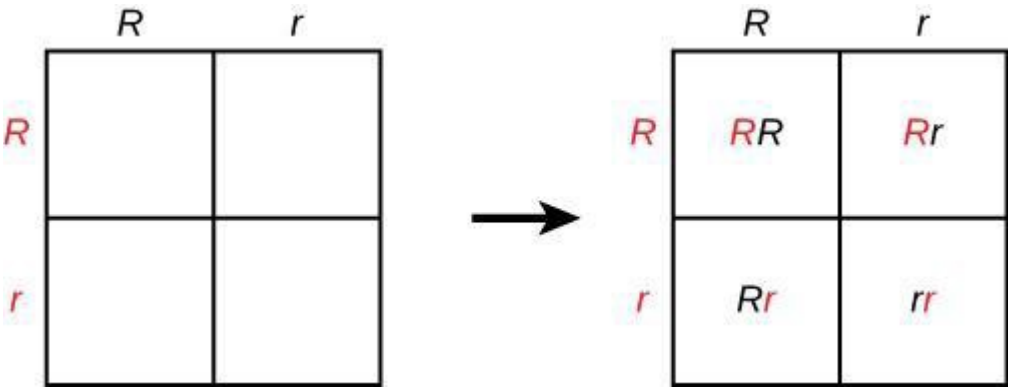


Figure 14.1: A Punnett square is used as a visual representation of crossed traits and the results of the crosses. Capital R represents the dominant trait, and lowercase r represents the recessive trait. The first square is a cross between the two dominant traits R and R. RR is the result.

Safety Precautions

- Do not let the flies fly off.
- Inform your teacher immediately of any broken glassware, as it could cause injuries.
- Clean up any spilled fluids to prevent other people from slipping.
- Wash your hands with soap and water after completion of the activity.

For this activity, you will need the following:

- Fruit flies
- Glass vials
- Fly food
- Paint brushes for manipulating flies
- Foam plugs
- Stereomicroscope
- Fly anesthetizing substance

For this activity, you will work *in pairs.*

Structured Inquiry

Step 1: Hypothesize/Predict: In this activity, you will perform crosses using the fruit fly *Drosophila melanogaster*. Your teacher will indicate the phenotypes that you will monitor in your crosses and will show you photographs of the traits (e.g., eye color vermillion/sepia, body color gray/ebony, normal wings/curly wings). You will begin by crossing a wild-type parent with a recessive parent. Knowing the dominant and recessive traits of the parent, predict the genotype and phenotype of the first-generation offspring using Punnett squares (see Figure 14.1). Draw the two Punnett squares in your notebook. Add your predictions to the first data table. You will fill the second one with your actual data later.

Step 2: Student-Led Planning: Listen carefully to your teacher's instructions on how to handle, anesthetize, determine the sex of, and count your flies. Practice, with your partner, how best to use the stereomicroscope. After you understand the procedure, draw a table in your notebook to record your data (sex and phenotype of parent, first generation, and second generation.)

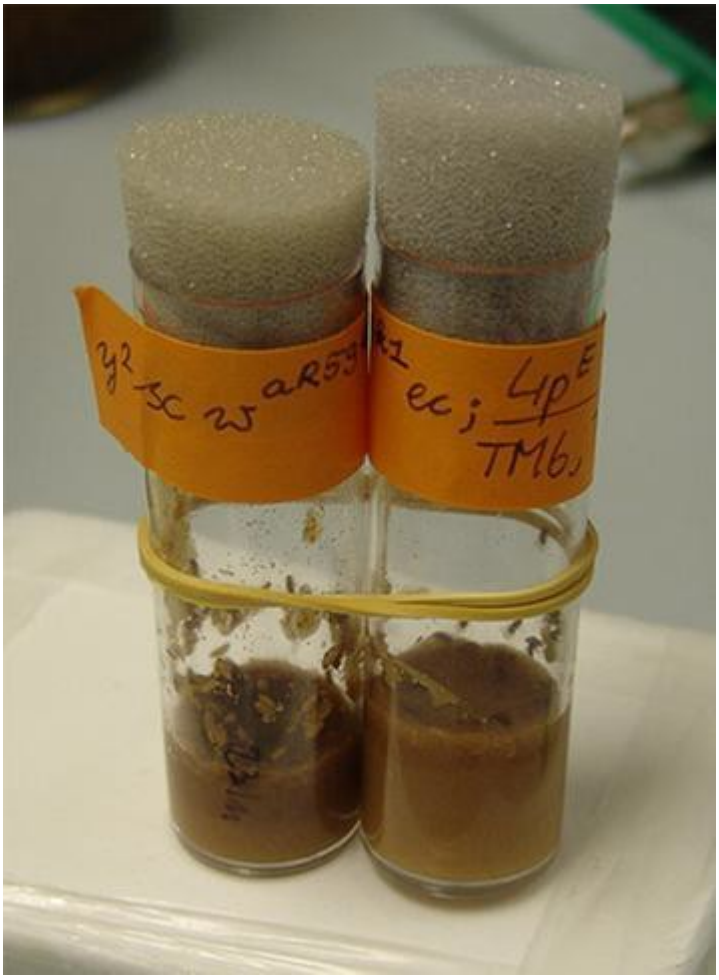


Figure 14.2: A typical vial of Drosophila melanogaster. The food medium is at the bottom of the vial, with fly larvae (maggots) on its surface. The fruit fly pupae can be seen adhered to the inner wall of the vial.

Step 3: Cross the wild-type parent and recessive parents as described by your teacher in Step 1. Then incubate

your vials. After the flies have laid eggs, anesthetize and re-collect the parent generation. Remove all the adult flies.

Step 4: After seven days, anesthetize the adult flies from the vials prepared in Step 3. Separate the male and female flies and count the phenotypes present, for each sex. Record your counts in your notebook.

Step 5: Repeat Steps 3 and 4 for the F2 generation. Record your counts in your notebook.

Step 6: Critical Analysis: Are the predictions you made in Step 1 supported by your data? Why or why not? What methods could you use to improve your results? Discuss with your partner and then write your answers in your notebook.

Guided Inquiry

Step 1: Hypothesize/Predict: Predict the original cross and the phenotypic frequencies of an already-crossed group of flies. The phenotypic frequency can be found by dividing the number of flies of a particular trait by the total number of flies. Using flies you already have available, examine the flies and record the sex and traits. Prepare data tables (to record sex and dominant and recessive traits) and Punnett squares.

Step 2: Student-Led Planning: Plan how you will cross your flies. After your teacher has approved, make your crosses for at least two generations. Record your data in your lab notebook.

Step 3: Critical Analysis: Are the predictions you made in Step 2 supported by your data? Why or why not? What methods could you use to improve your results? Discuss with your partner and then write your answers in your notebook.

Assessments

1. You are analyzing the inheritance of a new gene and find that it does not have a clear dominant and recessive allele. Instead, both alleles seem to be expressed when both are present. Does this gene follow a Mendelian inheritance pattern? Why or why not?
2. Answer the following about the assumptions of Mendelian inheritance:
    1. Do most traits in humans follow a Mendelian inheritance pattern? Why or why not?
    2. How could you determine that a trait does or does not follow the assumptions of Mendelian inheritance?

TEST FOR INDEPENDENCE (CHI-SQUARE TEST)

Learning Objectives

After completing the lab, the student will be able to:

1. Statistically test the probability that the difference between an observed and an expected result is due to chance.

Activity 2: Pre-Assessment

1. What does the chi-square test tell you?
2. Explain the difference between expected and observed data.
3. What is a null hypothesis?
4. Discuss the answers to questions 1, 2, and 3 with the class.

Activity 2: Test for Independence (Chi-Square Test)

The **chi-square** test is an independence test for the likelihood that an observed distribution is due to chance. Chi-square is calculated using the following equation

$$X_c^2 = \sum \frac{(O_i - E_i)^2}{E_i}$$

where X is the chi-square test statistic, c is the significant level of the test (we will use 0.05), O is the observed value for variable *i*, and E is the expected value for variable *i*.

To interpret the data, use a standard chi-square table, as provided by your teacher. On the left side of the table is the **degree of freedom** (*df*), which is calculated by subtracting 1 from the number of categories in the data. Across the top is the **probability** (*p*-value) or the probability that the observed value matches the expected value. It is used to determine whether the **null hypothesis** should be accepted or rejected. The null hypothesis states that there is no significant difference between the groups being measured.

Safety Precautions

- None

For this activity, you will need the following:

- Standard chi-square test table
- Calculator

For this activity, you will work *in pairs*.

Structured Inquiry

Step 1: Use chi-square analysis to determine if the data from the Activity 1 Structured Inquiry is independently assorted. Watch the video to learn how to perform a chi-square test:

One or more interactive elements has been excluded from this version of the text. You can view them online here: https://louis.pressbooks.pub/generalbiology1lab/?p=236#oembed-1

Create a data table for your analysis, and show your calculations in your notebook.

Step 2: Hypothesize/Predict: Predict whether you will accept or reject your null hypothesis (i.e., if they are independently assorted). What is your alternative hypothesis? Add your predictions to the data table you created in Step 1.

Step 3: Student-Led Planning: Using the data you collected in the previous activity, perform a chi-square test. Work with your partner.

Step 4: Critical Analysis: Based on your chi-square test, will you accept or reject your hypothesis? Why or why not? What methods could you use to improve your results? Discuss with your partner, and then write your answers in your notebook.

Guided Inquiry

Step 1: Now, use chi-square analysis to determine if the data from the Activity 1 Guided Inquiry is independently assorted. Create a data table for your analysis, and show your calculations in your notebook.

Step 2: Hypothesize/Predict: Predict whether you will accept or reject your null hypothesis (i.e., are your observed results significantly different from your expected results?). What is your alternative hypothesis? Add your hypotheses to the data table you created in Step 1.

Step 3: Student-Led Planning: Using the data you collected in the previous activity, perform a chi-square test. Work with your partner. Your expected data would be the number of flies that should possess the different phenotypes based on the ratios predicted by Mendelian inheritance for each cross.

Step 4: Critical Analysis: Based on your chi-square test, will you accept or reject your hypothesis? Why or why not? What does this tell you about the validity of your data? Discuss with your partner, and then write your answers in your notebook.

Assessments

1. What does the chi-square test tell you about a set of observed versus expected results?
2. Describe another situation, outside the realm of science, where a chi-square test would be useful.
3. What would change the degree of freedom in a chi-square test?

GENETICS OF AN EAR OF CORN

Learning Objectives

After completing the lab, the student will be able to:

1. Explain basic principles of inheritance, the definition of genotype and phenotype, and the assumptions of Mendelian inheritance.
2. Use a Punnett square.
3. Statistically test the probability that the difference between an observed and an expected result is due to chance.

Activity 1 Alternative: Pre-Assessment

1. How would you know if a trait follows a Mendelian inheritance pattern, assuming you know and can track the genotypes and phenotypes of an organism as it produces offspring?
2. What is a Punnett square, and what does it show? Using a Punnett square, predict the offspring of a cross between two heterozygous parents for gene A (Aa × Aa).
3. Discuss the answers to questions 1–2 with the class.

Activity 1 Alternative: Genetics of an Ear of Corn

Photograph of corn entitled "Yellow and purple!_Gabi" by nist6ss is marked with CC BY-SA 2.0.

Activity 1: Single Trait Inheritance

Based on the principles set forth by Mendel, we can predict what genotypes and phenotypes offspring will have based on the genotypes and phenotypes of their parents. One efficient way to do this involves using a Punnett square. A Punnett square is a grid where all the alleles of one parent are provided as the column headers, while all of the alleles from the second parent are provided as the row headers (Figure 14.1). When the alleles from the two parents are combined in the grid, the internal squares predict the genotypes of their offspring. In addition, multiple Punnett squares can predict offspring genotypes across several generations. The first filial generation (F1) is the offspring that results from crossing the original, parental generation. The second filial generation (F2) is the offspring that results from crossing F1 individuals.

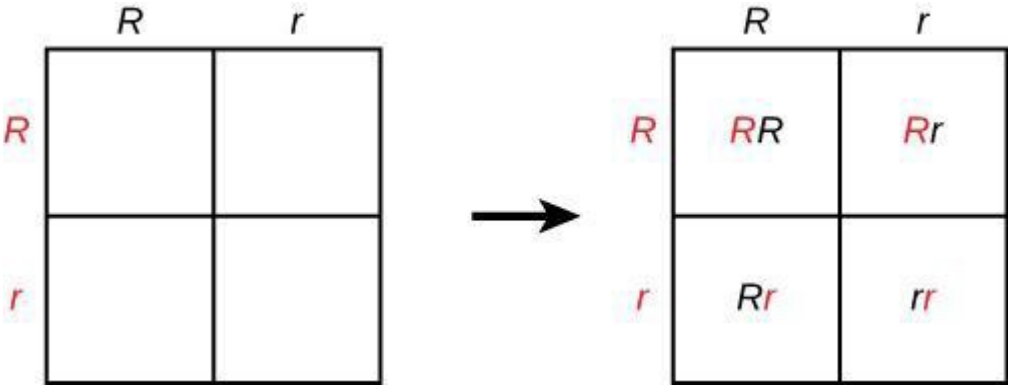
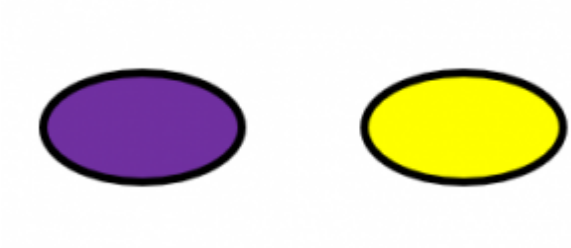


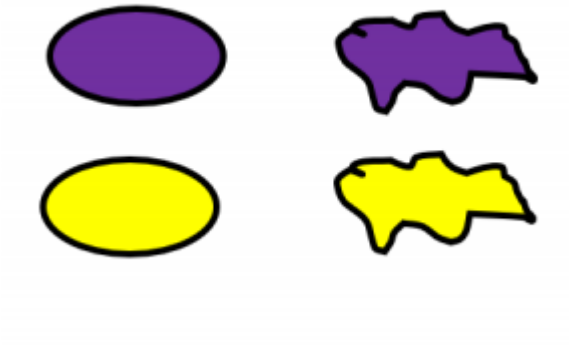
Figure 14.1: A Punnett square is used as a visual representation of crossed traits and the results of the crosses. Capital R represents the dominant trait, and lowercase r represents the recessive trait. The first square is a cross between the two dominant traits R and R. RR is the result.

Genes of corn kernel

For color of corn kernel (purple or yellow)



For shape of corn kernel (smooth or shrunken)



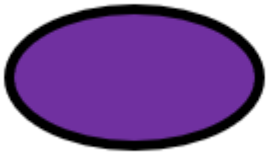
Alleles of corn kernel

Alleles are versions of a gene. Dominant alleles control the appearance or phenotype of the organism. Recessive

alleles disappear or recede and do not control the phenotype, unless the organisms has only recessive alleles for that gene

- Purple (P) is dominant allele
- Yellow (p) is recessive allele
- Smooth (S) is dominant allele
- Shrunken (s) is recessive allele

Phenotype of corn



Purple and smooth:

Purple and shrunken:

Yellow and smooth:

Yellow and shrunken:

Safety Precautions

- **Add**

For this activity, you will need the following:

- Corn cobs with different genotypes and phenotypes
  - Can be acquired from commercial sources. Carolina Biological Supply and Ward's Science are examples of companies selling corn ears for genetic studies to high schools and universities.

For this activity, you will work *in pairs.*

Structured Inquiry

Genotypes of corn

The corn has two parental versions of the same gene, one inherited from the female plant, the other from the male plant. For example, a corn could have Pp as a genotype for the color of the corn kernel. P from the female plant, p from the male plant. In regard to corn kernel shape, it could be SS: S from the female plant, S from the male plant.

Interactive question 1: What could be the genotype of a corn when it has a purple phenotype?

1. PP only
2. Pp only
3. Pp or PP
4. pp or PP
5. pp or Pp

Interactive question 2: What could be the genotype of a corn when it has a shrunken phenotype?

1. SS only
2. Ss only
3. Ss or SS
4. ss or SS
5. ss only

Homozygous means that an organism has the same allele (or version) for a gene. Heterozygous means, that an organism has different versions for an allele of the same gene. Taking the example of the corn kernel, what would a heterozygous genotype look like for the color of the corn kernel?

Interactive question 3: What would be a possible genotype for the color of a corn kernel if it would be heterozygous for color?

1. PP
2. pp
3. Pp or pP
4. All of the choices are correct

Interactive question 4: What would be a possible genotype for the shape of a corn kernel if it would be homozygous for shape?

1. Ss only
2. SS or ss
3. SS only
4. ss only
5. None of the choices are correct

A corn kernel heterozygous for color and shape of the corn kernel would be PpSs.

Interactive question 5: If both parents are heterozygous and crossed for offspring, what could be the alleles of the female and male reproductive cells (gametes)?

1. PS
2. Ps
3. pS
4. ps
5. all choices are possible genotypes that female and male reproductive cells (gametes) donate.

A Punnett square allows visualization of the genotypes of the parents and possible genotypes of the offspring.

Interactive question 6: What is the Punnett square for the cross of PpSs with PpSs?

Fill in the possible genotypes for the gametes

Gametes				

Interactive question 7: What is a correct Punnett square for the crossing mentioned above?

A)

Gametes	p	s	S	p
P				
S				
p				
s				

B)

Gametes	pp	Pp	SS	ss
Pp				
PP				
Ss				
ss				

C)

Gametes	PS	Ps	pS	ps
PS				
Ps				
pS				
ps				

D)

Gametes	P	p	Sp	SP
Ps				
pp				
Sp				
ss				

Once you have the genotypes of the gametes filled into the Punnett square, you can visualize the possible genotypes of the offspring.

Interactive question 8. Below is listed a Punnett square to be used. What would be the possible genotypes of the offspring? Spend 5 minutes to fill in the empty cells.

Gametes	PS	Ps	pS	ps
PS				
Ps				
pS				
ps				

Interactive question 9. Identify a correct Punnett square for the cross of PpSs with PpSs.

A)

Gametes	PS	Ps	pS	ps
PS	PPSS	PsSS	PPSS	ppSS
Ps	ppSS	PsPs	ppSS	PsPs
pS	ppSS	PsPs	ppSs	PPss
ps	ssPP	ppPP	PpSS	ppss

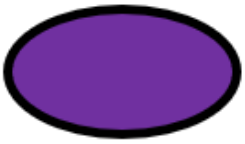
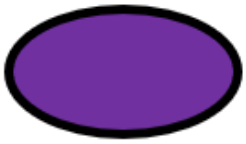
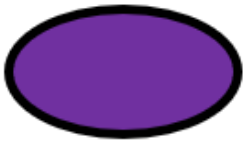
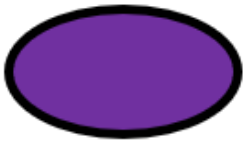
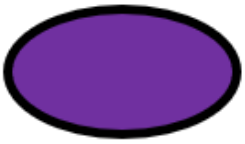
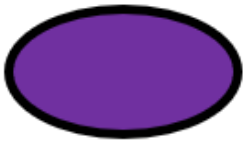
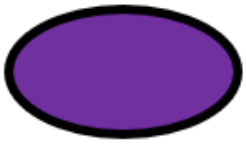
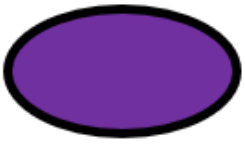
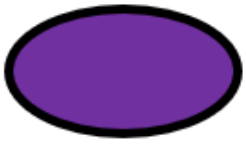
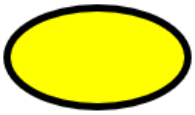
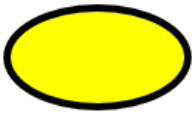
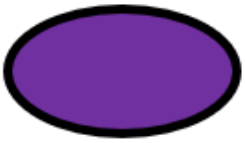
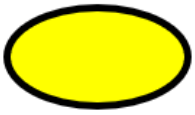
B)

Gametes	PS	Ps	pS	ps
PS	PPss	PPss	ppSS	ppss
Ps	PPSs	PPSs	PsSs	Psss
pS	PsSS	PsSs	ppSS	ppss
ps	PsSs	Ppss	ppss	ppss

C)

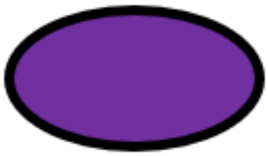
Gametes	PS	Ps	pS	ps
PS	PPSS	PPSs	PpSS	PpSs
Ps	PPSs	PPss	PpSs	Ppss
pS	PpSS	PpSs	ppSS	ppSs
ps	PpSs	Ppss	ppSs	ppss

Using the genotypes, you can calculate the phenotypes.

Gametes	PS	Ps	pS	ps
PS	PPSS 	PPSs 	PpSS 	PpSs 
Ps	PPSs 	PPss 	PpSs 	Ppss 
pS	PpSS 	PpSs 	ppSS 	ppSs 
ps	PpSs 	Ppss 	ppSs 	ppss 

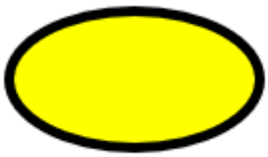
The Punnett square shows a total of 16 genotypes for the possible offspring. The ***different*** genotypes are: PPSS (1), PPSs (2), PpSS (2), PpSs (4), PPss (1), Ppss (2), ppSS (1), ppSs (2) and ppss (1). Overall, there are ***9 different*** genotypes for this cross. Mathematically the ratios are 1:2:2:4:1:2:1:2:1 for the genotypes.

One can also count the phenotypes. E.g. how many purple & smooth out of all possible 16 offspring? 9 out of 16 are purple & smooth. The information can be listed as follows:



9/16 Purple and Smooth

3/16 Purple and Shrunken



3/16 Yellow and Smooth

1/16 Yellow and Shrunken

However, to simplify matters the information for the frequency of phenotypes is normally listed in the following way: 9:3:3:1.

Mathematical calculations (algebra) can predict the phenotype ratios:

One starts for one gene or trait. E.g. Color of corn kernel: 3 P (purple) and 1 p (yellow)

Then the other gene or trait. E.g. shape of corn kernel: 3 S (smooth) and 1 s (shrunken)

Then the two traits are combined: (3P + 1p) x (3S + 1s) = 9 PS + 3PS + 3pS + 1ps

Chi Square Problem: An ear of corn has a total of 381 grains, including 215 Purple & Smooth, 80 Purple & Shrunken, 65 Yellow & Smooth, and 21 Yellow & Shrunken. These phenotypes and numbers are entered in Columns 1 and 2 of the following Table 2.

Your Tentative Hypothesis: This ear of corn was produced by a dihybrid cross (PpSs x PpSs) involving two pairs of heterozygous genes resulting in a theoretical (expected) ratio of 9:3:3:1. See dihybrid cross in Table 1.

Objective: Test your hypothesis using **chi square** and **probability** values. In order to test your hypothesis, you must fill in the columns in the following Table 2.

1. For the observed number (Column 2), enter the number of each grain phenotype counted on the ear of

corn.

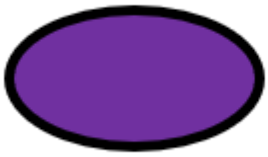
2. To calculate the observed ratio (Column 3), divide the number of each grain phenotype by 21 (the grain phenotype with the lowest number of grains).
3. For the expected ratio (Column 4), use 9:3:3:1, the theoretical ratio for a dihybrid cross. The fractional ratios for these four phenotypes are 9/16, 3/16, 3/16, and 1/16.
4. To calculate the expected number (Column 5), multiply the number of each grain phenotype by the expected fractional ratio for that grain phenotype.
5. In the last column (Column 6), for each grain phenotype take the observed number of grains (Column 2) and subtract the expected number (Column 5), square this difference, and then divide by the expected number (Column 5). Round off to three decimal places.
6. To calculate the chi square value, add up the four decimal values in the last column (Column 6).

Grain Phenotype (Column 1)	**Observed Number (Column 2)**	**Observed Ratio (Column 3)**	**Expected Ratio (Column 4)**	**Expected Number (Column 5)**	**[Obs No. – Exp No.]^2 ÷ Expected No. (Column 6)**
Purple and Smooth	215	10.23	9	**381 x 9/16 = 214**	**1 ÷ 214 = 0.0048**
Purple and Shrunken	80	3.81	3	**381 x 3/16 = 71**	**81/71 = 1.1408**
Yellow & and Smooth	65	3.09	3	**381 x 3/16 = 71**	**36/71 = 0.5070**
Yellow and Shrunken	21	1.0	1	**381 x 1/16 = 24**	**9/24 = 0.375**
Total Number:	381	—		**Chi Square Value:**	**1.9976**

Determine the degree of freedom

For dihybrid crosses the degree of freedom (df) is (number of phenotypes –1)

Only **4 different** phenotypes were observed (purple & smooth, purple & shrunken, yellow & smooth, yellow& shrunken)



9/16 Purple and Smooth:

3/16 Purple and Shrunken:

3/16 Yellow and Smooth:

1/16 Yellow and Shrunken:
degree of freedom (df) is (number of phenotypes −1)

- df = 4-1 = 3

The degree of freedom is 3

Use your own corn cob and calculate your numbers:

Chi Square Problem: An ear of corn has a total of ___ grains, including ___ Purple & Smooth, __ Purple & Shrunken, __ Yellow & Smooth, and __ Yellow & Shrunken. These phenotypes and numbers are entered in Columns 1 and 2 of the following Table 2.

Grain Phenotype (Column 1)	Observed Number (Column 2)	Observed Ratio (Column 3)	Expected Ratio (Column 4)	Expected Number (Column 5)	[Obs No. – Exp No.]^2 ÷ Expected No. (Column 6)
Purple and Smooth					
Purple and Shrunken					
Yellow & and Smooth					
Yellow and Shrunken					
Total Number:					

Some ideas and information were obtained from

https://www2.palomar.edu/users/warmstrong/lmexer4.htm

KEY TERMS

chi-square test
: an independence test for the likelihood that an observed distribution is due to chance

degree of freedom
: the number of independent categories in an experiment

null hypothesis
: states that there is no significant difference between the groups being measured

probability
: the likelihood that a hypothesis, statement, explanation, or value is correct

LAB 14: PLANT TRANSPIRATION

MEASUREMENT OF TRANSPIRATION AS WATER LOSS UNDER SELECTED CONDITIONS

Learning Objectives

After completing the lab, the student will be able to:

1. Measure the rate of transpiration.
2. Test and predict the way in which an environmental change affects the rate of transpiration.

Activity 1: Pre-Assessment

1. What environmental conditions do you think will most likely cause stomata to open? Which will most likely cause stomata to close? Explain your answer.
2. Why do you think the suction caused by transpiration is considered a negative-feedback mechanism?
3. Discuss the answers to questions 1 and 2 with the class.

Activity 1: Measurement of Transpiration as Water Loss under Selected Conditions

You will conduct this first activity in three parts. First, you will assemble an apparatus called a potometer, illustrated in Figure 19.1. A **potometer** consists of a **plant cutting** stuck into one end of a U-shaped tube and a graduated cylinder stuck into the other end. This device creates water potential, which will allow you to measure **transpiration**. In the second part, you will collect data for transpiration under a set of control conditions. In the third part, you will choose from a list of variables to change the conditions for the plant, and then you will collect transpiration data again using the modified apparatus.

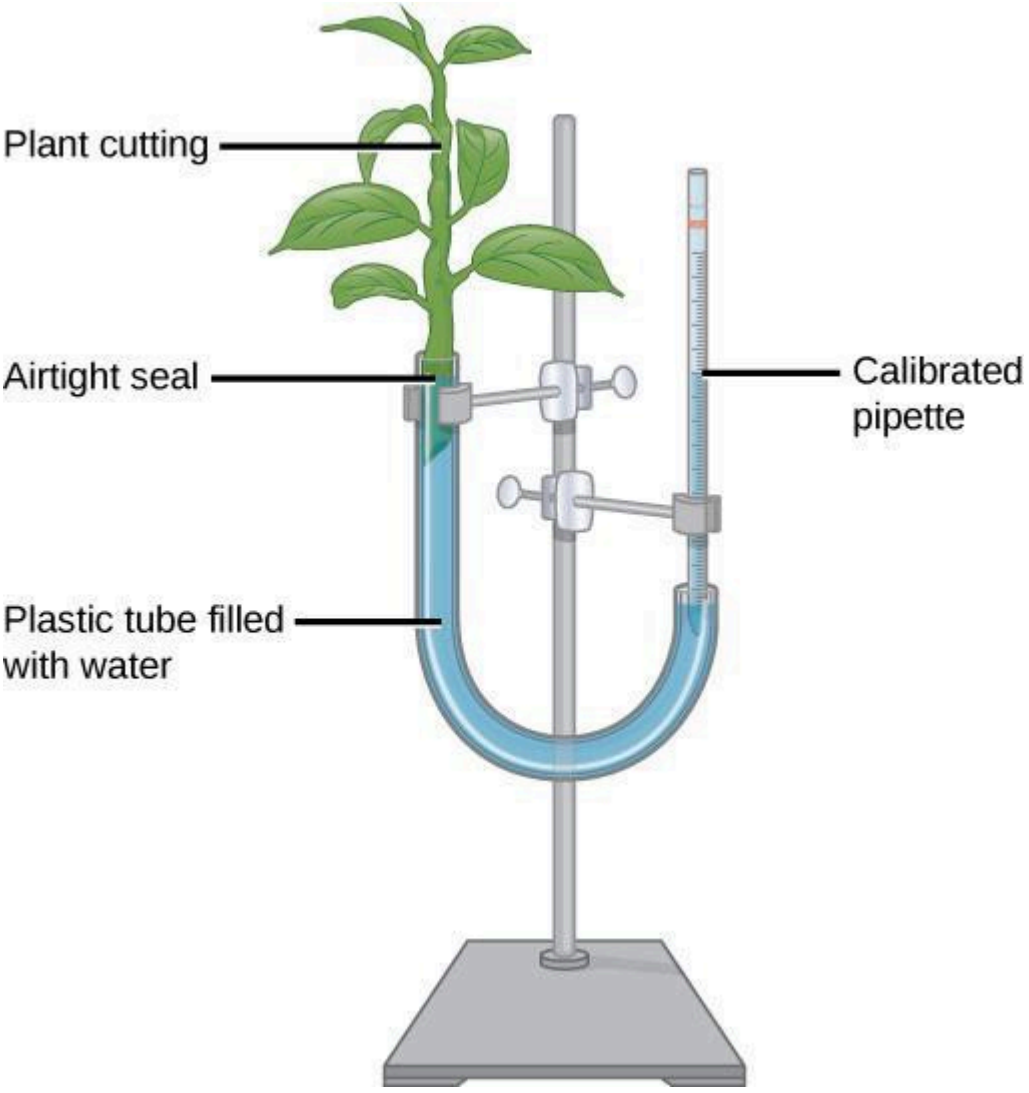


Figure 19.1: When transpiration takes place, water **evaporates** from open stomata. As the water potential difference increases between the bottom of the stem and the leaves, the stem draws in water from the tube, and the water level drops in the pipette

Safety Precautions

- Use care with all glassware.
- Clean up any spilled water immediately to avoid slippage.
- Insert the pipette carefully to avoid breaking the tip.
- Do not allow electric cords to get wet.
- Be careful when handling hot lamps.

For this activity, you will need the following:

- Ring stand
- Plant stalk with leaves
- Incandescent lamp

- Clamps
- Plastic tubing
- Basin half-filled with water
- Calibrated 1.0-mL pipette
- Basin
- Syringe
- Petroleum jelly
- Fluorescent light or water tank with incandescent light
- Fan
- Water sprayer
- Plastic bag
- Timer

For this activity, you will work *in pairs.*

Structured Inquiry

Step 1: Set up the **potometer**, using Figure 19.1 as a guide. To reduce the formation of bubbles, cut the twig and insert it into the water-filled plastic tubing while underwater. Add the **pipette** and adjust water level as necessary so the starting point is high enough to allow for readable decreases during the experiment. If there are any air bubbles, you can use the syringe to remove them. Use clamps to attach the potometer to the ring stand, making sure the heights of the plant relative to the pipette are similar to those in Figure 19.1.
Step 2: Hypothesize/Predict: After you insert the stem into the tube, how much water do you think will move out of the pipette? Write your predictions in your notebook.
Step 3: Student-Led Planning: You will start with measuring changes in the water level for the plant under the typical light conditions of the room. Before you begin, make a data table to collect information about the water level and the change in water level as a function of time. You will take data every five minutes, for a total of 20 minutes.
Step 4: When you are ready, insert the stem, making sure it is submerged, and take an initial reading of the water level at time zero and subsequent readings every five minutes. Then, record the water loss in your data table every five minutes, for a total of 20 minutes. After the 20-minute period, remove the leaves from the potometer and set them aside, as you will need to measure the area of the leaf measured in the next step.

Step 5: Critical Analysis: Discuss with your partner how the first trial worked and if any adjustments need to be made to the setup to ensure more accurate results. Are the predictions made in Step 2 supported by the data? Can your results be improved? After the data collection, you will need to calculate a rate of transpiration per minute. To do this, you will take a water level reading every five minutes for 20 minutes for each part of

the experiment. Then, you can graph the water measurements by time to estimate the rate of transpiration per minute. After this, you will need to determine the area of leaf surface measured. Since there are multiple leaves of different sizes, you will trace each leaf on graph paper and add up the total area. The final result of the experiments should show the rate of transpiration. Discuss with your partner and write your answers in your notebook.

Guided Inquiry

Step 1: Hypothesize/Predict: Choose from the following list of variables to alter the conditions for the plant:

- Use **fluorescent light** (if only an incandescent light is available, place a tank of water between the plant and the light source to absorb heat).
- Apply wind using a fan.
- Apply high moisture by spraying the plant with water and then enclosing it in a plastic bag.

How do you think your chosen variable will affect the rate of transpiration? Does the adjustment accurately mimic conditions in a natural environment? Why or why not?

Step 2: Student-Led Planning: How will you adjust your setup to incorporate your chosen variable? Create a plan with your partner. Then, have your teacher approve your plan before altering the setup. Once approved, alter your setup and create data tables for your experiment in your notebook.

Step 4: Collect the transpiration data using the potometer as in the Structured Inquiry. Record the measurements in your data table.

Step 5: Use the information in the Structured Inquiry to find the total surface leaf area of your plant sample and use it to calculate a final rate of transpiration for both trials. Graph your data in your notebook.

Step 6: Critical Analysis: How did the typical conditions compare with the altered conditions in terms of the rate of transpiration? Was the difference significant? Discuss your answers with your partner and write them in your notebook.

Assessments

1. One cutting has more leaves than another. Given this information, can you predict how the rate of transpiration would compare between the cuttings?
2. Explain whether your prediction in the Guided Inquiry was accurate. Include a reason based on how the variable affected transpiration.
3. How might drought or flooding induced by climate change affect transpiration of plants, and how, in turn, would this affect the ecosystem? Give an example.

COMPARISON OF OPENING OR CLOSING OF STOMATA UNDER SELECTED CONDITIONS

Learning Objectives

After completing the lab, the student will be able to:

1. Observe patterns of and predict percentages of open versus closed stomata under different conditions.

Activity 2: Pre-Assessment

1. What environmental conditions could be applied to a plant that would cause it to open more of its stomata?
2. Do you think there is a condition that would result in mostly closed stomata?
3. Discuss the answers to questions 1 and 2 with the class.

Activity 2: Comparison of Opening or Closing of Stomata under Selected Conditions

Plants will open or close stomata based on many factors. **Stomata** help regulate the water needs of the plant and exchange gases with the environment. If there is too much water inside the cells, stomata will open to release the pressure. If water pressure is low, stomata will remain closed to retain moisture. However, to keep water moving up from the roots and into the leaves, some stomata do need to open to build the negative pressure that allows roots to draw up water. Special cells, called **guard cells**, act like doors to open or close stomata.

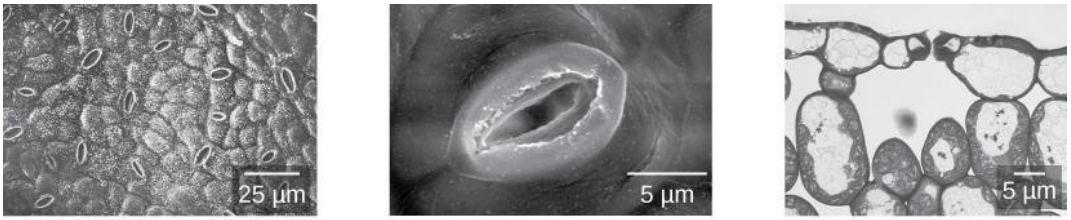


Figure 19.2: Transpiration takes place through open stomata, (a) generally scattered across the underside of leaves. (b) A single stoma. (c) A cross section of a stoma shows the two small guard cells above the large open air space within the leaf tissue. The guard cells contain darkened areas.

Safety Precautions

- Avoid directly inhaling nail polish fumes.
- Handle the microscope carefully and always carry it with two hands.

For this activity, you will need the following:

- Scissors
- Clear nail polish
- Empty microscope slides
- Microscope
- Leaves
- Clear cellophane tape
- Forceps

For this activity, you will work *in pairs*.

Structured Inquiry

Step 1: Prepare slides of leaf samples at normal room conditions. To do this, you will cut a sample of a leaf and coat both sides of the leaf with clear nail polish. Allow it to dry completely by holding the sample and waving it gently in the air.

Then, create wet mounts of the leaf samples and observe them under the microscope.

Step 2: Hypothesize/Predict: What percentage of open to closed stomata do you expect to find? Record your hypothesis in your notebook.

Step 3: Student-Led Planning: Prepare a data table to count the stomata. Then, place the slide under the microscope and count the number of stomata. Record your data in your table. If there is time, consider making more than one sample.

Draw sketches of the samples.

Step 4: Critical Analysis: Is it simple or complicated to count the stomata? Is it possible to count the same ones more than once? How did you ensure this did not happen? What did the results show when the leaves from the two different trials were compared?

Guided Inquiry

Step 1: Hypothesize/Predict: Choose a condition to which you will expose a leaf sample for 20 minutes. How do you think the altered condition will affect the stomata? Record your ideas in your notebook.

Step 2: Student-Led Planning: Prepare a slide of the leaf sample by cutting a leaf and coating both sides with clear nail polish. Allow the leaf sample to fully dry before placing it on the microscope slide. Prepare a data table and count the number of closed and open stomata. Draw sketches of the samples.

Step 3: Critical Analysis: Compare your results to the results from the Structured Inquiry. What conclusion can you draw from the data? Write your answer in your notebook.

Assessments

1. In a certain habitat, the plants can keep their stomata open for most of the day. What does this suggest about the characteristics of the habitat?
2. Could a researcher estimate how many plants in an enclosed area have their stomata open using the concentration of carbon dioxide in the air? Explain your answer.
3. How could a research scientist use the methods in this lab to determine how to care for a given species of plant?

KEY TERMS

airtight seal
: completely sealed so that no air can get in or out of it

calibrated pipette
: to prevent inaccuracies in order to attain better results, performance, and longevity of the pipette itself

evaporate
: change of water from liquid to gaseous state, often removing heat from a wet surface

fluorescent light
: a usually tubular electric lamp having a coating of fluorescent material on its inner surface and containing mercury vapor whose bombardment by electrons from the cathode provides ultraviolet light which causes the material to emit visible light

guard cells
: each of a pair of curved cells that surround a stoma, becoming larger or smaller according to the pressure within the cells

plant cutting
: a section of plant such as a modified stem, leaf, or root used for vegetative propagation that forms either adventitious shoots, adventitious roots (stem and single node cuttings), or both (root and leaf)

photometer
: an instrument for measuring the intensity of light

stomata
: any of the minute pores in the epidermis of the leaf or stem of a plant, forming a slit of variable width which allows movement of gases in and out of the intercellular spaces

transpiration
: (of a plant or leaf) the exhalation of water vapor through the stomata

LAB 15: STRAWBERRY DNA

DNA EXTRACTION FROM STRAWBERRIES

Learning Objectives

After completing the lab, the student will be able to:

- Extract DNA from the cells of strawberry fruit tissue.

Activity: Pre-Assessment

1. In a plant like the strawberry, where would you likely find DNA?
2. Discuss the answers to the questions with a partner (think, pair, share) and then the class.

Activity: DNA Extraction from Strawberries

The first step in working with nucleic acids (DNA and RNA) is to remove the molecules from inside the cell. Different types of cells need to be processed differently in order to release nucleic acids. All cells have a **cell membrane**, a **phospholipid bilayer** that separates the internal environment of the cell from the external environment. In eukaryotes, DNA is housed inside the nucleus of the cell, which is surrounded by the **nuclear membrane**, a second double-layered membrane, also composed largely of lipid molecules. When extracting DNA from plant cells, the **cell wall** must also be considered; some types of plant tissue require grinding or flash-freezing in order to break through the tough cell wall.

Strawberry fruit tissue is an excellent type of tissue to use for demonstration of **DNA extraction**. First, ripe strawberries are soft and juicy; as the fruit matures, the cells fill up with water and sugar, which make the fruit so delicious! Second, as the strawberry ripens, a series of **chemical reactions** take place within the cells that lead to the breakdown of long-chain polysaccharides, like cellulose and pectin, that make the cell wall tough. Lastly, cultivated strawberries (*Fragaria* x *ananassa*) are the product of a hybridization between two other strawberry

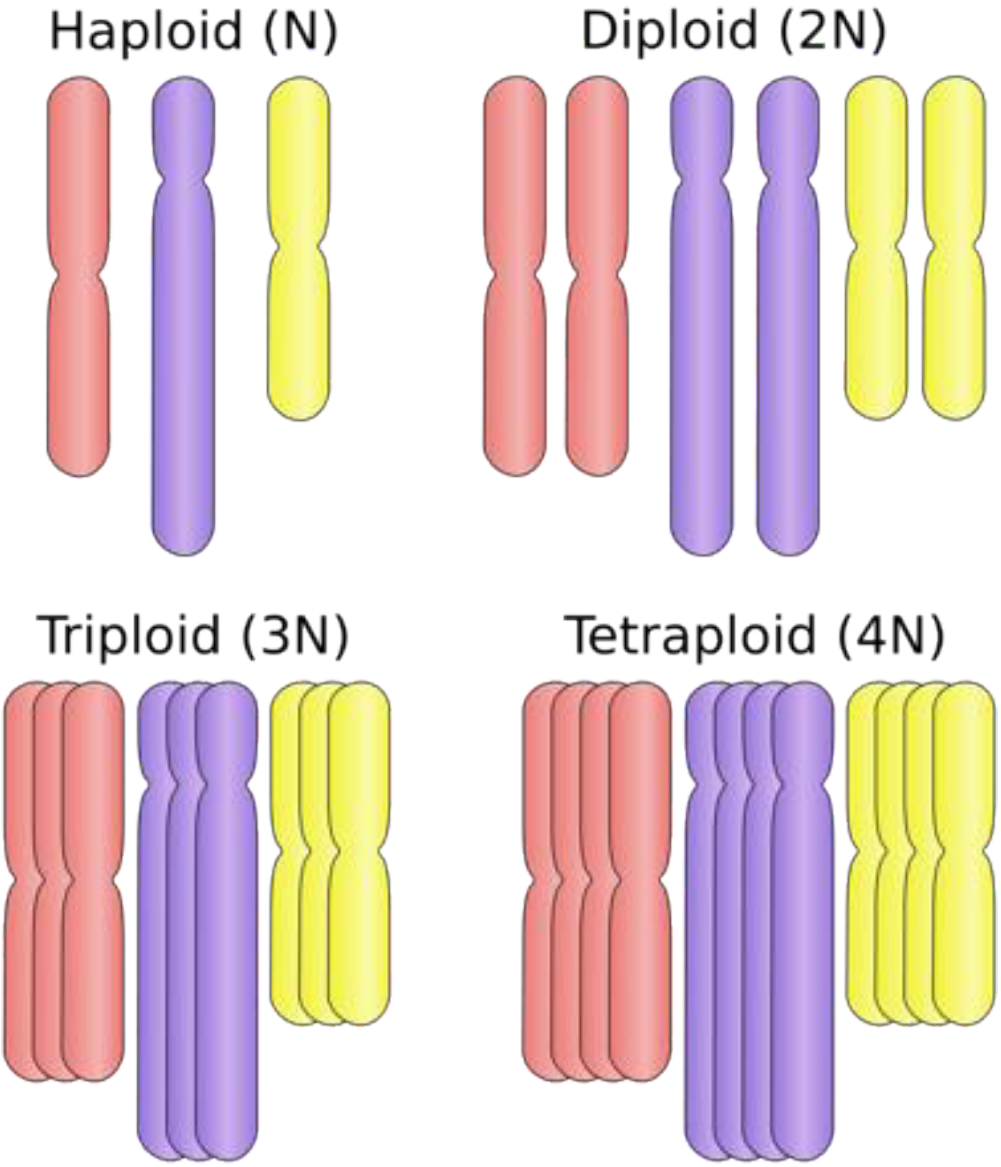
species, and they have an **octoploid** genome, meaning they have eight sets of chromosomes inside their cells. That translates to lots of molecules of DNA, which increases our yield and makes the DNA easier to visualize.

This protocol for extraction of DNA is based largely on the principle of **solubility**. Solubility refers to the ability of one substance (the solute) to dissolve in another substance (the solvent). Recall that polar substances dissolve easily in polar solvents, but do not dissolve easily in nonpolar solvents, a phenomenon commonly referred to as "like dissolves like." Water is a polar solvent, and molecules that dissolve easily in water are referred to as **hydrophilic**. DNA molecules are hydrophilic because the sugar-phosphate backbone of the molecules are highly polar. This means that DNA dissolves in water, so in this experiment, the DNA that is released when the cells are crushed dissolves in the juice/extraction **buffer** mixture.

Remember, there are two key ingredients in the DNA **extraction buffer** aside from the water: **dish soap** and **salt**. The dish soap acts to break up the lipid molecules that form the cell membrane and the nuclear membrane, which lyses the cell and releases the cellular contents, including DNA. The salt has two functions in the extraction process. It helps to neutralize the charge on the sugar-phosphate backbone, making DNA *less soluble* in water, and it also makes the DNA molecules stick to each other, so they are easier to visualize and remove from the solution.

Although the chemical reactions described above are all happening when you add the buffer and crush the strawberries, they are not visible to the naked eye. However, the addition of the cold ethanol caused a much more dramatic result! Ethanol is a nonpolar solvent, and when it is added to the solution of strawberry juice and extraction buffer, the DNA precipitates out of the solution. A **precipitation reaction** is a chemical reaction that causes a solid substance to emerge from a liquid solution. In this experiment, the addition of ethanol to the reaction forces DNA to precipitate out of solution, which we can then spool onto the wooden skewer.

Strawberry flowers; picture from Wikimedia Commons



Illustrations of **ploidy levels**; N=number of chromosomes in one set. Strawberries (*Fragaria* x *ananassa*) are **octoploid** (8N); illustration from Wikimedia Commons

Safety Precautions

For this activity, you will need the following:

- Plastic zipper bag
- 1 fresh strawberry
- 10 mL DNA extraction buffer
- Gauze squares
- Funnel
- Ice cold ethanol

- Plastic transfer pipettes
- Clear glass test tube
- Wooden skewer
- Microcentrifuge tubes

To make DNA extraction buffer (this part has been done for you), combine the following:

- 45 mL DI water
- 5mL liquid dish soap
- 0.75 g NaCl (table salt)

For this activity, you will work *in pairs*.

Structured Inquiry

Step 1: Place strawberry into plastic zipper bag and add 10mL of DNA extraction buffer. Seal the bag tightly. Gently, but thoroughly crush the strawberry inside the bag for about one minute.

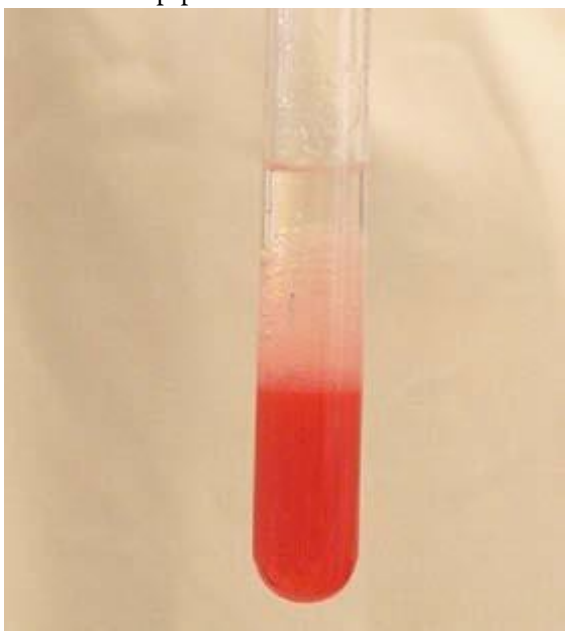
Line the funnel with a gauze square. Place the funnel into the test tube.

Pour the strawberry juice/DNA extraction buffer mixture into the funnel so the juice passes through the gauze and into the test tube. Use the gauze to strain the strawberry mixture so that only the juice flows into the tube and the pulp is retained in the gauze.

Discard the gauze and the strawberry pulp.

Step 2: Hypothesize/Predict: After straining the strawberry juice/DNA extraction buffer mix, do you anticipate being able to see DNA with the naked eye? What does DNA look like? Write your hypotheses and expectations in your notebook.

Layer an equal volume of ice cold ethanol on top of the strawberry juice mixture in the test tube using the plastic transfer pipette.



Step 3: Hold the tube still at eye level (DO NOT shake) and observe what happens at the interface of the alcohol and the strawberry juice when you twirl your wooden skewer through the interface of the solutions.

Use your wooden skewer to transfer your strawberry DNA into a microcentrifuge tube. Add a small amount of ethanol to the tube to prevent your DNA from drying out.

Step 4: Critical Analysis: You now have a clump of aggregated DNA molecules that you can see! This DNA contains all the genes within the strawberry genome. Given what you know about DNA, what could the DNA be used for after it has been extracted?

Discuss with your partner and then write your answers in your notebook.

References: Jones, Carolyn. DNA Extraction from Strawberries. https://www.murdoch.edu.au/Biotech-out-of-the-box/_document/Kit-Handout-Sheets/DNA-extraction-from-strawberries.pdfOpenStax CNX. 2018. https://cnx.org/contents/k6E8Y6SA@8/Primary-DNA-Molecular-Structure University of Missouri, Division of Plant Sciences. 2018. Strawberry: A Brief History. https://ipm.missouri.edu/meg/2012/5/Strawberry-A-Brief-History/

KEY TERMS

buffer
: substance that resists a change in pH by absorbing or releasing hydrogen or hydroxide ions

cell membrane
: the semipermeable membrane surrounding the cytoplasm of a cell

cell wall
: rigid cell covering comprised of various molecules that protects the cell, provides structural support, and gives shape to the cell

chemical reactions
: a process that involves rearrangement of the molecular or ionic structure of a substance, as opposed to a change in physical form or a nuclear reaction

dish soap and salt
: any chemical compound formed from the reaction of an acid with a base, with all or part of the hydrogen of the acid replaced by a metal or other cation

DNA extraction
: a method to purify DNA by using physical and/or chemical methods from a sample separating DNA from cell membranes, proteins, and other cellular components

extraction buffer
: a buffer solution used for the purpose of breaking open cells for use in molecular biology experiments that analyze the compounds of the cells

hydrophilic
: having a tendency to mix with, dissolve in, or be wetted by water

nuclear membrane
: is made up of two lipid bilayer membranes that in eukaryotic cells surround the nucleus, which encloses the genetic material. The nuclear envelope consists of two lipid bilayer membranes: an inner nuclear membrane and an outer nuclear membrane

phospholipid bilayer
: a two-layered arrangement of phosphate and lipid molecules that form a cell membrane, the hydrophobic lipid ends facing inward and the hydrophilic phosphate ends facing outward

precipitation reaction
: precipitation is the process of transforming a dissolved substance into an insoluble solid from a super-saturated solution. The solid formed is called the precipitate

solubility

the ability to be dissolved, especially in water

APPENDIX A: CHECKLIST FOR ACCESSIBILITY

Organizing Content

- Content is organized under headings and subheadings.
- Headings and subheadings are used sequentially (e.g., Heading 1, Heading 2).

Images

- Images that convey information include alternative text (alt text) descriptions of the image's content or function.
- Graphs, charts, and maps also include contextual or supporting details in the text surrounding the image.
- Images do not rely on color to convey information.
- Images that are purely decorative do not have alt text descriptions. (Descriptive text is unnecessary if the image doesn't convey contextual content information).

Links

- The link text describes the destination of the link and does not use generic text such as "click here" or "read more."
- If a link will open or download a file (like a PDF or Excel file), a textual reference is included in the link information (e.g., [PDF]).
- Links do not open in new windows or tabs.
- If a link must open in a new window or tab, a textual reference is included in the link information (e.g., [NewTab]).
- For citations and references, the title of the resource is hyperlinked, and the full URL is not hyperlinked.

Tables

- Tables are used to structure information and not for layout.

- Tables include row and column headers.
- Row and column headers have the correct scope assigned.
- Tables include a caption.
- Tables avoid merged or split cells.
- Tables have adequate cell padding.

Multimedia

- All audio content includes a transcript. The transcript includes all speech content and relevant descriptions of non-speech audio and speaker names/headings where necessary.
- Videos have captions of all speech content and relevant non-speech content that has been edited by a human for accuracy.
- All videos with contextual visuals (graphs, charts, etc.) are described audibly in the video.

Formulas

- Equations written in plain text use proper symbols (i.e., −, ×, ÷).[1]
- For complex equations, one of the following is true:
  - They were written using LaTeX and are rendered with MathJax (Pressbooks).
  - They were written using Microsoft Word's equation editor.
  - They are presented as images with alternative text descriptions.
- Written equations are properly interpreted by text-to-speech tools.[2]

Font Size

- Font size is 12 point or higher for body text in Word and PDF documents.
- Font size is 9 point for footnotes or endnotes in Word and PDF documents.
- Font size can be enlarged by 200 percent in webbook or ebook formats without needing to scroll side to side.

Learn more about Pressbooks' commitment to Accessibility.

1. For example, a hyphen (-) may look like a minus sign (−), but it will not be read out correctly by text-to-speech tools.
2. Written equations should prioritize semantic markup over visual markup so text-to-speech tools will read out an equation in a way that makes sense to auditory learners. This applies to both equations written in LaTeX and equations written in Microsoft Word's equation editor.