SCHOOL LAUNCH · LIFE SCIENCE K–8

Grade 7 Life Science: Cells and Microbes

A year of true stories about the small world that was always there, from Leeuwenhoek squinting through a lens no bigger than a pea at the “animalcules” in a drop of water to Schleiden and Schwann naming the cell, Semmelweis and the washbasin, Pasteur’s swan-necked flasks, Fleming’s moldy dish and van Helmont’s willow in its pot of earth, each one taken apart to see how it was figured out and then tried again at the kitchen table with a microscope, a ruler and a notebook.

First edition

> *“And God made the beast of the earth after his kind, and cattle after their kind, and every thing that creepeth upon the earth after his kind: and God saw that it was good.” (Genesis 1:25, KJV)*

Course guide

This course takes a seventh grader into the world of living things too small to see: the microscope and how it opened that world, the cell and its parts, bacteria, protists and yeast, germs and the long fight against disease, photosynthesis and respiration, the fungi, and finally the bodies of whole animals, from the gills of a fish to the long neck of a giraffe. Each unit is built around real moments in the history of science: Antonie van Leeuwenhoek's letters to the Royal Society in 1676, Robert Hooke's drawings in *Micrographia* (1665), Matthias Schleiden and Theodor Schwann declaring in 1838 and 1839 that plants and animals alike are built of cells, Ignaz Semmelweis ordering the doctors of a Vienna hospital to wash their hands in 1847, Louis Pasteur's flasks, Alexander Fleming's penicillin in 1928, and Jan Baptist van Helmont weighing a willow tree and its soil over five years.

Every lesson follows the same path. First comes **the story**. Then **how they figured it out**: what question was asked, what was tried, what was measured, what failed and what was changed. Then **the big idea**, explained at middle-school depth and building toward high-school Biology: cell theory, organelles, diffusion, fermentation, the germ theory of disease, the inputs and outputs of photosynthesis and respiration, gas exchange and circulation. Last, the student **observes and measures** for himself or herself: drawing what is under the lens to scale, counting yeast bubbles, timing floating leaf disks, graphing results and asking whether the numbers support the idea.

A single thread runs through the year: **the closer we look, the more order we find**. A drop of pond water holds whole communities; a single cell is busier than a city; a leaf turns light, air and water into food; a bird's lungs and a fish's gills are each fitted exactly to the life they live. The year moves from the smallest living things to whole animals, ends with a dissection lab and a lab practical, and closes with an optional **Nature Lab** that takes the notebook outdoors, to a farm or zoo, or to a museum.

The course is written from a Christian view of creation. The world of cells and microbes was hidden for almost all of history, yet it was ordered and good from the beginning: "And God made the beast of the earth after his kind, and cattle after their kind, and every thing that creepeth upon the earth after his kind: and God saw that it was good." (Genesis 1:25) Many of the scientists in these lessons worked in the conviction that the world could be understood because a wise Creator made it. The student learns to measure carefully, report honestly and wonder freely, and to see careful science as a way of tracing the handiwork of God.

WHO IT IS FOR

Written for students in grade 7 (about ages 12–13) who read on their own, can work with decimals, ratios and percents, and can read and make simple bar and line graphs. The course builds toward a high-school Biology course. A parent or teacher reads along, checks answers, and supervises every experiment, especially the bacteria cultures, any use of stains, heat or blades, and the dissections. Most experiments use household things; the main

purchases are a student microscope with slides, a few agar plates and a simple dissection kit with preserved specimens, and a virtual dissection may stand in for the frog.

HOW TO USE THIS COURSE

The weekly rhythm. The year has 32 lessons, one a week, in eight units of four, plus the optional Nature Lab. Session 1 (about 45 minutes) is the warm-up, the video, the core ideas, the picture study and a worked example. Session 2 (about 45 minutes) is the experiment, the nature notebook and the practice set. In the last week of each unit, add a third session for the unit test and the unit project. The Nature Lab can be used over the summer, on good-weather days through the year, or after the last unit.

The ★ quarter path. Ten lessons are marked ★: Leeuwenhoek, Schleiden and Schwann, bacteria, protists, Semmelweis, van Helmont's willow, the leaf disk lab, mushrooms, how a fish breathes and the earthworm dissection. Families who want a one-quarter version can do only those ten, one a week, and still cover the microscope, the cell, microbes and disease, photosynthesis, fungi and animal bodies. Units 7 and 8, marked 🐾, turn from cells to whole animals.

The nature notebook and the experiments. Use one composition book for the whole year. Every experiment ends with something drawn, counted or measured: a labeled drawing with its magnification, a data table with units, a graph, and one sentence on what the results mean and what you would change. All measurements are metric (millimeters, micrometers, milliliters, grams, degrees Celsius). An adult supervises every experiment. Wash hands before and after lab work. Bacteria plates are taped shut once they are streaked and are never opened again; grow them at room temperature, not in a warm place, and let an adult soak them in bleach before throwing them away. Never eat or taste anything in a lab, and never eat a wild mushroom. Use gloves and goggles for stains and dissections, and cut away from the body.

Worked examples and practice. Each lesson has three worked examples that walk step by step through the numbers of biology: magnification and the true size of a cell, rates of bubbles or floating disks per minute, ratios, reading a growth curve, doubling of bacteria and simple Punnett-style counting. Then come ten practice problems, including two data-reading questions in the style of the SAT and ACT science sections and one in which the student designs an experiment with a clear variable and control. Answers are given; check them together, and have the student explain any wrong answer before moving on.

Unit tests and unit projects. Each unit ends with a test of 60 points in 10 to 12 problems (multiple choice, short answer and one diagram to label or interpret) and a unit project that pulls the unit together, such as a set of scale drawings from pond water, an edible cell model with a key, a fermentation graph, a handwashing experiment, a photosynthesis report or a lab practical. Keep the unit projects in one folder for the year.

A word about time. The course is written from a Christian view of creation (Genesis 1:1) and the Flood (Genesis 6–9). Deep-time names such as "Jurassic" or "Ice Age" are taught as museum words so students can read any sign or textbook. Wherever they come up, a ⏳

Thinking About Time box sets out what the sign says, what we observe, and how Christians read it.

SCOPE AND SEQUENCE

Unit 1: The Microscope (1–4)

Leeuwenhoek's single-lens microscopes and his "animalcules"; the parts and care of a compound microscope; magnification and field of view; making wet-mount slides; and scale drawings of the life in a drop of pond water.

Unit 2: The Cell (5–8)

Hooke names the cell and Schleiden and Schwann show that all plants and animals are made of cells; the organelles and their jobs; how plant and animal cells differ; and an edible cell model.

Unit 3: Single-Celled Life (9–12)

Bacteria, their shapes and how fast they divide; the protists of pond water; yeast, fermentation and the rising of bread; and how to grow bacteria safely in sealed dishes.

Unit 4: Germs and Disease (13–16)

Semmelweis and the washbasin in Vienna; Pasteur's swan-necked flasks and the end of spontaneous generation; Fleming's discovery of penicillin; and how the body's defenses fight infection.

Unit 5: Photosynthesis and Respiration (17–20)

Van Helmont weighs a willow and its soil; the floating leaf disk lab; respiration as the other half of the cycle; and how light, water and air become food.

Unit 6: Fungi (21–24)

Mushrooms and the hidden network beneath them; molds on bread and fruit; spore prints; and why no one should ever eat a wild mushroom.

Unit 7: Animal Physiology 🐾 (25–28)

How gills take oxygen from water; the one-way airflow of a bird's lungs and air sacs; how insects breathe through tubes and wear their skeletons outside; and how a giraffe moves blood up a long neck.

Unit 8: Dissection Lab 🐾 (29–32)

Careful, respectful dissection of an earthworm and a frog (or a virtual frog); an owl pellet review of skeletons and food chains; and a lab practical that brings the year together.

Unit 9: Nature Lab (optional) (Any time)

Take the nature notebook outdoors: a journal walk, a measured backyard survey, a visit to a zoo, farm or museum, and a small, labeled specimen collection.

MATERIALS

- **Nature notebook (a graph-paper composition book)**: One for the whole year: labeled drawings with magnifications, data tables, graphs and conclusions.
- **A student compound microscope (about 40× to 400×) with blank slides and cover slips**: The heart of Units 1–3. A sturdy beginner microscope with an LED light is enough; a clip-on phone lens helps for a few lessons but cannot replace it.
- **Iodine solution and methylene blue (or food coloring), droppers and toothpicks**: For staining onion, cheek and pond-water slides. Wear gloves; iodine stains skin and cloth.
- **Prepared nutrient agar plates (or plain gelatin and broth) and clear packing tape**: For growing bacteria and molds in Units 3, 4 and 6. Plates are sealed once streaked and never reopened; an adult disposes of them with bleach.
- **Kitchen supplies: dry yeast, sugar, flour, balloons, baking soda, spinach leaves, a plastic syringe without a needle, a hole punch and clear cups**: For the yeast, bread and floating leaf disk experiments.
- **A metric ruler, a kitchen scale that reads grams, a thermometer in degrees Celsius, measuring cups in milliliters and a timer**: Everything in the course is measured in metric units.
- **A simple dissection kit, safety goggles and disposable gloves**: Scissors, forceps, probes, pins and a tray. A craft knife or scalpel is used only by or with an adult.
- **Preserved specimens: an earthworm, a frog and an owl pellet from a science supplier**: For Unit 8. A free virtual frog dissection may replace the real frog.
- **A pond-water jar, a magnifying glass, paper bags or envelopes and colored pencils**: For pond-water slides, spore prints, the Nature Lab and notebook drawings.
- **Optional: one of the books or resources listed in a unit**: Never required. See the list in each unit.

GRADING

Grades come from the unit tests, the experiments, the nature notebook and the unit projects, because science is learned both by understanding ideas and by observing and measuring carefully. An experiment that did not turn out as predicted earns full credit when the data are honestly recorded and explained.

- Unit tests (60 points each): 30%
- Experiments (prediction, careful measurement, safety and the improve-it step): 30%
- Nature notebook (dated pages, labeled drawings, data tables and graphs): 20%
- Unit projects: 20%

A 90–100, B 80–89, C 70–79, D 60–69. Below 60: review the unit with help and retake the test or redo the experiment.

- Grade the process, not just the result. Did the student predict, control variables, measure carefully, record honestly, explain the data and suggest an improvement?
- A notebook drawing earns full marks when it has a date, a title, labels, the magnification and an estimated true size in micrometers or millimeters.
- A data table earns full marks when it has a title, labeled columns with metric units, correct calculations and one sentence about what the numbers mean.
- Practice sets are for learning, not grading; check them together and correct mistakes. The unit tests may be done orally for a student who writes slowly.
- Safety is part of the grade. A student who skips goggles, opens a sealed culture plate or handles a blade carelessly repeats the safety review before the next lab.

A NOTE TO PARENTS

You do not need to be a biologist to teach this course. Read the story together, look at the picture, and then be the lab partner: hold the timer, read the scale, help focus the microscope. Ask “What do you predict?”, “How did you measure it?” and “What would you change next time?” Those three questions are most of what a good science teacher does.

Your job in the lab is mostly safety. Be present for every experiment, keep bacteria plates sealed and dispose of them with bleach, handle stains and blades with care, and settle in advance whether your family will do the frog dissection or the virtual one. The dissections are taught with respect: the animal is studied carefully, nothing is wasted or mocked, and the student learns how wonderfully it was made.

Above all, the course hopes to raise a young person who looks closely, measures honestly and is not afraid to say “I was wrong; the data say otherwise.” The microscope shows that the smallest corners of creation are full of order and beauty. As the student learns how a cell works, how bread rises and how a bird breathes, we hope wonder grows alongside knowledge, and gratitude to the One who made it all.

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★ quarter path · ⏳ Thinking About Time box

UNIT 1

Microscope

In 1676 a cloth merchant in Delft, Holland, looked through a lens no bigger than a pea at a drop of pepper water and saw it swarming with living creatures. Antonie van Leeuwenhoek had found a world that had been there since the creation but that no human eye had ever seen. This unit begins Grade 7 with the tool that opened that world: the microscope. Everything else in this course, from cells and bacteria to germs, yeast and molds, depends on knowing how to use it well.

You will repeat Leeuwenhoek's pepper-water experiment and learn how the Royal Society checked his claim. You will meet Robert Hooke and his lamp-lit microscope, Ernst Abbe and the limits of light, Jan Swammerdam and his tiny scissors, and the naturalists Müller and Ehrenberg, who drew the creatures of the pond. Along the way you will learn the parts of a compound microscope, focus from low power to high, make wet mounts, dry mounts and stained slides, and finish with a pond survey and a set of careful scientific drawings.

This is also a unit of numbers. You will convert millimeters to micrometers, calculate total magnification and field of view, measure cells, mix stains by ratio, draw scale bars and estimate how many creatures live in a milliliter of water. Most of all, you will practice the scientist's habit of separating what you see from what you think it means.

BEFORE YOU BEGIN

- Can measure in millimeters and centimeters and multiply and divide by 10, 100 and 1,000
- Can find an average and a percent, and read a simple data table and bar graph
- Can name the independent and dependent variables in a simple experiment
- Can follow written safety directions and handle glass carefully

MASTERY CHECKLIST

- I can tell how Leeuwenhoek discovered microorganisms in 1676 and why the Royal Society asked Hooke to repeat his observation.
- I can name the parts of a compound microscope, carry it correctly and focus from low power to high power.
- I can calculate total magnification and field of view and use them to estimate the size of a cell.
- I can make a wet mount without bubbles, a dry mount and a stained slide.
- I can identify common pond organisms and make a biological drawing with ruled labels, the magnification and a scale bar.
- I can convert between millimeters and micrometers and use ratios to mix a stain or find a drawing's magnification.

OPTIONAL BOOKS AND RESOURCES

- **Foldscope paper microscope** (https://foldscope.com/): A low-cost folding paper microscope (about 140×) designed at Stanford University. A good choice for families without a compound microscope, and it fits in a pocket for pond trips. A compound microscope reaching 400× is still best for this unit.
- ***Micrographia* by Robert Hooke (free at Project Gutenberg)** (https://www.gutenberg.org/ebooks/15491): Free. Hooke's 1665 book with his drawings of the flea, the louse, mold and his own microscope (Lesson 1.2). The old English is hard going, so read a page or two of a chosen observation aloud with a parent.

1.1 Leeuwenhoek's "Animalcules" (1676) ★

OBJECTIVES

- Tell how Antonie van Leeuwenhoek, a cloth merchant of Delft, discovered living things too small to see and how the Royal Society checked his claim.
- Convert between millimeters and micrometers and use magnification to find how large a tiny object appears.
- Explain why a scientific discovery must be repeated by other people before it is accepted.

Warm-up. Lay a sheet of plastic wrap flat over a page of newspaper. With a straw or fingertip, place one small, round drop of water on the wrap over a single letter. Look straight down. Is the letter under the drop bigger or smaller than its neighbors? Now try a bigger, flatter drop and a smaller, rounder one. Which drop magnifies more? Write one sentence about what shape a strong lens must have.

Video. Coming soon. Antonie van Leeuwenhoek single-lens microscope and his "animalcules": search museum channels (the Rijksmuseum Boerhaave in Leiden, the Royal Society), microscopy channels such as Journey to the Microcosmos, and homeschool science channels such as Homeschool Pop (Alternate) for a short history of the first look at microbes.

KEY TERMS

animalcule
"Little animal": the English word used in the 1600s and 1700s for the tiny moving creatures Leeuwenhoek saw. Today we call them microorganisms.

microorganism
A living thing too small to see clearly without a microscope, such as a bacterium, a protist or a yeast cell. Also called a microbe.

simple microscope
A microscope with only one lens. Leeuwenhoek's instruments were simple microscopes with a single, tiny, very curved lens.

magnification
How many times larger an image looks than the real object. Magnification = image size ÷ actual size.

micrometer (µm)
One thousandth of a millimeter. 1 mm = 1,000 µm. Bacteria are usually about 1 to 5 µm long.

replication
Repeating an observation or experiment, often by a different person, to check that the result is real.

CORE IDEAS

The story. In the autumn of 1676, a cloth merchant in the Dutch city of Delft sat down to write a very long letter to the Royal Society of London. His name was Antonie van Leeuwenhoek. He had been soaking crushed peppercorns in water, because he wanted to know why pepper tastes so sharp on the tongue. He guessed that pepper might be made of tiny spiky particles. When he looked at a drop of the pepper water through one of his homemade lenses, he found no spikes. Instead the water was swarming with living creatures, many of them far smaller than anything anyone had ever seen. Some darted, some tumbled, some crept. In his letter, dated October 9, 1676, he described these "little animals" (in Dutch, *diertgens*), and English readers soon called them **animalcules**. He had also found them in rainwater, well water and sea water.

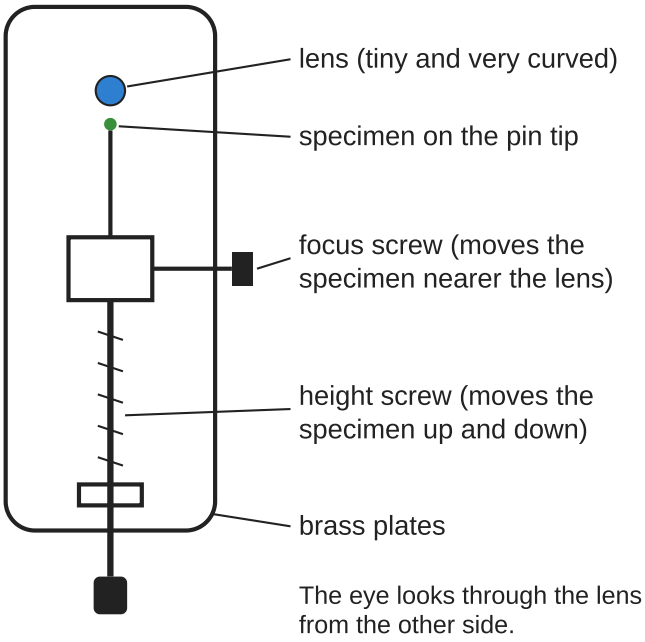
How they figured it out. Leeuwenhoek never went to a university and could not read Latin, the language of learned men in his day. He ran a cloth shop and held a job at Delft's city hall. But he had a craftsman's hands and endless patience. He made tiny glass lenses, some no bigger than a small pea, and mounted each one in a hole between two thin brass plates. The specimen sat on the point of a pin behind the lens, and two screws moved the pin up and down and nearer or farther until the view was sharp (see the diagram). His best surviving microscope magnifies about 270 times. He made hundreds of these instruments over his long life and kept his best lens-making method secret. The members of the Royal Society were amazed and a little doubtful: could a drop of water really hold so many living things? So they asked their own curator of experiments, Robert Hooke, to try it. In November 1677 Hooke showed the animalcules in pepper water to the members in London. The discovery had been **replicated**, and in 1680 the Society made Leeuwenhoek a Fellow.

The big idea. Leeuwenhoek opened a whole world of **microorganisms**, living things too small to see clearly with the eye alone. To talk about them we need a small unit. A **micrometer** (µm) is one thousandth of a millimeter: 1 mm = 1,000 µm. A careful eye can just make out a speck about 0.1 mm (100 µm) across. Many of the animalcules Leeuwenhoek saw were protists 50 to 300 µm long, near the edge of what an eye can see. But others were bacteria only 1 to 5 µm long, so he was almost certainly the first person in history to see bacteria. A lens helps because of **magnification**: magnification = image size ÷ actual size. A bacterium 2 µm long, magnified 270 times, makes an image 540 µm, or about half a millimeter, long. That is big enough for the eye to notice as a moving dot.

In the world today. Over fifty years Leeuwenhoek sent the Royal Society nearly two hundred letters. He described red blood cells, the fibers of muscle, and blood flowing through tiny vessels in the tail of an eel. In 1683 he scraped the white matter from between his own teeth and found it full of moving creatures. He also watched fleas and grain weevils hatch from eggs, which showed that they do not spring up from dust or rotting grain, as many people then believed. Today we know microbes live in soil, water, air and our own bodies. Most are harmless or helpful: they make yogurt and bread, break down dead leaves and enrich the soil. A few cause disease, a link that later scientists such as Pasteur worked out, as you will see in Unit 4.

God’s handiwork. Before 1676 no one knew that a single drop of rain held a crowd of living creatures, each one moving, feeding and growing. They had been there all along, made by God and unseen by man. The Bible says, “It is the glory of God to conceal a thing: but the honour of kings is to search out a matter” (Proverbs 25:2). Leeuwenhoek was no king, only a shopkeeper with a lens and a question about pepper. Yet by searching patiently, he found glory hidden in a drop of water.

Try it and see. In this lesson you will repeat the pepper-water experiment, counting the moving things in a drop each day for five days. Like the Royal Society, you will check a claim made 350 years ago with your own eyes.



Leeuwenhoek’s simple microscope, seen from the specimen side. The viewer held the plate right up to the eye on the other side.

PICTURE STUDY



Antonie van Leeuwenhoek, Jan Verkolje, about 1680–1686 · Oil on canvas · Rijksmuseum, Amsterdam

Jan Verkolje was a painter who worked in Delft, the same city where Leeuwenhoek lived. He painted this portrait sometime between about 1680 and 1686, a few years after the pepper-water letter, when Leeuwenhoek was around 50 years old and already famous across Europe. By then he was a Fellow of the Royal Society, and learned visitors came to Delft to look through his lenses.

Look at how Verkolje shows him: not as a nobleman or a soldier, but as a respectable townsman of Delft at his table. A painted portrait like this was costly. It tells us that a shopkeeper who never went to a university had become a person worth remembering.

Look closely

- Look at his face. What kind of person do you think he was: patient, proud, curious, cheerful? What clues do you see?
- Look at his clothes and his wig. What do they tell you about his place in Delft society?
- Find his hands. What are they doing? Why might a painter take care with the hands of a man famous for fine handwork?
- Find where the light falls in the picture. What does the painter want you to look at first?

Talk about it

1. Leeuwenhoek’s instruments were smaller than his hand. If you painted his portrait, what objects would you put on the table to tell his story?
2. Why do you think the Royal Society listened to a man who had no university degree?
3. What would you want to ask him if you could visit his shop in Delft?

> **Safety first.** Glass slides and coverslips break into sharp pieces: hold them by the edges and sweep up broken glass with a brush, never your fingers. Pepper water grows bacteria. Never drink or taste it, keep it away from food, wash your hands after every look, and at the end pour it down the drain with plenty of running water and wash the jars in hot soapy water. A microscope lamp can get warm.

EXPERIMENT: PEPPER WATER, DAY BY DAY

You will repeat the experiment that started microbiology. Your question: **How does the number of tiny moving things in pepper water change over five days, compared with plain water?** Before you begin, write a prediction in your lab notebook.

What we change: what is in the jar (pepper or no pepper) and how many days it has soaked. What we keep the same: the kind and amount of water, the jar size, the place the jars sit, the size of the drop and the magnification. What we measure: the average number of moving things in one field of view.

You will need

- A microscope that reaches 100× and 400× (or a Foldscope paper microscope)
- Two clean glass jars of the same size, labeled A and B
- About 1 tablespoon of whole black peppercorns, crushed in a plastic bag with a rolling pin
- 500 mL of rainwater, pond water or bottled spring water (city tap water has chlorine, which can slow the microbes)
- Glass slides, coverslips and a dropper
- Paper towels, lab notebook, pencil and graph paper

Steps

1. Pour 250 mL of water into each jar. Add the crushed pepper to jar A. Jar B gets water only; it is your **control**.
2. Cover each jar loosely with a paper towel held by a rubber band, and set both in the same spot out of direct sunlight.
3. On Day 0, make a wet mount from each jar: one drop of water from just below the surface, then a coverslip lowered at an angle.
4. Scan at 100× to find movement, then switch to 400×. In three different fields of view, count every moving thing. Write the three counts and their average in a table with columns Day, Jar A average, Jar B average, Notes.
5. Repeat at about the same time each day through Day 5. Take your drop from the same depth every day.
6. Draw one of the animalcules each day. Note its shape and how it moves: darting, spinning, wriggling or gliding.
7. Make a line graph with Day on the x-axis and average count on the y-axis. Draw both jars on the same graph with different colors.

8. Write a conclusion: Was your prediction right? What did the control jar show? What might explain the change you saw?

Tips

- Bacteria at 400× look like tiny jiggling dots or rods. Larger protists are easy to see even at 100×.
- If the creatures move too fast to count, use a smaller drop or count only the ones that cross the middle of the view.
- Dust and bits of pepper drift but do not swim on their own. Count only things that change direction by themselves.
- Microbes need time to multiply. If you see little on the first days, be patient and keep counting.

Check your work

- I set up a control jar and kept everything the same except the pepper.
- I recorded three counts and an average each day and made a line graph.
- I can explain what my control jar shows and why it matters.

NATURE NOTEBOOK

1. Draw Leeuwenhoek’s microscope from the diagram, full page, and label the lens, the specimen pin, the focus screw and the height screw.
2. Make a size ladder: draw a 10 cm line and mark where a grain of salt (about 0.3 mm), a hair’s width (about 0.08 mm), a red blood cell (about 0.008 mm) and a bacterium (about 0.002 mm) would fall if the line went from 0 to 0.3 mm. Which ones crowd together near zero?
3. Copy Proverbs 25:2 at the top of a page. Below it, list three things you have never looked at closely but would like to put under a lens.

> **From history.** Leeuwenhoek was born in Delft in October 1632, the same month that the painter Johannes Vermeer was baptized in the same city. After Vermeer died in 1675, the city appointed Leeuwenhoek as the trustee to settle Vermeer’s debts and estate. No one knows for certain whether the two men were friends, but both spent their lives studying light, one with paint and the other with lenses. Leeuwenhoek lived to the age of 90 and was still sending letters about his observations to London in the last years of his life.

WORKED EXAMPLES

Example 1. Leeuwenhoek's best surviving microscope magnifies about 270 times. A bacterium is 2 µm long. How long does its image appear, in micrometers and in millimeters? Is that big enough to notice if the eye can just make out about 0.1 mm?

1. Image size = actual size × magnification = 2 µm × 270 = 540 µm.
2. Convert: 540 µm ÷ 1,000 µm per mm = 0.54 mm.
3. Compare: 0.54 mm is more than 5 times the 0.1 mm the eye can just make out.

Answer: The image is 540 µm, or 0.54 mm, long, more than five times the smallest speck the eye can see, so Leeuwenhoek could see it as a tiny moving dot.

Example 2. A paramecium (a pond protist) is about 0.2 mm long. A bacterium is about 2 µm long. How many bacteria lined up end to end would equal the length of one paramecium?

1. Put both in the same unit: 0.2 mm × 1,000 µm per mm = 200 µm.
2. Divide: 200 µm ÷ 2 µm = 100.
3. Check the ratio another way: the paramecium is 100 times longer, so its length to the bacterium's is 100 : 1.

Answer: About 100 bacteria end to end would match one paramecium.

Example 3. Scientists estimate large numbers by counting a small sample and multiplying. A student counts animalcules in three fields of view of a drop of pepper water: 12, 15 and 9. The coverslip covers about 50 fields of view. Estimate how many animalcules are under the coverslip.

1. Average count per field: (12 + 15 + 9) ÷ 3 = 36 ÷ 3 = 12.
2. Multiply by the number of fields: 12 × 50 = 600.
3. This is an estimate, so round sensibly and say how it was made.

Answer: About 600 animalcules (12 per field × 50 fields).

PRACTICE

1. EASY What was Leeuwenhoek's trade, and in what city did he live?
2. EASY Convert: (a) 0.5 mm to micrometers; (b) 3,000 µm to millimeters.
3. EASY Why is Leeuwenhoek's instrument called a simple microscope?
4. MEDIUM A protist 30 µm long is viewed with a lens that magnifies 250 times. How long is the image in millimeters?

5. MEDIUM A student kept pepper water for five days and recorded the average number of moving things per field of view at 400×:
   Day 1: 0 · Day 2: 3 · Day 3: 25 · Day 4: 60 · Day 5: 55
   (a) On which day was the count highest? (b) Between which two days did the count rise the most, and by how much? (c) Describe the overall trend in one sentence.
6. MEDIUM The unaided eye can just make out an object about 0.1 mm (100 µm) wide. Use the table to decide which objects need a microscope to be seen at all.
   Grain of table salt: 300 µm · Human hair (width): 80 µm · Paramecium: 200 µm · Red blood cell: 8 µm · Bacterium: 2 µm
7. MEDIUM Leeuwenhoek watched fleas and grain weevils hatch from eggs. What common belief of his day did this observation argue against?
8. HARD A red blood cell is about 8 µm across and a bacterium about 2 µm long. How many times longer is the red blood cell? If both were magnified 400 times, how long would each image be in millimeters?
9. HARD When Leeuwenhoek's letter reached London, the Royal Society asked Robert Hooke to see the animalcules for himself before they accepted the claim. Explain in two or three sentences why this was good science and not an insult.
10. CHALLENGE Design an experiment to test this question: Does boiling the water and pepper first change how many animalcules appear after four days? Name the independent variable, the dependent variable, at least three things to keep the same and a control.

COMMON MISTAKES

- Thinking Leeuwenhoek invented the microscope. Microscopes with two or more lenses already existed, and Robert Hooke published drawings made with one in 1665. Leeuwenhoek's achievement was making single lenses so good that they showed more than anyone had seen before.
- Thinking that all microbes are harmful germs. Most microbes are harmless or helpful; they make bread and yogurt, break down dead things and enrich the soil. Only some cause disease.
- Mixing up millimeters and micrometers. A micrometer is a thousand times smaller than a millimeter, so 2 µm is 0.002 mm, not 0.2 mm or 2 mm.

> **Make it a game · Bigger or Smaller?.** Write each of these on its own card with its size: grain of salt 300 µm, paramecium 200 µm, human hair width 80 µm, cheek cell 60 µm, red blood cell 8 µm, yeast cell 5 µm, bacterium 2 µm. Shuffle the cards and deal two. The first player to say which is bigger, and how many times bigger (rounded to a whole number), wins the pair. When all cards are used, race to lay the whole set in order from largest to smallest.

> **Scripture.** "It is the glory of God to conceal a thing: but the honour of kings is to search out a matter." (Proverbs 25:2, KJV)

God filled every drop of water with living creatures long before anyone could see them. Searching them out with patience and careful work, as Leeuwenhoek did, is an honorable

calling.

Practice answers

1. He was a cloth merchant (a draper) who also held a city job, and he lived in Delft in the Netherlands.
2. (a) 0.5 × 1,000 = 500 µm. (b) 3,000 ÷ 1,000 = 3 mm.
3. Because it has only one lens. A compound microscope uses two or more lenses, one after the other.
4. 30 µm × 250 = 7,500 µm = 7.5 mm.
5. (a) Day 4, with 60. (b) Between Day 3 and Day 4, a rise of 35 (Day 2 to 3 rose only 22). (c) The count started near zero, rose quickly from Day 2 to Day 4, and then leveled off and dropped slightly on Day 5.
6. The hair's width (80 µm), the red blood cell (8 µm) and the bacterium (2 µm) are smaller than 100 µm and need a microscope to be seen as separate objects. The salt grain and the paramecium can just be seen as specks, though a microscope is needed to see any detail. (A single hair is long, so we can see its length, but not measure its width without help.)
7. The belief that small creatures spring up by themselves from dust, mud or rotting grain (spontaneous generation). He showed they come from eggs laid by parents of their own kind.
8. 8 ÷ 2 = 4 times longer. Red blood cell image: 8 × 400 = 3,200 µm = 3.2 mm. Bacterium image: 2 × 400 = 800 µm = 0.8 mm. (The ratio is still 4 : 1.)
9. A claim that no one else has seen could be a mistake, a flaw in the lens or a misunderstanding. When a different person, with different equipment, sees the same thing (replication), we have much better reason to believe it is real. Hooke's success in 1677 confirmed the discovery, and the Society then honored Leeuwenhoek by making him a Fellow.
10. Independent variable: whether the pepper water was boiled (boiled vs. not boiled). Dependent variable: the average number of moving things per field of view at 400× on Day 4. Keep the same: amount and kind of water, amount of pepper, jar size, cover, place and temperature, drop size, magnification and time of day of counting. Let the boiled jar cool before setting it out. Control: the unboiled pepper water jar (a plain-water jar is a useful second control). Use at least two jars of each kind and count three fields per jar to make the result more reliable.

1.2 Using a Microscope

OBJECTIVES

- Name the parts of a compound light microscope and tell what each part does.
- Carry, focus and adjust a microscope correctly, starting on the lowest power.
- Calculate total magnification, find the field of view at each power and use it to estimate the size of a cell.

Warm-up. Hold a magnifying glass (or the lens from old reading glasses) at arm's length and look at a window or lamp across the room through it. What do you notice about the image? Now hold the lens close to a printed word and move it slowly away from the page. At what point does the word blur? Then flip over? Write down what you saw. You have just found two facts you will meet again today: lenses have a focus, and some images are upside down.

Video. Coming soon. How to use a compound light microscope (parts, total magnification, focusing on low power first, field of view): search Amoeba Sisters, microscope makers' education pages, university biology lab channels and homeschool science channels for a clear beginner demonstration.

KEY TERMS

compound microscope
A microscope that uses two sets of lenses, the objective and the eyepiece, so that the second lens magnifies the image made by the first.

objective lens
The lens closest to the specimen. Most school microscopes have three, usually 4×, 10× and 40×, on a revolving nosepiece.

eyepiece (ocular)
The lens you look into at the top of the microscope, usually 10×.

total magnification
Eyepiece power × objective power. A 10× eyepiece with a 40× objective gives 400×.

field of view
The circle of the specimen you can see through the microscope. The higher the magnification, the smaller the field of view.

resolution
The ability to show two points close together as separate points. Magnification makes things bigger; resolution makes them sharp.

CORE IDEAS

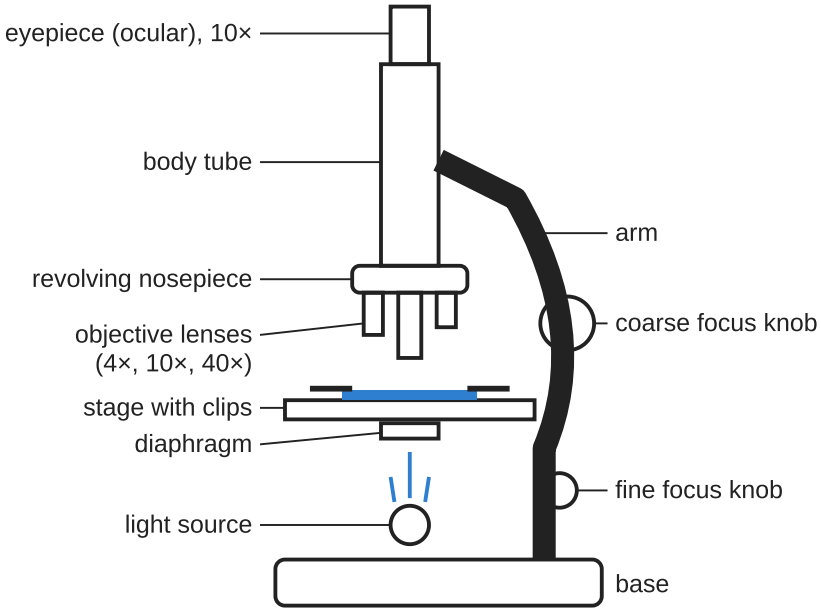
The story. In 1665, eleven years before Leeuwenhoek's pepper-water letter, a London scientist named Robert Hooke published a large and beautiful book called *Micrographia*, which means "small drawings." It held careful engravings of a flea, a louse, the point of a needle, mold and a thin slice of cork. Near the front, Hooke drew his own microscope. It was a **compound microscope**: a long tube with one lens near the bottom, close to the specimen, and more lenses near the top, close to the eye. Hooke found that the hardest part was not the lenses but the light. Tiny things seen at high power look dim, so he set an oil lamp beside the microscope and placed a glass globe full of salt water between them to gather the lamplight and throw a bright spot onto the specimen.

How they figured it out. Two lenses in a row multiply each other's power, which is why compound microscopes can magnify so much. But the early ones had a problem: every lens bent light a little unevenly, so images were blurry and edged with colored fringes. Leeuwenhoek's single tiny lens often gave a sharper view than the compound microscopes of his day. For nearly two hundred years opticians improved lenses by trial and error. Then in the 1870s, in the German town of Jena, a physicist named Ernst Abbe, working with the instrument maker Carl Zeiss, worked out the mathematics of how a microscope forms an image. Abbe showed that light itself sets a limit: no light microscope, however well made, can separate two points closer together than about 0.2 µm. Zeiss microscopes built on Abbe's rules became the best in the world, and that limit still holds for light microscopes today.

The big idea. Light from the **light source** passes up through the **diaphragm**, which controls how much light gets through, then through the specimen on the **stage**. The **objective lens** makes a magnified image, and the **eyepiece** magnifies that image again. So **total magnification** = eyepiece × objective. With a 10× eyepiece, the 4×, 10× and 40× objectives give 40×, 100× and 400×. As magnification goes up, the **field of view** shrinks in the same proportion: ten times more magnification shows a circle one tenth as wide. If the field is 4.5 mm across at 40×, it is 1.8 mm at 100× and 0.45 mm (450 µm) at 400×. Knowing the field of view lets you estimate the size of anything you see. Remember too that magnification is not everything. Making a blurry image bigger only gives a bigger blur. What lets you see fine detail is **resolution**.

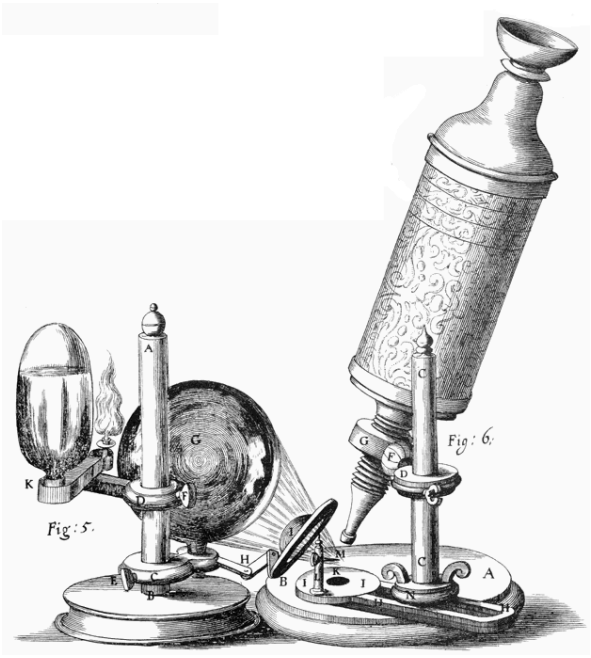
Try it and see. Good habits protect the microscope and make you faster. Carry it upright with two hands, one on the **arm** and one under the **base**. Always start with the lowest-power objective clicked into place. Put the slide under the stage clips with the specimen over the hole. Watching from the side, use the **coarse focus knob** to bring the objective close to the slide, then look through the eyepiece and focus slowly *away* from the slide until the image appears. Center what you want to see, then turn the nosepiece to the next objective. On medium and high power use only the **fine focus knob**, because the high-power objective almost touches the slide and the coarse knob could crack it. Adjust the diaphragm: high power usually needs more light. You will notice that the image is upside down and backward, and that when you push the slide to the left, the image moves to the right. Clean lenses only with lens paper.

God’s handiwork. Every part of the microscope has a partner in your own eye. The eye has a lens that focuses light, an iris that opens and closes like the diaphragm, and a retina that receives the image, and it focuses on its own, near and far, thousands of times a day. Inventors needed centuries to build a good microscope. The Psalmist asks, “He that formed the eye, shall he not see?” The One who made the eye sees perfectly what our best lenses only begin to show.



The parts of a compound light microscope. Light travels upward from the light source, through the specimen, the objective and the eyepiece to the eye.

PICTURE STUDY



Hooke's Microscope, from Micrographia, Robert Hooke, 1665 · Engraving (printed illustration in the book Micrographia) · Published in London by the printers to the Royal Society; copies survive in many rare-book libraries, and scans are free online.

Robert Hooke was the Royal Society's curator of experiments, the man whose job was to show the members something new at their meetings. In *Micrographia* (1665) he drew the instrument he used for all his tiny pictures. The same Hooke, twelve years later, would confirm Leeuwenhoek's animalcules.

Hooke was an excellent artist as well as a scientist. Everything in the book was drawn by hand while looking through the lens, then carefully engraved onto copper plates for printing. Samuel Pepys, the famous London diarist, wrote that he sat up late reading it and called it the most ingenious book he had ever read in his life.

Look closely

- Find the long tube of the microscope. Where would the eye go, and where would the specimen go?
- Find the oil lamp and the glass globe. Trace with your finger the path light would take from the flame to the specimen.
- Compare this microscope to the diagram of a modern one. Which parts can you match? Which parts are missing?
- Look at the decoration on the instrument. Why might a maker in 1665 have made a scientific tool beautiful?

Talk about it

1. Hooke had no electric light. Why do you think lighting was the hardest problem for early microscope users?
2. A modern microscope has a diaphragm and a built-in lamp. Which part of Hooke's setup did each of these replace?

> **Safety first.** Carry the microscope with two hands. Glass slides and coverslips can break; hold them by the edges. Never focus the coarse knob toward the slide while looking through the eyepiece on high power. A built-in lamp can get warm. If your microscope has a mirror instead of a lamp, never aim it at the sun: reflected sunlight through the lenses can badly hurt your eyes. Use a desk lamp or a bright window instead.

EXPERIMENT: MEASURING THE FIELD OF VIEW

Your question: **How does the width of the field of view change as the magnification goes up?** Write a prediction first: if magnification goes up 10 times, what happens to the width you can see?

What we change: the objective lens (4×, 10×, 40×). What we keep the same: the eyepiece, the slide, the light setting as far as possible. What we measure: the diameter of the field of view in millimeters, and how many letters of print fit across it.

You will need

- Compound microscope with 4×, 10× and 40× objectives and a 10× eyepiece
- A clear plastic metric ruler with millimeter marks (thin enough to fit on the stage), or a strip of millimeter graph paper
- A slide made from a small piece of newspaper with the letter "e" (see the tips), and a coverslip
- Lab notebook, pencil and colored pencils

Steps

1. Carry the microscope to the table with two hands and plug it in with the cord out of the way.
2. Click the 4× objective into place. Lay the ruler on the stage so its millimeter marks cross the middle of the hole.
3. Focus with the coarse knob, then the fine knob. Line one millimeter mark up with the left edge of the circle and count how many millimeters fit across. Estimate to the nearest half millimeter. Record it as the field of view at 40× total.
4. Calculate total magnification for each objective and write it in a table with columns Objective, Total magnification, Field of view (mm), Field of view (µm).
5. You cannot measure the high-power field well with a ruler, so calculate it: multiply your 40× field by 40 and divide by the new total magnification. Fill in the table.
6. Now place the letter "e" slide on the stage right side up. Focus at 40×. Draw what you see. Which way is the "e" facing?

7. While looking, move the slide slowly to the left, then away from you. Record which way the image moves.
8. Switch to 100× and 400×, using only the fine knob. Draw the “e” at each power the size it fills the circle. Note how much of the letter you can see and whether the view gets brighter or darker.
9. Write a conclusion: What happened to the field of view as the magnification went up? Was the relationship what you predicted?

Tips

- To make the “e” slide, cut out one small lowercase “e” from a newspaper, lay it right side up on the slide, add one drop of water and a coverslip.
- If you see nothing at all, go back to the lowest power, make sure the slide is centered over the light, and open the diaphragm.
- A dark circle with a bright rim in the view is usually an air bubble, not a cell.

Check your work

- I can carry, set up and focus a microscope starting on low power.
- I measured the field of view at 40× and calculated it at 100× and 400×.
- I can explain why the “e” looks upside down and backward and which way the image moves.

NATURE NOTEBOOK

1. Draw a large, labeled side view of your own microscope from life (not from the diagram). Label at least ten parts and draw an arrow for the path light takes.
2. Draw three circles the same size. In each, draw how the newspaper “e” looked at 40×, 100× and 400×, and write the total magnification and field of view under each.
3. Write the microscope rules you learned as a short checklist titled “Before I Focus,” and tape a copy near the place you keep the microscope.

> **From history.** Ernst Abbe did more than solve the mathematics of the microscope. When Carl Zeiss died in 1888, Abbe set up the Carl Zeiss Foundation in 1889 to own the company. It was known for treating its workers well, with an eight-hour workday, paid holidays and a pension, at a time when such things were rare. Zeiss lenses were soon used in many of the great laboratories of Europe, including those of scientists studying the germs you will meet later in this course.

WORKED EXAMPLES

Example 1. A microscope has a 10× eyepiece and objectives of 4×, 10× and 40×. Find the total magnification with each objective.

(Use the map or drawing "The eyepiece and the objective in use multiply together." shown earlier in this lesson.)

1. Total magnification = eyepiece × objective.
2. Low power: 10 × 4 = 40×.
3. Medium power: 10 × 10 = 100×.
4. High power: 10 × 40 = 400×.

Answer: 40×, 100× and 400×.

Example 2. At 40× a student measures the field of view as 4.5 mm across. What is the field of view at 400×, in millimeters and micrometers?

1. Field of view gets smaller in the same proportion that magnification gets larger.
2. The magnification increases by 400 ÷ 40 = 10 times.
3. So the field of view is 10 times smaller: 4.5 mm ÷ 10 = 0.45 mm.
4. Convert: 0.45 mm × 1,000 = 450 µm.
5. Check: field × magnification should stay the same. 4.5 × 40 = 180 and 0.45 × 400 = 180.

Answer: 0.45 mm, or 450 µm.

Example 3. At 400× (field of view 450 µm), a student sees cheek cells lying edge to edge straight across the middle of the circle. About 9 cells fit across. Estimate the width of one cheek cell.

1. Size of one cell ≈ field of view ÷ number of cells across.
2. 450 µm ÷ 9 = 50 µm.
3. Convert if needed: 50 µm = 0.05 mm.

Answer: About 50 µm (0.05 mm) across. (Real cheek cells are roughly 50 to 60 µm, so the estimate is reasonable.)

PRACTICE

1. EASY Which knob should you use to focus when the 40× objective is in place, and why?
2. EASY What does the diaphragm do?

3. EASY A microscope has a 15× eyepiece and a 40× objective. What is the total magnification?
4. MEDIUM Put these steps in the right order: (A) switch to the 10× objective; (B) center the specimen; (C) click the 4× objective into place; (D) focus with the coarse knob; (E) place the slide under the stage clips; (F) sharpen with the fine knob.
5. MEDIUM Looking through a microscope, you see a paramecium near the lower-left edge of the field. Which way should you move the slide to bring it to the center?
6. MEDIUM A student recorded these results with the same slide and the same diaphragm setting:
   Total magnification 40× · field of view 4.5 mm · brightness: very bright · onion cells seen across: 15
   Total magnification 100× · field of view 1.8 mm · brightness: bright · cells across: 6
   Total magnification 400× · field of view 0.45 mm · brightness: dim · cells across: 1.5
   (a) What happens to brightness as magnification increases? (b) Use the 100× row to estimate the length of one onion cell. (c) Do the three rows agree?
7. MEDIUM Graph-reading: A student plots field of view (y-axis) against total magnification (x-axis) for 40×, 100× and 400×. Which description fits the graph: (a) a straight line rising to the right; (b) a straight line falling to the right; (c) a curve that falls steeply at first and then flattens? Explain.
8. HARD Why does making the image bigger not always let you see more detail? Use the words magnification and resolution.
9. HARD At 100× the field of view is 1.8 mm. A water flea (Daphnia) stretches across about two thirds of the field. About how long is it in millimeters? Could you see it without a microscope?
10. CHALLENGE Design an experiment to test whether the diaphragm setting changes how many details a student can see in an unstained onion skin at 100×. Name the independent and dependent variables, what you would keep the same, and how you would make the result fair.

COMMON MISTAKES

- Starting on high power. The field of view at 400× is so small that it is very hard to find anything. Always find and center the specimen on low power first.
- Thinking the image moves the same way the slide moves. In a compound microscope the image is upside down and backward, so it moves the opposite way.
- Thinking higher magnification always means a better view. Higher power shows a smaller area, is dimmer, and cannot show detail finer than the resolution of the lens allows.

> **Make it a game · Name That Part.** One player points to a part of the microscope (or the diagram) and the other must name it and tell its job in one sentence within five seconds: “Diaphragm: controls the light.” Then switch to math cards: “10× eyepiece, 40× objective?” “400×!” or “Field is 2 mm at 100×; what at 400×?” “0.5 mm!” Score one point for each correct answer and play to fifteen.

> **Scripture.** “He that planted the ear, shall he not hear? he that formed the eye, shall he not see?” (Psalm 94:9, KJV)

The best microscope ever built copies ideas already found in the human eye: a lens, an iris and a screen for the image. The God who formed the eye sees all things perfectly.

Practice answers

1. The fine focus knob only. The high-power objective is very close to the slide, and the coarse knob moves the stage so far that it could crash the lens into the slide and break it.
2. It controls how much light passes up through the specimen. Opening it makes the view brighter; closing it makes it dimmer but can increase contrast for clear, pale specimens.
3. 15 × 40 = 600×.
4. C, E, D, B, A, F. (Start on low power, place the slide, focus with coarse, center, switch to the next objective, then use only the fine knob.)
5. Move the slide toward the lower left. The image moves opposite to the slide, so to move the image up and to the right, toward the center, you push the slide down and to the left.
6. (a) The view gets dimmer as magnification increases. (b) 1.8 mm ÷ 6 = 0.3 mm = 300 µm. (c) Yes: 4.5 ÷ 15 = 0.3 mm and 0.45 ÷ 1.5 = 0.3 mm, so all three give about 300 µm.
7. (c). Field of view is inversely proportional to magnification (field × magnification stays the same), so it drops from 4.5 mm to 1.8 mm between 40× and 100×, then only to 0.45 mm by 400×. An inverse relationship gives a curve that falls quickly and then levels off.
8. Magnification only makes the image larger. Resolution is the ability to show two close points as separate. If the lens cannot resolve two points, magnifying more just makes a larger blur (“empty magnification”). A light microscope cannot separate points closer than about 0.2 µm, no matter how much the image is enlarged.
9. Two thirds of 1.8 mm = 1.2 mm. Yes, 1.2 mm is much larger than the 0.1 mm the eye can make out, so it can be seen as a tiny moving speck without a microscope, though not in detail.
10. Independent variable: the diaphragm setting (for example fully open, half open, nearly closed). Dependent variable: a count of a detail you can see, such as the number of cells in the field whose nucleus is visible. Keep the same: the slide and spot on it, the objective (100×), the lamp brightness, the focus method and the observer. To be fair, test each setting three times in mixed order, have a second person count without knowing the setting, and average the counts.

1.3 Making Slides

OBJECTIVES

- Prepare a wet mount and a dry mount without trapping air bubbles, and tell when to use each.
- Stain a specimen by drawing stain under the coverslip, and explain why stains help us see cell parts.
- Use ratios to mix a dilute stain and use the field of view to measure cells on your own slides.

Warm-up. Hold a slice of bread, a leaf and a sheet of tissue paper in turn in front of a flashlight or a bright window. Which lets light through? Now tear the thinnest edge you can from the bread and the leaf and try again. Write one sentence: what must a specimen be like for light to pass through it into a microscope?

Video. Coming soon. How to make a wet mount slide and stain onion and cheek cells (lowering the coverslip at an angle, drawing iodine or methylene blue under the coverslip): search university biology lab channels, Amoeba Sisters, microscope makers' education pages and homeschool science channels.

KEY TERMS

specimen
The thing being studied under the microscope, such as a piece of onion skin or a drop of pond water.

wet mount
A slide on which the specimen sits in a drop of water (or stain) under a coverslip. Used for living things and soft tissue.

dry mount
A slide on which a dry specimen, such as a hair, a feather barb or salt grains, is placed under a coverslip without water.

coverslip
A very thin square of glass or plastic laid over the specimen to flatten it, keep it from drying and protect the objective lens.

stain
A dye that colors some cell parts more than others so they stand out. Iodine and methylene blue are common stains.

prepared slide
A permanent slide, usually of a very thin, stained slice of tissue sealed under a coverslip, made to last for years.

CORE IDEAS

The story. In the 1660s and 1670s, in Amsterdam, a young Dutchman trained as a doctor, Jan Swammerdam, spent long days bent over a lens, taking apart insects. He did not have modern slides or stains. He made his own tiny scissors, knives and lancets, so small that he sharpened them while looking through a magnifier, and he used fine glass tubes to blow air into the delicate vessels of insects so he could follow them. With this patient work he showed that the wings and legs of a butterfly are already forming, folded up, inside the caterpillar before it becomes a chrysalis, and that the large “king” bee of the hive is really a queen, the mother of the colony. His work showed something every microscope user learns quickly: what you see depends on how carefully you **prepare** what you look at.

How they figured it out. Leeuwenhoek glued dry specimens onto the point of his pin and sealed liquids inside thin glass tubes. In the 1700s many microscope owners used “sliders”: thin strips of ivory or bone with round holes, each holding a specimen between two little discs of clear mica. The slider slid through a slot in the microscope, which is where our word **slide** comes from. Glass slides took over in the 1800s, and in 1840 the Microscopical Society of London adopted a standard size of 3 inches by 1 inch (about 76 mm by 26 mm). Slides are still made that size today, so a slide prepared in one country fits a microscope in any other. Workers learned to seal thin slices under glass with Canada balsam, a clear resin from the balsam fir, making **prepared slides** that last for generations.

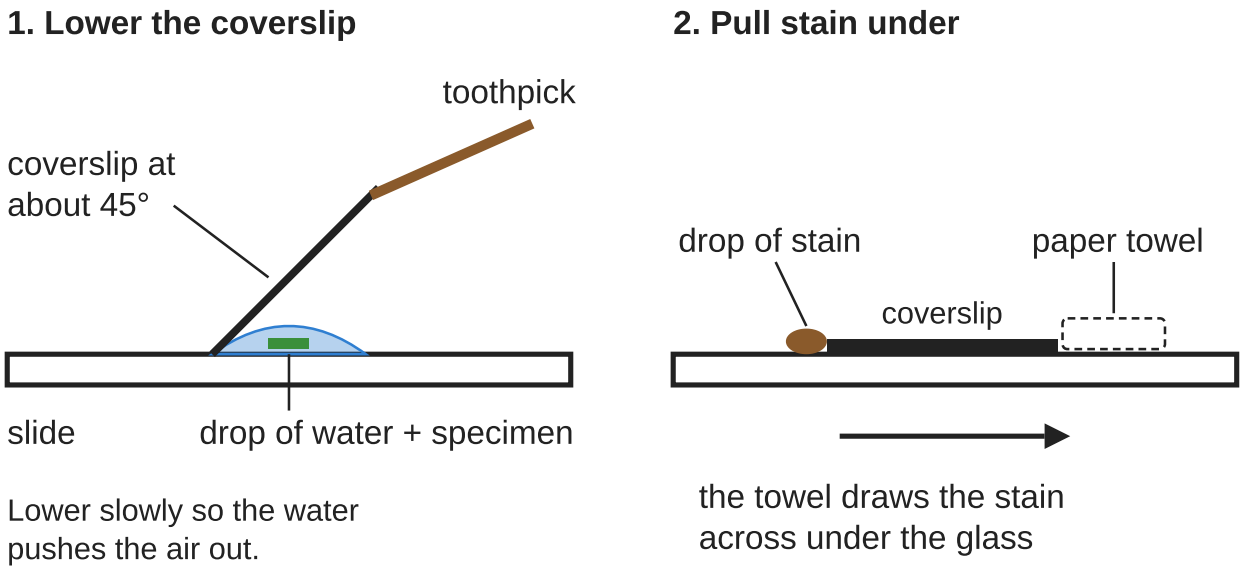
The big idea. A light microscope shines light *through* the specimen, so a good specimen must be thin, flat and clear, one layer of cells if possible. A thick chunk just looks black. For living things and soft tissue we make a **wet mount**: a drop of water, the specimen, then a **coverslip**. The coverslip flattens the specimen, keeps it from drying out and keeps the lens clean. For dry things like hair, salt or a feather barb, a **dry mount** is enough. Many cells are nearly colorless, so we add a **stain**. Iodine turns starch blue-black and makes the nucleus of an onion cell stand out as a darker dot. Methylene blue colors the nucleus of an animal cell, such as a cheek cell, deep blue. Each stain sticks more to some parts of the cell than others, and that difference is what we see.

Try it and see. To make a wet mount, place one drop of water in the middle of a clean slide. Lay the specimen flat in the drop. Hold the coverslip by its edges, touch one edge to the slide beside the drop at about a 45-degree angle, and lower it slowly with a toothpick so the water spreads out and pushes the air ahead of it (see the diagram). Bubbles appear as perfect circles with thick dark rims; they are not cells. To stain a slide that is already made, put a drop of stain touching one edge of the coverslip and hold a torn corner of paper towel against the opposite edge. The towel soaks up water and pulls the stain across under the glass.

In the world today. Hospital laboratories still prepare slides every day. A drop of blood is smeared thin and stained so that a technician can count the different blood cells. In 1884 a Danish doctor, Hans Christian Gram, invented a stain that colors some kinds of bacteria purple and leaves others pink; the Gram stain is still one of the first tests used to identify bacteria. Doctors who study diseased tissue slice it thinner than a hair with a machine called

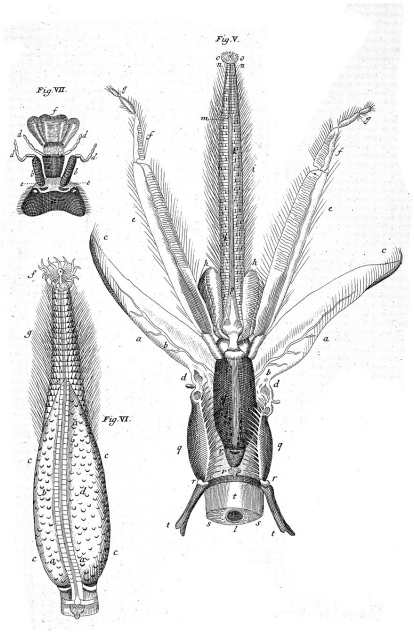
a microtome and stain it before looking. The skills are the same ones you practice at the kitchen table: thin, flat, clear and carefully stained.

God's handiwork. Swammerdam was a man of deep faith who saw the work of God in the smallest creatures. He wrote that in the anatomy of a tiny louse he could see the very finger of God. Careful slide-making is a lesson in faithfulness in small things: a careless coverslip hides the beauty that a patient one reveals.



Making a wet mount (left) and drawing a stain under the coverslip (right).

PICTURE STUDY



Mouthparts of the Honeybee and the Wasp, from Biblia Naturae, Jan Swammerdam (drawings), engraved for printing, 1737–1738 (published after Swammerdam's death in 1680) · Copperplate engraving in the book Biblia Naturae ("The Bible of Nature") · Published in Leiden, edited by Herman Boerhaave; copies survive in many rare-book libraries, and scans are free online.

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Jan Swammerdam died in 1680, at only 43, and most of his finest work was never printed in his lifetime. Nearly sixty years later the famous Leiden physician Herman Boerhaave bought his papers and published them in Dutch and Latin as *Biblia Naturae*, "The Bible of Nature." The plates of the honeybee are among the most famous: Swammerdam dissected bees under the microscope and drew their mouthparts, sting, eyes and inner organs.

Look closely and you will see that each figure is numbered and each part is marked with a small letter that is explained in the text. Scientific drawings still use that method today.

Look closely

- Count the separate figures on the plate. Why would an artist draw one animal in so many pieces?
- Find the small letters beside the parts. How do they help a reader who was not there to see the dissection?
- Choose one figure and guess which part of the bee it shows. What clues helped you?
- Notice the fine lines used for shading. How did the engraver show that a part is round or hollow without using color?

Talk about it

1. Swammerdam had to prepare each tiny part before he could draw it. What would happen to a dissected bee part if it dried out under the lens?

2. Why do you think Swammerdam's editor chose the title "The Bible of Nature"?

> **Safety first.** Ask an adult to cut the onion; knives and broken glass are sharp. Iodine and methylene blue are poisonous if swallowed and stain skin, clothes and counters: wear old clothes and safety glasses, work on newspaper, keep the bottles capped and away from younger children, and wash your hands when done. Scrape only the inside of your own cheek, gently, with the flat side of a clean toothpick, and throw the toothpick away at once. Never share toothpicks.

EXPERIMENT: DOES A STAIN HELP? ONION AND CHEEK CELLS

Your question: **Does staining make the nucleus easier to see?** Write a prediction: in an unstained and a stained onion slide, in what fraction of cells will you be able to see the nucleus?

What we change: stain or no stain. What we keep the same: the onion layer, the size of the piece, the magnification (100×), the light setting and the person counting. What we measure: out of 20 cells, how many show a clear nucleus.

You will need

- Compound microscope, glass slides, coverslips, a dropper and toothpicks
- An onion, a cutting board and a knife (adult use)
- Tweezers
- Lugol's iodine from a science supplier, or tincture of iodine from a pharmacy mixed 1 part to 4 parts water
- Methylene blue (sold at pet stores for aquariums) or iodine for the cheek slide
- Paper towels, newspaper, safety glasses, lab notebook

Steps

1. Have an adult cut the onion into quarters. Peel one thick layer and snap it backward; a thin, clear skin will peel off the inner side. Use tweezers to pull off a piece about 1 cm square.
2. Make a wet mount with plain water. Lay the skin flat without folds, lower the coverslip at an angle and check for bubbles at 40×.
3. At 100×, look at 20 cells in a row. Tally how many show a clear nucleus. Record in a table with columns Slide, Cells checked, Nucleus visible, Percent.
4. Now stain the same slide: put one drop of diluted iodine at one edge of the coverslip and draw it through with a paper towel on the other side. Wait one minute.
5. Count 20 cells again in the same area. Calculate each percent: (nucleus visible ÷ 20) × 100.
6. Draw three stained onion cells at 400×. Label the cell wall, the cytoplasm and the nucleus.

7. Make a cheek slide: put a drop of methylene blue (or iodine) on a new slide, gently scrape the inside of your cheek with a flat toothpick, swirl the toothpick in the drop and add a coverslip. Find the scattered, flat cells at 100× and draw one at 400×.
8. Make a dry mount of a hair or a few grains of salt and draw it.
9. Write a conclusion: Did the stain help? By how many percentage points? How do the onion cells differ in shape from the cheek cells?

Tips

- If the onion skin folds over, start again with a smaller piece. One layer is the goal.
- Too much stain makes everything dark. Draw a little more plain water through to rinse it.
- Cheek cells are flat and clear and drift alone or in small clumps. Look for them at low power first.

Check your work

- I made a wet mount with no large bubbles and a dry mount.
- I stained a slide by drawing stain under the coverslip.
- I counted, calculated percents and compared stained with unstained cells.

NATURE NOTEBOOK

1. Draw the four steps of making a wet mount as a comic strip of four boxes, with one sentence under each.
2. Draw one stained onion cell and one stained cheek cell side by side at the same scale. Label each and write two differences you see.
3. Draw an air bubble as it looks under the microscope and write a note to your future self: “How to tell a bubble from a cell.”

> **From history.** Swammerdam’s discovery that the large bee in the hive is a female, the mother of all the others, overturned an idea that had been taught since ancient times, when writers called the leader of the hive a “king.” When he dissected her under the microscope, he found her ovaries full of eggs. Shakespeare, writing before Swammerdam, still calls the leader of the bees a king in his play *Henry V*.

WORKED EXAMPLES

Example 1. A stain recipe says to mix 1 part iodine tincture with 4 parts water. How much of each do you need to make 10 mL of stain? What fraction of the mixture is iodine tincture?

1. Total parts = 1 + 4 = 5 parts.
2. Each part = 10 mL ÷ 5 = 2 mL.
3. Iodine tincture = 1 × 2 mL = 2 mL. Water = 4 × 2 mL = 8 mL.
4. Check: 2 mL + 8 mL = 10 mL. Fraction of iodine tincture = 1/5 = 20%.

Answer: 2 mL of iodine tincture and 8 mL of water; the mixture is 1/5, or 20%, iodine tincture.

Example 2. At 100× the field of view is 1.8 mm across. A student sees 5 onion cells lying end to end across the middle of the field. Estimate the length of one onion cell in millimeters and micrometers.

(Use the map or drawing "A flat, single layer of onion skin under a coverslip makes cells easy to count a" shown earlier in this lesson.)

1. Length of one cell ≈ field of view ÷ number of cells.
2. 1.8 mm ÷ 5 = 0.36 mm.
3. Convert: 0.36 mm × 1,000 = 360 µm.

Answer: About 0.36 mm, or 360 µm, long.

Example 3. A microtome cuts tissue into slices 10 µm thick for prepared slides. How many slices can be cut from a piece of tissue 2 mm thick?

1. Put both in the same unit: 2 mm × 1,000 = 2,000 µm.
2. Number of slices = 2,000 µm ÷ 10 µm = 200.

Answer: 200 slices.

PRACTICE

1. EASY Why must a specimen for a light microscope be thin?
2. EASY Would you use a wet mount or a dry mount for (a) pond water, (b) a strand of hair, (c) onion skin?
3. EASY Give two jobs of the coverslip.
4. MEDIUM A student sees several perfect circles with thick black rims and bright centers on a wet mount. What are they, and how could the student avoid them next time?

5. MEDIUM Methylene blue stock is diluted 1 part to 9 parts water. How much stock is in 20 mL of the diluted stain?
6. MEDIUM A class tested a stain on onion skin. Each student checked 20 cells.
   Unstained slides: nucleus visible in 4, 6, 5 and 5 cells.
   Stained slides: nucleus visible in 17, 18, 19 and 18 cells.
   (a) Find the average number for each group. (b) Express each average as a percent of 20 cells. (c) What can the class conclude?
7. MEDIUM Data-reading: Look again at the results in problem 6. (a) What is the range (highest minus lowest) in each group? (b) Do any unstained results overlap with any stained results? (c) What do your answers say about how much to trust the conclusion?
8. HARD Where does the word “slide” come from, and why was it useful for scientists to agree on a standard slide size in 1840?
9. HARD At 400× the field of view is 0.45 mm. A stained cheek cell fills one ninth of the field’s width. About how wide is the cell in micrometers? Is this larger or smaller than the onion cell length of 360 µm, and by about how many times?
10. CHALLENGE Design an experiment to find out which works better on onion skin: iodine or food coloring. Name the independent variable, the dependent variable, what you keep the same and how many trials you would run.

COMMON MISTAKES

- Using a piece of specimen that is too thick. A thick piece looks dark and blurry at any power; one thin layer of cells is best.
- Mistaking air bubbles for cells. Bubbles are perfect circles with thick dark rims that change as you focus; cells have walls or membranes, contents and often a nucleus.
- Thinking a stain shows the true colors of a cell. Most cells are nearly colorless; the stain is a dye added by the scientist to make certain parts stand out.

> **Make it a game · Mystery Slide.** One player secretly makes a dry or wet mount of something from the kitchen or yard: salt, sugar, a hair, a thread, a feather barb, a flake of pepper, a bit of leaf. The other player may look at 40× and 100× and ask three yes-or-no questions before guessing. A correct guess on the first look scores 3 points, after one question 2 points, after three questions 1 point. Then switch. Bonus point for naming a correct difference between salt and sugar crystals.

> **Scripture.** “He that is faithful in that which is least is faithful also in much: and he that is unjust in the least is unjust also in much.” (Luke 16:10, KJV)

Preparing a slide is a small job, but doing it carefully is what makes the whole observation trustworthy. Faithfulness in small tasks builds the habits of a good scientist.

Practice answers

1. Light has to pass through the specimen to reach the lenses. A thick specimen blocks the light and looks dark, and its layers of cells pile on top of each other.

2. (a) Wet mount (it is already liquid with living things). (b) Dry mount. (c) Wet mount (soft tissue that would dry and wrinkle).
3. Any two: it flattens the specimen into one even layer; it keeps the specimen from drying out; it keeps liquid off the objective lens; it holds the specimen still.
4. They are air bubbles. Lower the coverslip at an angle, touching one edge to the drop first and letting it down slowly, so the water pushes the air out ahead of it.
5. Total parts = 10. Each part = 20 ÷ 10 = 2 mL. Stock = 2 mL (and water = 18 mL).
6. (a) Unstained: (4 + 6 + 5 + 5) ÷ 4 = 5. Stained: (17 + 18 + 19 + 18) ÷ 4 = 18. (b) Unstained: 5 ÷ 20 = 25%. Stained: 18 ÷ 20 = 90%. (c) Staining made the nucleus visible in far more cells (about 90% compared with 25%), and all four students got similar results, so the stain clearly helps.
7. (a) Unstained: 6 − 4 = 2. Stained: 19 − 17 = 2. (b) No. The highest unstained result (6) is far below the lowest stained result (17). (c) Each group’s results are close together and the groups are far apart, so the difference is much larger than the normal variation between students. The conclusion that the stain helps can be trusted.
8. From the 1700s “sliders,” strips of ivory or bone holding specimens that slid through a slot in the microscope. A standard size (3 by 1 inches, about 76 by 26 mm) meant any slide would fit any microscope stage and storage box, so scientists could trade and send slides to one another.
9. 0.45 mm ÷ 9 = 0.05 mm = 50 µm. It is smaller: 360 ÷ 50 = 7.2, so the onion cell is about 7 times longer.
10. Independent variable: the kind of stain (iodine, blue food coloring, and plain water as a control). Dependent variable: the number of cells out of 20 with a clearly visible nucleus at 100×. Keep the same: onion layer and piece size, amount of stain, waiting time (for example one minute), magnification, light setting and the person counting. Run at least three slides of each and average the results; ideally the counter should not know which stain is on which slide.

1.4 Pond-Water Drawings

OBJECTIVES

- Collect pond water safely and find, identify and describe at least four kinds of pond microorganisms.
- Make a scientific drawing that follows the rules: clean lines, ruled labels, a title, the magnification and a scale bar.
- Calculate drawing magnification and scale-bar length, and estimate how many organisms live in a milliliter of water.

Warm-up. Look at a leaf, a coin or a key for one full minute. Then put it out of sight and draw it from memory. Now bring it back and draw it again while looking. Compare the two drawings. What details did you miss the first time? Write one sentence about why scientists draw from life and not from memory.

Video. Coming soon. Pond water under the microscope (paramecium, amoeba, euglena, volvox, rotifers, water fleas, diatoms) and how to make a biological drawing: search Journey to the Microcosmos, nature and microscopy footage channels, university biology lab channels and homeschool science channels.

KEY TERMS

protist
A single-celled (or simple many-celled) living thing with a nucleus that is not a plant, animal or fungus. Paramecium, amoeba and euglena are protists.

cilia
Many short, hair-like structures that beat together to move a cell or sweep food toward it. A paramecium is covered with them.

flagellum
A long, whip-like structure that a cell lashes to swim. Euglena has one (plural: flagella).

pseudopod
“False foot”: a bulge of cytoplasm that an amoeba pushes out to creep along and to surround its food.

biological drawing
A clear, accurate pencil drawing of a specimen made from life, with ruled label lines, a title, the magnification and a scale.

scale bar
A short line on a drawing marked with the real length it stands for, such as 50 µm, so a reader can measure the specimen.

CORE IDEAS

The story. More than a century after Leeuwenhoek, a Danish naturalist named Otto Friedrich Müller spent years drawing the tiny creatures of ponds, ditches and the sea around Copenhagen. He was one of the first to try to sort them into kinds and give them names, as Linnaeus had done for plants and animals. Müller died in 1784 before his great book was finished, and it was published two years later, in 1786, with the title *Animalcula Infusoria*. It was filled with plates of his careful drawings. Because his drawings were accurate, later scientists could look at a creature in their own drop of water, compare it to Müller's plates and know which creature he meant.

How they figured it out. In 1838 the German scientist Christian Gottfried Ehrenberg published an enormous book on the same tiny creatures, with hundreds of beautiful drawings. His drawings were excellent, but some of his explanations were wrong. He believed that every animalcule had a stomach, a gut and other organs like a large animal's, and he drew the round food bubbles inside them as many little stomachs. Other microscopists soon showed that those bubbles were food vacuoles inside a single cell. Ehrenberg's story teaches an important lesson: **what you see** and **what you think it means** are two different things. A good drawing records only what is seen, so that others can check it even if the explanation turns out to be wrong.

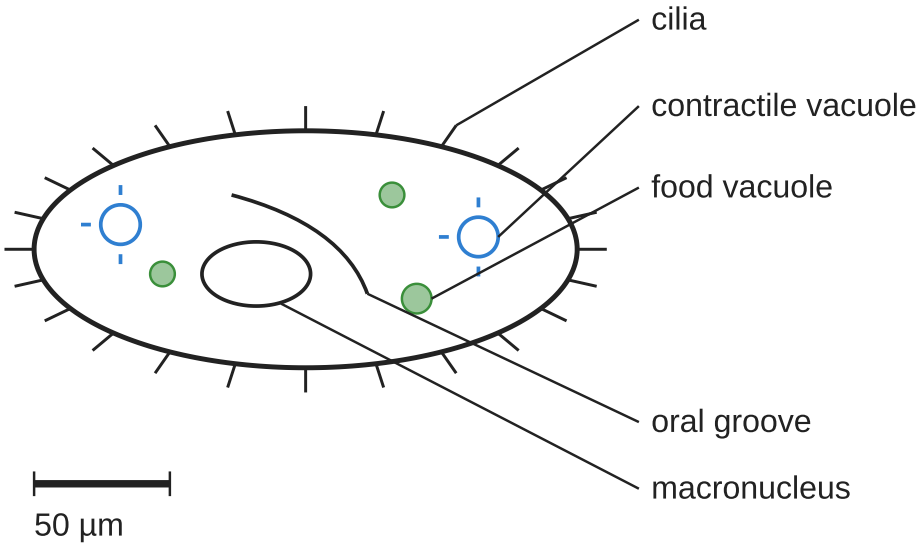
The big idea. A **biological drawing** follows simple rules so that anyone can read it. Use a sharp pencil and draw large, at least half a page. Draw single, clean outlines without sketchy strokes, and use dots (stippling) instead of shading. Draw only what you actually see, in the right proportions. Rule straight label lines with a ruler; they should never cross, and the labels should line up at one side. Give the drawing a title, the date, where the sample came from and the magnification. Add a **scale bar**: a short line marked with the real length it stands for. The drawing's own magnification is drawing size ÷ real size, so a paramecium 0.2 mm long drawn 80 mm long is drawn at 400×.

Who lives in a pond. A spoonful of pond water can hold many kinds of living things. A **paramecium** (about 0.2 mm long) is shaped like a slipper and glides and spins by beating thousands of **cilia**. An **amoeba** creeps slowly by pushing out **pseudopods** and wraps them around its food. A **euglena** (about 0.05 mm) is green with chloroplasts, has a red eyespot, swims with a **flagellum** and moves toward light. **Volvox** is a hollow green ball made of hundreds or thousands of cells, sometimes large enough to see as a green dot. **Diatoms** are single cells inside glassy shells shaped like boats, discs or stars. You may also find tiny many-celled animals: **rotifers**, with a crown of beating cilia that looks like spinning wheels, and **water fleas** (*Daphnia*), small crustaceans 1 to 5 mm long whose beating hearts you can watch through their clear shells.

In the world today. Biologists still count and draw the tiny living things in ponds and streams. The kinds and numbers present tell them about the health of the water, because some creatures need clean water rich in oxygen while others can live in muddy, still water. Wildlife managers, fish farmers and water-treatment workers all use these surveys to care for the water God has entrusted to us.

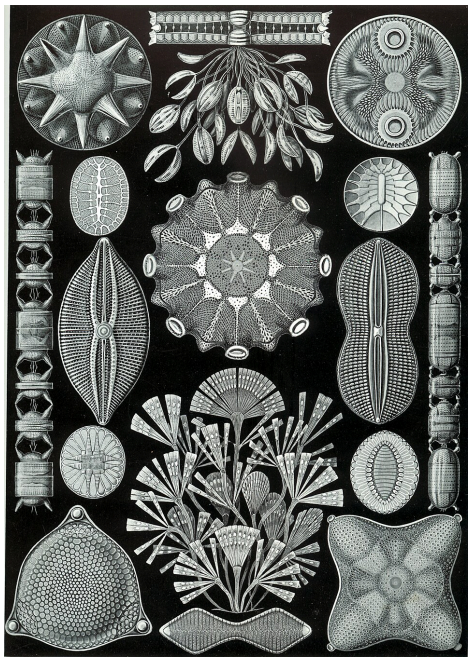
God’s handiwork. Long before anyone had a microscope, the Psalmist looked at the sea and praised God for “things creeping innumerable, both small and great beasts.” A pond in your own neighborhood is a small sea of that kind. Every creature in it was made after its kind, with its own way of moving, feeding and living, and drawing them carefully is a way of paying attention to what God has made.

Paramecium, ×400 (pond water, surface sample)



A model biological drawing: single clean outlines, ruled label lines that do not cross, a title with the magnification and a scale bar.

PICTURE STUDY



Diatomea, Plate 4 from Kunstformen der Natur, Ernst Haeckel (drawings); lithograph by Adolf Giltsch, 1899–1904 (published in parts) · Color lithograph · From the book Kunstformen der Natur (“Art Forms in Nature”), published in Leipzig and Vienna by the Bibliographisches Institut; copies in many libraries, and scans are free online.

Ernst Haeckel was a German zoologist who spent much of his life studying and drawing tiny sea creatures under the microscope. His book *Art Forms in Nature* was printed in parts from 1899 to 1904 and contains one hundred plates. Haeckel’s ideas about the origin of living things were much argued over in his own day, and some of his drawings of embryos were later shown to be inaccurate, but his plates of microscopic creatures are still admired for their beauty.

This plate shows diatoms, single cells that live inside two glassy shells fitting together like the top and bottom of a pillbox. Every kind of diatom has its own shell pattern of tiny holes and ridges, so precise that people once used diatom shells to test the quality of microscope lenses.

Look closely

- Find a diatom shaped like a disc, one shaped like a boat and one shaped like a star.
- Look at the patterns of dots and lines on the shells. Are they regular or random?
- Notice how Haeckel arranged the diatoms on the page. Is this how they would look in a drop of water? Why might he have arranged them this way?
- A diatom looks different from the top than from the side, like a pillbox. Can you find shapes that might be the same kind of diatom seen from two sides? Why would a scientist draw both views?

Talk about it

1. Haeckel made his drawings beautiful. Your drawings should be accurate first. What is one rule of biological drawing that Haeckel's decorated page does not follow?
2. If you find a diatom in your pond water, how will its shell help you tell which kind it is?

> **Safety first.** Collect pond water only with an adult beside you, and stay back from steep or slippery banks; never go out on ice. Pond water can carry germs: do not drink it, cover any cuts, wear gloves if you can, keep it away from food and wash your hands well after collecting and after every look. Handle slides by the edges. When you are done, return living samples to the pond or pour them outdoors on the ground.

EXPERIMENT: POND SURVEY: TOP, MIDDLE AND BOTTOM

Your question: **Do different kinds and numbers of tiny creatures live at the surface, in the open water and in the bottom muck of the same pond?** Write a prediction: which sample will hold the most living things?

What we change: where the sample is taken. What we keep the same: the pond, the day, the jar size, the drop size, the magnification and the number of drops checked. What we measure: how many organisms of each kind are found in three drops from each sample.

You will need

- Three clean jars with lids, labeled Surface, Middle and Bottom
- A long-handled ladle, or a cup taped to a stick
- Compound microscope, slides, coverslips and a dropper
- A small piece of cotton fibers or a drop of thick clear gel (optional, to slow fast swimmers)
- Sharp pencil, ruler, eraser and plain drawing paper
- Gloves, paper towels and soap

Steps

1. With an adult, visit a pond, ditch or slow stream. Skim the Surface jar along the top, including a bit of floating green scum. Hold the Middle jar under water about 20 cm down. For the Bottom jar, scoop gently along the bottom near a plant to get a little muck and water.
2. Leave the lids loose and keep the jars cool and out of direct sun. Look at them within a day.
3. Make a wet mount from one drop of the Surface jar. Scan the whole coverslip at 40×, then look closer at 100× and 400×.
4. Use the identification notes from the lesson to name each creature you can. Tally every organism in a table with columns Sample, Paramecium, Amoeba, Euglena, Volvox, Diatoms, Rotifers, Water fleas, Other.
5. Check three drops from each jar the same way and add the tallies.

6. Choose the two most interesting creatures and make full biological drawings of each, following all the rules. Measure each creature with your field of view and add a scale bar.
7. Make a bar graph of the total organisms found in each sample.
8. Write a conclusion: Which sample held the most life? Which held the most kinds? Why might different creatures live in different places in the same pond?

Tips

- Fast swimmers like paramecia can be slowed by adding a few cotton fibers to the drop, which give them a tangle to bump into.
- Look along the edges of bits of plant and muck; many creatures feed there.
- If a creature leaves the field, do not chase it at high power. Go back to 40×, find it and center it.
- Draw what you see, not what a textbook picture shows.

Check your work

- I collected three samples safely and checked three drops from each.
- I identified at least four kinds of organisms and recorded tallies in a table.
- My two drawings have a title, date, sample place, magnification, ruled labels and a scale bar.

NATURE NOTEBOOK

1. Make a full-page biological drawing of one pond creature, following every rule in the lesson. Ask someone to check it against the rules list.
2. Draw a “Field Guide Page” with small boxes for paramecium, amoeba, euglena, volvox and a diatom. In each, draw the creature and write one way to recognize it.
3. Draw a small map of the pond where you collected, marking where each sample came from, with notes on the weather, water color and any plants or animals you saw.

> **From history.** Ehrenberg did not only study ponds. He also collected dust that fell on ships at sea and soil from many lands, and he found it full of the glassy shells of diatoms. Some rocks and soils are made almost entirely of diatom shells; this fine white powder, called diatomaceous earth, is dug up today and used in water and swimming-pool filters and as a gentle polishing powder.

WORKED EXAMPLES

Example 1. A paramecium is 0.2 mm long. In your notebook you draw it 80 mm long. What is the magnification of your drawing?

(Use the map or drawing "A paramecium drawn at ×400." shown earlier in this lesson.)

1. Magnification = drawing size ÷ real size.
2. Both are in millimeters: 80 mm ÷ 0.2 mm = 400.
3. Write it under the drawing as "×400."

Answer: The drawing is magnified 400 times (×400).

Example 2. Your drawing is at ×400. How long should you draw a scale bar that stands for 50 µm?

1. Length on the drawing = real length × magnification.
2. 50 µm × 400 = 20,000 µm.
3. Convert: 20,000 µm ÷ 1,000 = 20 mm = 2 cm.

Answer: Draw the scale bar 20 mm (2 cm) long and label it "50 µm."

Example 3. A dropper makes drops of about 0.05 mL. A student counts all the paramecia under the coverslip in three drops of pond water and finds 6, 9 and 3. About how many paramecia are in 1 mL of that pond water?

1. Average per drop: (6 + 9 + 3) ÷ 3 = 18 ÷ 3 = 6.
2. Drops in 1 mL: 1 ÷ 0.05 = 20.
3. Paramecia per mL ≈ 6 × 20 = 120.

Answer: About 120 paramecia per milliliter.

PRACTICE

1. EASY Match each organism with how it moves: paramecium, amoeba, euglena. Choices: flagellum, cilia, pseudopods.
2. EASY List four rules of a good biological drawing.
3. EASY A green, oval cell with a red spot swims toward the window side of the drop, lashing a long whip. What is it most likely to be?
4. MEDIUM A euglena 50 µm long is drawn 30 mm long. What is the magnification of the drawing?
5. MEDIUM A student drew a water flea at ×20. The drawing is 50 mm long. How long is the real water flea?

6. MEDIUM A pond survey found these totals in three drops from each sample:
   Surface: Euglena 14, Volvox 6, Paramecium 3, Diatoms 5, Rotifers 1 (total 29)
   Middle: Euglena 2, Volvox 1, Paramecium 4, Diatoms 2, Rotifers 0 (total 9)
   Bottom: Euglena 1, Volvox 0, Paramecium 11, Diatoms 9, Rotifers 4 (total 25)
   (a) Which sample had the most organisms? (b) In which sample were green, light-using organisms (euglena and volvox) most common? (c) Suggest one reason.
7. MEDIUM Using the same table, what fraction of the bottom sample's organisms were paramecia? Which sample had the most rotifers?
8. HARD Ehrenberg drew the round food bubbles inside protists and called them stomachs. What part of his work was observation and what part was interpretation? Why is it still useful to have his drawings?
9. HARD Three drops of 0.05 mL each from a stream held 2, 0 and 4 rotifers. Estimate the number of rotifers in 1 liter of stream water. (1 L = 1,000 mL)
10. CHALLENGE Design an experiment to test whether euglena gather toward light. Describe the setup, the independent and dependent variables, what you keep the same and what result would support the idea.

COMMON MISTAKES

- Drawing what a textbook picture shows instead of what is on the slide. A scientific drawing records only what you actually observe.
- Thinking everything in pond water is a single cell. Rotifers and water fleas are tiny many-celled animals with mouths, guts and muscles.
- Leaving out the magnification or scale bar. Without them no one can tell whether the creature was 2 µm or 2 mm long.

> **Make it a game · Pond Creature Charades.** Write paramecium, amoeba, euglena, volvox, rotifer, water flea and diatom on slips of paper. One player draws a slip and acts out how that creature moves (spinning and gliding, oozing out a pseudopod, whipping a flagellum toward a flashlight, rolling slowly as a ball, hopping like a water flea) without speaking. The others guess. The guesser earns a point by naming one more true fact about it. Play until each creature has been acted twice.

> **Scripture.** "So is this great and wide sea, wherein are things creeping innumerable, both small and great beasts." (Psalm 104:25, KJV)

The Psalmist could not count the creatures of the sea, and we cannot count those in a single pond. A careful drawing is one way to give thanks for each kind God has made.

Practice answers

1. Paramecium: cilia. Amoeba: pseudopods. Euglena: flagellum.
2. Any four: use a sharp pencil; draw large; use single clean lines; use stippling instead of shading; draw only what you see, in proportion; rule straight label lines that do not cross; line up labels at the side; include a title, date, sample source and magnification; add a scale bar.

3. A euglena: it has chloroplasts (green), a red eyespot and a flagellum, and it moves toward light.
4. Convert 30 mm to 30,000 µm. 30,000 ÷ 50 = 600, so ×600.
5. Real size = drawing size ÷ magnification = 50 mm ÷ 20 = 2.5 mm.
6. (a) The surface sample, with 29. (b) The surface, with 20 of the 29 (14 + 6). (c) Euglena and volvox use light to make food, and the surface gets the most sunlight; euglena also swims toward light.
7. 11 out of 25 = 11/25 = 44%. The bottom sample had the most rotifers (4).
8. The observation was that there are round bubbles inside the cells, which he drew accurately. The interpretation was that they were stomachs, which turned out to be wrong; they are food vacuoles. Because his drawings honestly recorded what he saw, later scientists could use them to identify the same creatures and correct his explanation.
9. Average per drop = (2 + 0 + 4) ÷ 3 = 2. Drops per mL = 1 ÷ 0.05 = 20, so 2 × 20 = 40 per mL. Per liter: 40 × 1,000 = 40,000 rotifers.
10. Setup: fill two identical clear jars or test tubes with the same euglena-rich pond water. Wrap one half of each jar in foil (or cover half a dish with dark paper) and set them by the same window. Independent variable: light or dark half. Dependent variable: the number of euglena per drop taken from the lit side and from the dark side after a set time, such as 2 hours. Keep the same: the water, jar size, temperature, time and drop size. Count three drops from each side and average. Supporting result: clearly more euglena per drop on the lit side than on the dark side in both jars (and again if the foil is switched to the other side).

Unit 1 review

Answer each question. Show your work for calculations, include units, and use complete sentences for explanations. For multiple-choice questions, write the letter of the best answer. The test is worth 60 points.

1. (4 pts) Multiple choice. In his letter of 1676, Leeuwenhoek described tiny living creatures he found in (a) a slice of cork; (b) water in which he had soaked pepper; (c) a drop of his own blood; (d) the eye of a fly.
2. (5 pts) Why did the Royal Society ask Robert Hooke to look for the animalcules himself, and what happened in 1677? Explain why this step matters in science.
3. (5 pts) A bacterium is 3 µm long. (a) Write its length in millimeters. (b) Seen through a lens that magnifies 270 times, how long is its image in millimeters?
4. (4 pts) Multiple choice. You switch from the 10× objective to the 40× objective. Which of these happens? (a) The field of view gets larger and brighter. (b) The field of view gets smaller and dimmer. (c) The field of view stays the same size. (d) The image turns right side up.
5. (6 pts) A microscope has a 10× eyepiece. At 40× total magnification the field of view is 4.5 mm. (a) What is the total magnification with the 10× objective? (b) What is the field of view at that magnification? (c) At 400×, 9 cells fit across a 0.45 mm field. How wide is one cell in micrometers?
6. (5 pts) A student puts a slide on the stage, clicks the 40× objective into place and turns the coarse knob while looking into the eyepiece. Name two mistakes and say what the student should do instead.
7. (4 pts) Multiple choice. The best way to avoid air bubbles in a wet mount is to (a) drop the coverslip flat from high above; (b) press hard on the coverslip with a finger; (c) touch one edge of the coverslip to the drop and lower it slowly at an angle; (d) use no water.
8. (5 pts) A stain is mixed 1 part iodine tincture to 4 parts water. (a) How much of each is needed to make 15 mL? (b) Why do we stain onion skin before looking at it?
9. (6 pts) Data reading. Students checked 20 onion cells per slide.
   Unstained: 3, 5, 4 cells with a visible nucleus.
   Stained: 18, 17, 19.
   (a) Find the average for each group. (b) Give each as a percent. (c) Name the independent and dependent variables.
10. (6 pts) Diagram item. Draw a biological drawing of a paramecium (from memory or from your notebook) at least 8 cm long. Label the cilia, oral groove, contractile vacuole, food vacuole and macronucleus with ruled lines, and add a title, the magnification and a scale bar.
11. (5 pts) Match each pond organism to its description: (1) paramecium, (2) amoeba, (3) euglena, (4) water flea. Descriptions: (A) green cell with a red eyespot and a flagellum; (B) many-celled crustacean whose heart can be seen beating; (C) slipper-shaped cell covered with cilia; (D) cell that creeps with pseudopods.

12. (5 pts) Three drops of pond water, each 0.05 mL, held 4, 7 and 4 euglena. (a) Estimate the number of euglena per milliliter. (b) Euglena move toward light. In which part of a pond would you expect to find the most, and why?

UNIT PROJECT: MY MICROSCOPE FIELD JOURNAL

Assemble a bound field journal of your microscope work from this unit. Include your pepper-water table and line graph, your field-of-view table and the three drawings of the letter “e,” your stained and unstained onion counts with percents, and at least four full biological drawings of different pond organisms, each with a title, date, sample place, magnification, ruled labels and scale bar. Finish with a one-page cover essay: tell the story of Leeuwenhoek’s discovery and explain, using your own observations, what you learned about how to see small things truly.

- **Observation and drawing**: Drawings are made from life, large and clean, with single outlines, ruled labels that do not cross, the magnification and a correct scale bar.
- **Data and calculations**: Tables are complete and dated; averages, percents, field of view, magnification and sizes are calculated correctly with units.
- **Experimental thinking**: Each experiment states its question, prediction, variables and control, and the conclusions match the data.
- **Explanation and care**: The cover essay tells Leeuwenhoek’s story accurately and separates observation from interpretation; the journal is neat and organized.

Answer key

1. (b) Water in which he had soaked pepper.
2. The claim was new and surprising, so the Society wanted someone else, with a different microscope, to see the creatures before accepting it. In November 1677 Hooke showed the animalcules in pepper water to the members. Replication by another observer gives good reason to trust that a result is real and not a mistake.
3. (a) 3 ÷ 1,000 = 0.003 mm. (b) 3 µm × 270 = 810 µm = 0.81 mm.
4. (b) The field of view gets smaller and dimmer.
5. (a) 10 × 10 = 100×. (b) 4.5 mm × 40 ÷ 100 = 1.8 mm. (c) 0.45 mm ÷ 9 = 0.05 mm = 50 µm.
6. Mistake 1: starting on high power; start with the lowest-power (4×) objective, find and center the specimen, then switch up. Mistake 2: using the coarse knob on high power while looking through the eyepiece; this can crash the objective into the slide. Use coarse focus only on low power (watching from the side when moving toward the slide), and only the fine knob on high power.
7. (c) Touch one edge of the coverslip to the drop and lower it slowly at an angle.
8. (a) 5 parts total, so each part is 3 mL: 3 mL iodine tincture and 12 mL water. (b) Onion cells are nearly colorless; the stain colors some parts more than others, so the nucleus and the cell walls stand out clearly.
9. (a) Unstained: 12 ÷ 3 = 4. Stained: 54 ÷ 3 = 18. (b) Unstained 4/20 = 20%; stained 18/20 = 90%. (c) Independent: whether the slide was stained. Dependent: the number (or percent) of cells with a visible nucleus.
10. Full marks for: a slipper-shaped or oval outline drawn with a single clean line; short cilia around the edge; five correct labels (cilia on the edge, oral groove as a curved channel on one

side, contractile vacuoles near the ends, small round food vacuoles inside, a large oval macronucleus near the middle); ruled label lines that do not cross, with labels lined up at one side; a title, a magnification and a scale bar. Example: a drawing 80 mm long of a 0.2 mm paramecium is ×400, and a 50 µm scale bar should be 20 mm long.

11. 1–C, 2–D, 3–A, 4–B.
12. (a) Average = 15 ÷ 3 = 5 per drop; 1 ÷ 0.05 = 20 drops per mL; 5 × 20 = 100 euglena per mL. (b) Near the sunny surface, because euglena use chloroplasts to make food from light and swim toward light.

UNIT 2

The Cell

In Unit 1 you learned to use a microscope like Leeuwenhoek. Now you will ask the question that puzzled scientists for more than 170 years after Hooke first saw cork "cells": what are living things made of? You will sit down (in imagination) at a Berlin dinner table in 1838, where the botanist Matthias Schleiden and the animal scientist Theodor Schwann realized that plants and animals are built on the same plan. Their work, corrected by Rudolf Virchow, became the **cell theory**, one of the foundations of all biology.

Then you will go inside the cell. You will meet Camillo Golgi, who found a mysterious net inside an owl's nerve cells, and the scientists who used the first electron microscopes to prove it was real. You will learn the job of each **organelle**, compare plant and animal cells with Robert Brown's orchids and your own cheek cells, and measure osmosis in potato strips. Finally you will build an edible scale model of a cell and test why fresh pineapple ruins gelatin.

Throughout the unit you will work like a real scientist: estimating sizes in micrometers, calculating scale factors and percent changes, reading data tables and graphs, and designing fair tests. And you will see, as David did, that we are "fearfully and wonderfully made" (Psalm 139:14), from the whole body down to its smallest living part.

BEFORE YOU BEGIN

- Can focus a microscope at low and high power and make a simple wet-mount slide (Unit 1)
- Can multiply and divide decimals and convert between meters, centimeters and millimeters
- Can find a percent of a number and read a simple bar graph
- Knows the difference between living and nonliving things

MASTERY CHECKLIST

- I can tell the story of Schleiden and Schwann and state the three parts of the cell theory.
- I can estimate the size of a cell in micrometers from the field of view of a microscope.
- I can name the main organelles of a cell and describe the job of each.
- I can compare plant and animal cells and explain osmosis and turgor pressure.
- I can calculate a scale factor, build a scale model of a cell and name its limitations.
- I can read a data table or graph from a cell experiment and design a fair test.

OPTIONAL BOOKS AND RESOURCES

- **Robert Hooke, *Micrographia* (free at Project Gutenberg)** (https://www.gutenberg.org/ebooks/search/?query=micrographia): Free. Read Hooke's

own description of the cork cells (Observation XVIII) aloud with Lesson 2.1. The spelling is old-fashioned, so a parent may want to read it first.

- **Cells Alive** (https://www.cellsalive.com/): Free. Long-running educational site with interactive animal and plant cell diagrams you can click part by part. Useful for review with Lessons 2.2 and 2.3.
- **Harvard Museum of Natural History** (https://hmnh.harvard.edu/): The home of the Blaschka Glass Flowers from Lesson 2.4. The museum's site has photographs and background on the collection; worth a visit if you are ever near Cambridge, Massachusetts.

2.1 Schleiden and Schwann (1838–1839) ★

OBJECTIVES

- Tell how Matthias Schleiden and Theodor Schwann, building on Hooke and Robert Brown, concluded that plants and animals are both built of cells.
- State the three parts of the cell theory and explain how Rudolf Virchow corrected Schleiden and Schwann's idea about where new cells come from.
- Estimate the size of a cell in micrometers from a microscope's field of view, and use surface area to volume ratios to explain why cells are small.

Warm-up. Put a few grains of table salt and a small piece of onion skin side by side on a dark plate. Look at both with a magnifying glass. One was never alive and one was. Write down every difference you can see in five minutes. Which one do you think is built of tiny repeating units, and which one is built of solid crystals?

Video. Coming soon. History of the cell theory (Hooke, Schleiden, Schwann, Virchow) and real microscope footage of onion and cheek cells; search Amoeba Sisters, Homeschool Pop (Alternate), SciShow Kids, Smithsonian, and microscope channels such as Journey to the Microcosmos.

KEY TERMS

cell
The smallest unit of a living thing that can carry on all the processes of life. Every living thing is made of one or more cells.

cell theory
The scientific theory that all living things are made of cells, that the cell is the basic unit of structure and function in living things, and that all cells come from cells that already exist.

nucleus
The large, rounded structure inside many cells that holds the cell's instructions. Robert Brown named it in 1831.

unicellular
Made of only one cell, such as a bacterium or an amoeba.

multicellular
Made of many cells that work together, such as an oak tree, a frog or a person.

micrometer (µm)
One-thousandth of a millimeter. Most cells are between about 10 and 100 micrometers across.

CORE IDEAS

The story. In the autumn of 1838, two young German scientists sat down to dinner together in Berlin. Matthias Schleiden had studied law and worked as a lawyer in Hamburg, given it up, and become a botanist. Theodor Schwann worked for the great physiologist Johannes Müller and spent his days at the microscope studying animal tissues. Schleiden talked about his plants. Every part of a plant he had examined, root, stem, leaf and flower, was built of cells, and inside young plant cells he kept finding a dark, rounded body, the **nucleus**. Schwann sat up. He had seen bodies just like that inside the cells of the notochord, the stiff rod along the back of a tadpole. The two men went straight to Schwann's laboratory, and Schleiden looked. They were the same kind of thing.

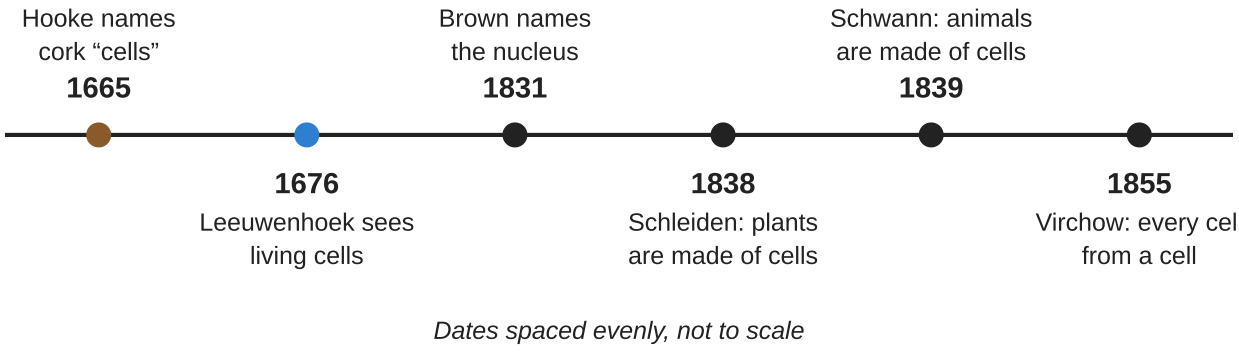
How they figured it out. Neither man started from nothing. In 1665 Robert Hooke had looked at a thin slice of cork and named the little boxes he saw "cells." In 1676 Antonie van Leeuwenhoek saw living single-celled creatures swimming in water. In 1831 the Scottish botanist Robert Brown noticed a dark spot in the cells of orchid leaves and called it the nucleus. Around the same years, lens makers learned to build microscope lenses that cut down the blurry colored fringes of older instruments. In 1838 Schleiden published his conclusion that plants are made of cells. Schwann then spent a year looking at cartilage, muscle, nerve, fat, feathers and growing eggs, and he kept finding cells with nuclei. In 1839 he published a book whose title, in English, is *Microscopical Researches into the Accordance in the Structure and Growth of Animals and Plants*. Its message was simple and enormous: plants and animals, so different on the outside, are built on the same plan inside.

The big idea. Their work, with one important correction, became the **cell theory**, one of the foundations of all biology. It has three parts. (1) All living things are made of one or more cells. (2) The cell is the basic unit of structure and function in living things. (3) All cells come from cells that already exist. Some living things are **unicellular**, a whole creature in one cell. Others are **multicellular**; your body has tens of trillions of cells. Most cells are tiny, about 10 to 100 **micrometers** across. A micrometer (µm) is one-thousandth of a millimeter, so about 100 cells of 20 µm would fit side by side across the head of a pin, and a cell of 20 µm is 50 times narrower than a millimeter.

The correction. Schleiden and Schwann got the third part wrong. They believed new cells formed out of a clear fluid, a little like crystals growing in salt water. Later microscopists, watching carefully, saw cells pinch in two. Robert Remak described cells dividing, and in 1855 the physician Rudolf Virchow made famous the Latin saying *omnis cellula e cellula*, "every cell from a cell." This is how science works at its best: a good idea is tested by more careful looking, and the part that does not match what is seen is replaced.

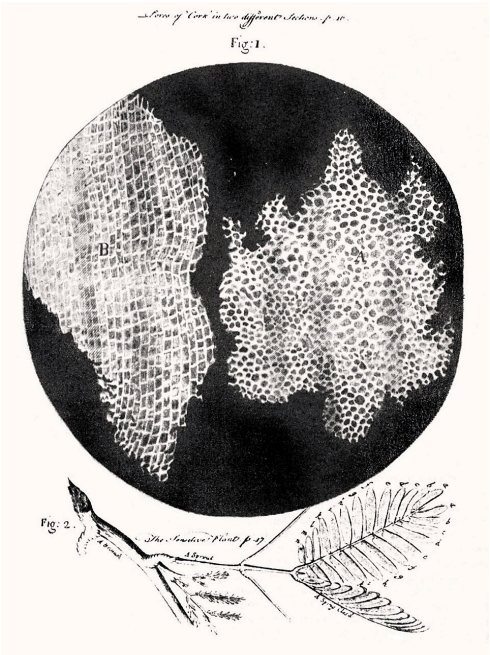
Try it and see. Why are cells so small? A cell takes in food and oxygen and gets rid of wastes through its outer surface, but it uses them throughout its whole volume. When a cube doubles in width, its surface grows 4 times but its volume grows 8 times, so each bit of the inside has less surface to serve it. A small cell has plenty of surface for its size; a huge one would starve in the middle. That is why an elephant is not built of giant cells. Its cells are about the same size as a mouse's. It simply has many more of them.

God’s handiwork. Theodor Schwann was a devout Catholic Christian all his life, and he gave his working years to the careful study of God’s creatures. The cell theory reveals an astonishing order: the rose, the robin and the child are each built from tiny living rooms that work, grow and divide. David did not know about cells, but he knew he was “fearfully and wonderfully made,” and the microscope has only made that sentence larger.



The cell theory was built by many people over almost two hundred years. (Dates are spaced evenly, not to scale.)

PICTURE STUDY



Cork Cells, from Micrographia (Observation XVIII), Robert Hooke, 1665 · Engraving printed in a book · Published in London for the Royal Society; copies are kept in many libraries

Robert Hooke drew his own pictures for *Micrographia*, the first great book of microscope drawings. Here he shows a thin slice of cork cut two ways. Cut straight across, the cork looks like a honeycomb. Cut the long way, the same chambers look like rows of little boxes.

Hooke called them “cells,” from the Latin word for a small room, like the rooms of monks in a monastery.

Hooke was seeing only the empty walls of dead bark cells. He had no idea that each box had once held a living cell with a nucleus. It took 173 more years, and better lenses, before Schleiden and Schwann understood what those little rooms really meant.

Look closely

- Find the view that looks like a honeycomb. Are all the openings the same size and shape?
- Find the view where the cells look like stacked boxes. How is it different from the honeycomb view?
- Look at the walls between the cells. Are they thick or thin compared with the empty spaces?
- Look for anything inside the cells. What do you see, and why?

Talk about it

1. Why do the same cells look so different when the cork is cut in two directions?
2. Hooke saw cells but did not discover the cell theory. What did Schleiden and Schwann add that Hooke did not know?
3. If you could show Hooke one thing through a modern microscope, what would you choose and why?

> **Safety first.** Glass slides and cover slips can break and cut; carry them by the edges and let an adult clean up any broken glass. For the cheek sample, use your own clean toothpick once, scrape gently, and throw it away. If you use iodine stain, an adult should handle the bottle: iodine stains skin and clothing and is poisonous if swallowed. Wash your hands when you finish.

EXPERIMENT: THE SCHWANN SURVEY: IS EVERYTHING THAT LIVES MADE OF CELLS?

Schwann did not prove the cell theory by looking at one thing. He looked at tissue after tissue and kept a careful record. In this experiment you will do the same with plant, animal and nonliving samples, and decide whether your results support the cell theory.

Question: Do samples from living things show cells, while samples that were never alive do not? **Prediction:** Write yours before you begin. **What we change:** the sample. **What we keep the same:** the magnification, the light, the stain and how thin the sample is. **What we measure:** whether cells are seen, how many fit across the field of view, and the estimated size of one cell.

You will need

- A microscope with 40×, 100× and 400× settings (a sturdy beginner microscope is fine)
- Glass slides, cover slips and an eyedropper of water

- Iodine stain from the pharmacy (adult handles) or a drop of blue food coloring
- Samples: a thin layer peeled from inside an onion, a sliver of cork from a bottle stopper, a smear of ripe banana, a thin lettuce or spinach leaf piece, your own cheek cells on a clean toothpick
- Nonliving controls: a few grains of salt and a small square of plastic wrap
- Clear plastic ruler with millimeter marks, pencil and lab notebook

Steps

1. Measure your field of view. Lay the clear ruler on the stage and look at 40×. Count how many millimeters fit across the circle of light and write it down. (Many microscopes show about 4 to 5 mm at 40×.)
2. Rule a table in your notebook with columns: Sample, Living or never alive?, Cells seen?, Number of cells across the field, Magnification, Estimated cell size (µm), Sketch.
3. Make a wet mount of the onion layer: lay it flat on a slide, add one drop of water or stain, and lower a cover slip at an angle so no bubbles form.
4. Look at 40×, then 100×. Count how many cells fit end to end across the field. Sketch three cells and label any nucleus you see.
5. Repeat with the cork, banana, leaf and cheek samples. For the cheek, scrape the inside of your cheek gently, stir the toothpick in a drop of stain on the slide, and add a cover slip.
6. Repeat with the salt grains and the plastic wrap, using the same magnification and light.
7. Calculate the size of one cell for each sample: field of view in micrometers divided by the number of cells across. Remember that 1 mm = 1,000 µm, and that the field at 100× is about four-tenths of the field at 40×.
8. Write a conclusion of three to five sentences: Did your results support the cell theory? Which sample surprised you? Why do the cork cells look empty?

Tips

- Thin is the secret. If you see a dark smear, your sample is too thick; tear a thinner piece.
- Air bubbles look like perfect black-edged circles. Cells have straighter walls or irregular outlines and often a nucleus.
- Always focus with the lowest power first, then switch to higher power and use only the fine focus knob.

Check your work

- I recorded every sample in a data table, including the nonliving controls.
- I estimated the size of at least two kinds of cells in micrometers and showed my math.
- I explained whether my results support the cell theory and why cork cells look empty.

NATURE NOTEBOOK

1. Draw three onion cells and three cheek cells side by side at the same magnification. Label the cell membrane or wall, the nucleus and the magnification, and write the estimated size of each cell in micrometers.
2. Make a timeline card of the six people in this lesson (Hooke, Leeuwenhoek, Brown, Schleiden, Schwann, Virchow). Under each name, write one sentence about what that person saw.
3. Draw a 1 cm cube and a 2 cm cube. Write the surface area and volume of each, and write one sentence explaining why the bigger cube would be a worse shape for a cell.

From history. Theodor Schwann made other discoveries besides the cell theory. In 1836 he found pepsin, the substance in the stomach that begins breaking down proteins, and he used a word for the chemical changes in cells that became our word “metabolism.” The cells that wrap around many nerve fibers are still called Schwann cells. In 1839 he left Berlin to teach anatomy at the Catholic University of Louvain in Belgium, and he later taught for many years at Liège.

WORKED EXAMPLES

Example 1. At 100× your microscope’s field of view is 1.5 mm across. You count 6 onion cells lined up end to end across the field. About how long is one onion cell in micrometers?

1. Change millimeters to micrometers: 1 mm = 1,000 µm, so 1.5 mm × 1,000 = 1,500 µm.
2. Six cells fill that width, so divide: 1,500 µm ÷ 6 = 250 µm.
3. Check: 250 µm is one-quarter of a millimeter, which matches the size of onion skin cells in most textbooks.

Answer: About 250 µm long.

Example 2. The same microscope is switched from 100× to 400×. What is the new field of view? A stained cheek cell fills about one-sixth of the field’s width. About how wide is the cheek cell?

1. When the magnification is 4 times greater (400 ÷ 100 = 4), the field of view becomes 4 times smaller.
2. New field of view: 1,500 µm ÷ 4 = 375 µm.
3. One-sixth of the field: 375 µm ÷ 6 = 62.5 µm, which rounds to about 60 µm.
4. Compare: the onion cell (250 µm) is about 4 times longer than the cheek cell.

Answer: The field is 375 µm across, and the cheek cell is about 60 µm wide.

Example 3. Model three cube-shaped “cells” with sides of 1, 2 and 4 µm. Find the surface area, the volume and the surface area to volume ratio of each. What happens to the ratio as the cell gets bigger?

1. Surface area of a cube = 6 × side × side. Volume = side × side × side.
2. 1 µm cube: surface area = 6 × 1 × 1 = 6 µm²; volume = 1 µm³; ratio = 6 ÷ 1 = 6 to 1.
3. 2 µm cube: surface area = 6 × 2 × 2 = 24 µm²; volume = 8 µm³; ratio = 24 ÷ 8 = 3 to 1.
4. 4 µm cube: surface area = 6 × 4 × 4 = 96 µm²; volume = 64 µm³; ratio = 96 ÷ 64 = 1.5 to 1.
5. Each time the side doubles, the ratio is cut in half. A bigger cell has less surface for each unit of volume it must feed.

Answer: The ratios are 6:1, 3:1 and 1.5:1. As the cube grows, the ratio shrinks, which is why cells stay small and large creatures have more cells, not bigger ones.

PRACTICE

1. EASY State the three parts of the cell theory.
2. EASY Which scientist concluded in 1838 that plants are made of cells, and which concluded in 1839 that animals are too?
3. EASY Convert 0.05 mm to micrometers. Is that a reasonable size for a cell?
4. MEDIUM What structure did Schleiden describe at dinner that made Schwann remember the cells of a tadpole’s notochord? Who had named it first?
5. MEDIUM What did Schleiden and Schwann believe about where new cells come from, and how was this corrected?
6. MEDIUM At 40× a field of view is 4.5 mm across, and 9 cells fit across it end to end. How long is each cell in millimeters and in micrometers?
7. MEDIUM A student recorded these results at 100×. Onion skin: cells seen, nucleus visible after stain. Cork: cells seen, empty walls only. Banana: loose cells seen. Cheek: cells seen, nucleus visible. Salt grain: no cells, sharp-edged cube. Plastic wrap: no cells, smooth. Which conclusion is best supported? (A) Only plants are made of cells. (B) Samples from living or once-living things showed cells; samples that were never alive did not. (C) All cells contain a visible nucleus. (D) Salt is made of very small cells.
8. HARD Cubes with sides of 1, 2, 3 and 4 µm have surface area to volume ratios of 6, 3, 2 and 1.5 to 1. (a) What pattern connects the side length and the ratio? (b) Predict the ratio for a 6 µm cube, then check by calculation.
9. CHALLENGE Design an investigation to test the claim “every part of a plant is made of cells.” Name the samples, a control, what you will keep the same, and what you will record.
10. HARD Hooke saw cork cells in 1665, but the cell theory was not proposed until 1838 and 1839. In three or four sentences, explain why it took so long.

COMMON MISTAKES

- Thinking Robert Hooke discovered the cell theory. Hooke named cells, but he saw only empty cork walls and did not know cells were the living units of all creatures.
- Thinking bigger animals have bigger cells. An elephant's cells are about the same size as a mouse's; the elephant simply has many more of them.
- Thinking new cells can form out of nonliving fluid. Schleiden and Schwann believed this, but every new cell comes from a cell that already exists.

Make it a game · Cell or Not?. A parent or friend names a thing: a mushroom, a pencil, a feather, a sugar cube, a slice of apple, a grain of sand, a bit of bread mold, a strand of your hair. You have five seconds to say "cell" (made of cells, or once was) or "not" (never alive), and one sentence of proof. Score a point for each correct answer and a bonus point if you can name which scientist would have tested it first. Play to ten points.

Scripture. "I will praise thee; for I am fearfully and wonderfully made: marvellous are thy works; and that my soul knoweth right well." (Psalm 139:14, KJV)

David praised God for the body he could see. Schleiden and Schwann showed that inside that body are tens of trillions of living cells, each one built with care. The closer we look, the more there is to praise.

Practice answers

1. (1) All living things are made of one or more cells. (2) The cell is the basic unit of structure and function in living things. (3) All cells come from cells that already exist.
2. Matthias Schleiden (plants, 1838) and Theodor Schwann (animals, 1839).
3. 0.05 × 1,000 = 50 µm. Yes; most cells are about 10 to 100 µm across.
4. The nucleus. Robert Brown named it in 1831 after seeing it in orchid leaf cells.
5. They thought new cells formed out of a clear fluid, somewhat like crystals. Later microscopists, including Robert Remak, saw cells divide, and in 1855 Rudolf Virchow made famous the idea that every cell comes from a cell (omnis cellula e cellula).
6. 4.5 mm ÷ 9 = 0.5 mm, which is 0.5 × 1,000 = 500 µm.
7. (B). Cheek cells rule out A, cork cells show no nucleus so C is not supported, and the salt showed crystals, not cells, so D is wrong.
8. (a) The ratio equals 6 divided by the side length; as the side grows, the ratio shrinks. (b) Predicted 6 ÷ 6 = 1 to 1. Check: surface area = 6 × 6 × 6 = 216 µm^2; volume = 216 µm^3; 216 ÷ 216 = 1 to 1.
9. Sample answer: Test thin slices or peels of root, stem, leaf, petal and fruit from one kind of plant (for example, a carrot plant or a celery stalk with leaves). Use a never-living control such as a salt grain or plastic wrap to show what "no cells" looks like. Keep the same microscope, magnification, light, stain and sample thickness. Record for each sample whether cells are seen, a sketch, the number of cells across the field and the estimated cell size. If every plant part shows cells and the control does not, the results support the claim.
10. Hooke saw only the empty walls of dead cork cells, so he did not know cells were living units. Early microscopes gave blurry, color-fringed images, and better lenses only came into wide use around the 1830s. The nucleus was not named until 1831, and it gave scientists a way to

recognize cells in very different tissues. Finally, someone had to compare plants and animals carefully and see that they were built on the same plan, which is what Schleiden and Schwann did.

2.2 Organelles: The Working Parts of a Cell

OBJECTIVES

- Name the main organelles of an animal cell (cell membrane, cytoplasm, nucleus, ribosomes, endoplasmic reticulum, Golgi apparatus, mitochondria, lysosomes, vacuoles) and describe the job of each.
- Tell how Camillo Golgi's stain and, later, the electron microscope revealed parts of the cell too small or too faint for ordinary microscopes.
- Use scale factors and microscope limits to compare the sizes of organelles, and test a model of a selectively permeable membrane.

Warm-up. Think about a busy bakery. Make a quick list of the jobs that must be done for a loaf of bread to reach a customer: keeping the recipe, mixing, baking, wrapping, delivering, taking out the trash, keeping the doors locked at night. Keep your list. By the end of this lesson you will match each job to a part of a living cell.

Video. Coming soon. Animal cell organelles and their jobs, with electron microscope images of mitochondria, endoplasmic reticulum and Golgi apparatus; search Amoeba Sisters, Homeschool Pop (Alternate), SciShow Kids, Smithsonian, and real cell footage from microscope channels.

KEY TERMS

organelle
A tiny structure inside a cell that does a particular job, as an organ does in the body. The word means "little organ."

cell membrane
The thin, flexible outer boundary of a cell, made of a double layer of fat-like molecules with proteins in it. It is selectively permeable: it lets some substances pass and keeps others out.

cytoplasm
Everything inside the cell membrane except the nucleus: a jelly-like fluid with the organelles held in it.

nucleus
The organelle that holds the cell's DNA, the instructions for building and running the cell. It is wrapped in a double membrane with pores.

ribosome
A tiny structure that builds proteins by following instructions copied from the DNA. Ribosomes float free in the cytoplasm or sit on the rough endoplasmic reticulum.

mitochondrion (plural: mitochondria)
The organelle that releases the energy stored in food, using oxygen, in the process called cellular respiration.

CORE IDEAS

The story. In 1898, in his laboratory at the University of Pavia in Italy, the physician Camillo Golgi was studying nerve cells from the brain of a barn owl. Twenty-five years earlier he had invented a stain, his “black reaction,” that used silver to turn a few nerve cells dark against a pale background. Now, inside the nerve cells, he saw something new: a fine, lacy network curled near the nucleus. He called it the “internal reticular apparatus,” meaning a little net inside the cell. Many scientists doubted him. They suspected the net was only a smudge left by the silver. For half a century people argued about whether it was real.

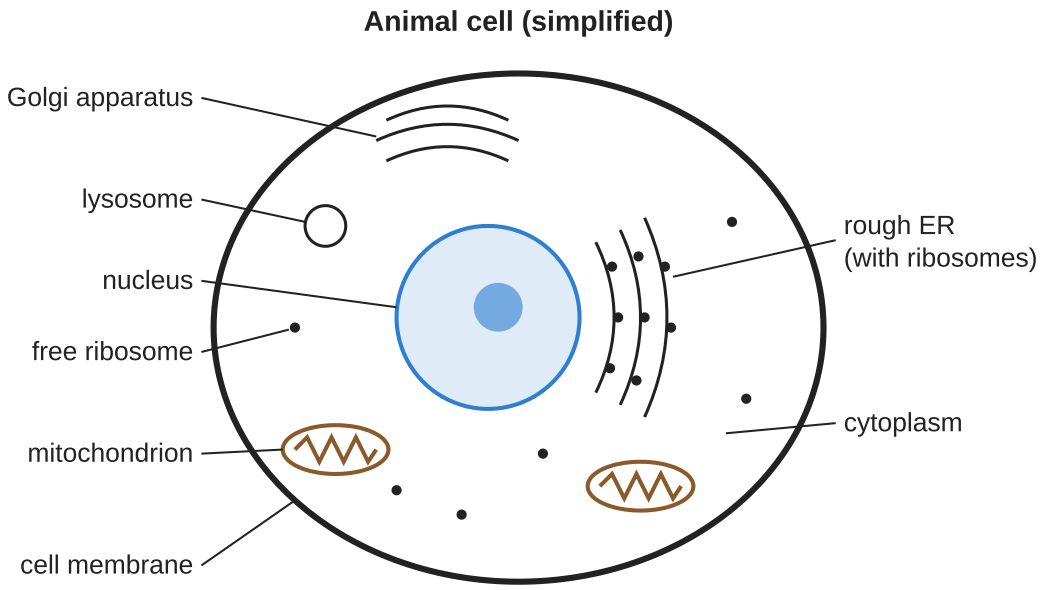
How they figured it out. A light microscope cannot show details much smaller than about 0.2 micrometers, no matter how good its lenses are, because light itself is too coarse. In 1931 Max Knoll and Ernst Ruska in Berlin built the first electron microscope, which uses a beam of electrons instead of light and can show far finer detail. In the 1940s and 1950s, scientists at the Rockefeller Institute in New York, including Albert Claude, Keith Porter and George Palade, used it to photograph the inside of cells. They saw folded sheets of membrane they named the *endoplasmic reticulum*, tiny grains now called ribosomes, and, sure enough, stacks of flattened sacs exactly where Golgi had seen his net. Today it is called the **Golgi apparatus**, and in 1974 Claude, Palade and Christian de Duve shared a Nobel Prize for showing how the cell is organized.

The big idea. A cell is not a bag of jelly. It is full of **organelles**, “little organs,” each with a job. Picture a cell as that bakery. The **cell membrane** is the door and walls: a thin double layer of fat-like molecules that lets water, oxygen and food in and wastes out, but keeps many other substances where they belong. We call it *selectively permeable*. The **cytoplasm** is the jelly-like floor of the shop where the work happens, crossed by a scaffolding of protein fibers. The **nucleus** is the office where the recipe book is kept: the DNA. Inside it a dark spot, the nucleolus, helps make ribosome parts.

The workers. Ribosomes are the bakers: they read copied instructions from the DNA and build proteins one link at a time. Many sit on the *rough endoplasmic reticulum* (rough ER), a system of folded membranes next to the nucleus; the *smooth ER*, without ribosomes, makes fats and helps break down harmful substances. The **Golgi apparatus** is the wrapping and shipping room: it sorts and packages proteins into tiny membrane bubbles called vesicles and sends them where they are needed. **Mitochondria** are the ovens and power plant: they use oxygen to release the energy stored in sugar, a process called cellular respiration, which you will study in Unit 5. **Lysosomes** are the cleanup crew, small sacs that digest worn-out parts and food particles. **Vacuoles** are storage rooms for water, food and wastes.

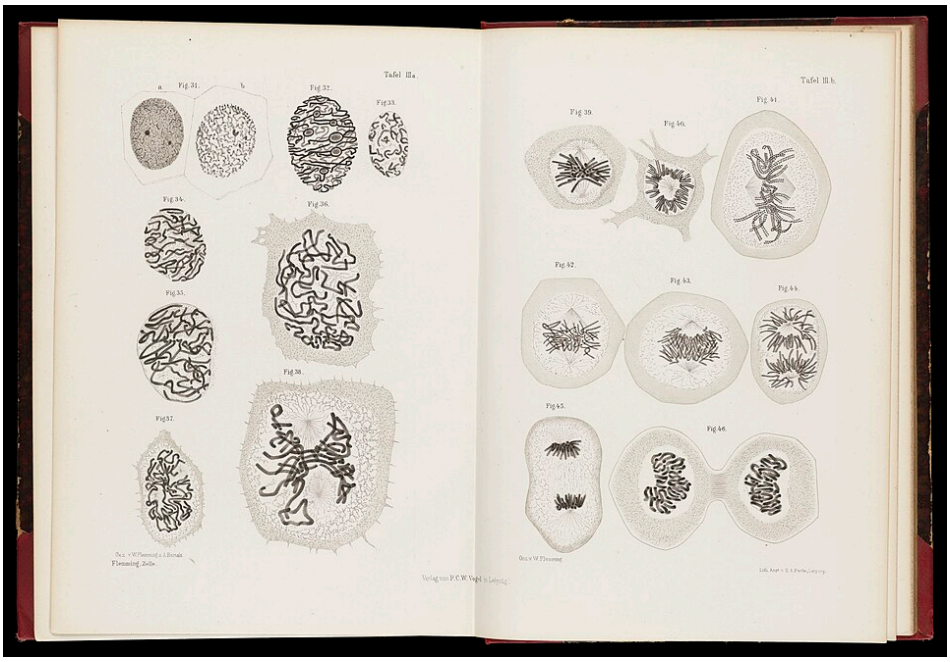
Try it and see. Function follows need. A muscle cell, which works hard all day, may hold hundreds or thousands of mitochondria. A cell that makes and ships a lot of protein, such as a cell in the pancreas that makes digestive juices, is crowded with rough ER and Golgi stacks. In this lesson’s project you will build a model membrane from a plastic bag and watch which molecules get through.

God’s handiwork. Paul wrote that God has “set the members every one of them in the body, as it hath pleased him.” The same is true inside each cell. No organelle works alone: the nucleus holds the plan, the ribosomes build, the Golgi ships, the mitochondria supply energy, and the membrane guards it all. Remove any one of them and the whole cell suffers. Order like this, packed into a space too small to see, is a fitting reason for wonder.



A simplified animal cell. Real cells are three-dimensional and far more crowded than any drawing.

PICTURE STUDY



Cells and Their Nuclei, plates from Zellsubstanz, Kern und Zelltheilung (Cell Substance, Nucleus and Cell Division), Walther Flemming, 1882 · Printed plates of the scientist's own microscope drawings · Published in Leipzig; copies are kept in many libraries

Photo: Wellcome Collection · CC BY 4.0 (https://creativecommons.org/licenses/by/4.0) · via Wikimedia Commons

Walther Flemming, a German anatomist, studied the cells of young salamanders, whose cells and nuclei are unusually large. He stained them with new dyes and found that something in the nucleus soaked up the color strongly. He named it chromatin, from the Greek word for color, and drew what happened to it as a cell divided, a process he named mitosis.

Flemming drew what he saw with great patience, cell after cell, and his drawings were so careful that biology textbooks still reprint them. They are some of the first pictures to show that the nucleus is not a still, simple spot, but a busy place where the cell's instructions are kept and passed on.

Look closely

- Find a cell where the nucleus looks like a loose tangle of threads. That is chromatin spread out while the cell is working.
- Find a cell where the dark threads are bunched or lined up in the middle.
- Find a cell where the threads have been pulled toward two ends. What do you think will happen next?
- Look at the cell outlines. Are all the cells the same size and shape?

Talk about it

1. Flemming used a light microscope. Which organelles from this lesson could he see well, and which were too small?

2. Why would a scientist choose salamander cells instead of human cells to study the nucleus?
3. How does Flemming’s work support the third part of the cell theory, that every cell comes from a cell?

> **Safety first.** Iodine is poisonous if swallowed and stains skin, clothes and counters. An adult should add the iodine, and you should work on newspaper or an old tray. Do not taste anything in this experiment, and wash your hands when you finish. Keep iodine away from anyone with an iodine allergy.

EXPERIMENT: A MEMBRANE IN A BAG

A cell membrane lets some molecules through and blocks others. You can model this with a thin plastic sandwich bag. Starch molecules are huge; iodine molecules are small. Iodine turns starch a deep blue-black, so the color will show you which way the molecules moved.

Question: Can small iodine molecules pass through a plastic bag “membrane” while large starch molecules cannot? **Prediction:** Write which liquid you think will turn blue-black. **What we change:** time (and, in a second trial, the kind of bag). **What we keep the same:** amount of starch, amount of water, number of iodine drops, temperature. **What we measure:** the color inside and outside the bag every 5 minutes, scored 0 (no change) to 4 (deep blue-black).

You will need

- 2 thin plastic sandwich bags (the cheap fold-top or zip kind), plus 1 thicker freezer bag for a second trial
- 1 tablespoon cornstarch and 120 mL warm water
- 2 clear glasses or jars, each with 250 mL of tap water
- Iodine (tincture from the pharmacy) and an eyedropper; adult handles
- Twist ties or rubber bands, a spoon, a timer, a white sheet of paper, lab notebook

Steps

1. Stir the cornstarch into the 120 mL of warm water until it is cloudy and smooth.
2. Pour half of the starch mixture into a sandwich bag. Squeeze out the air and close it tightly. Rinse the outside of the bag so no starch is on it.
3. Have an adult add 15 drops of iodine to one glass of water. It should turn a tea or amber color. Write down the starting colors: starch inside the bag (white) and water outside (amber).
4. Set the glass on the white paper and lower the bag into the iodine water. Start the timer.
5. Every 5 minutes for 30 minutes, record the color inside and outside the bag using the 0 to 4 scale.
6. Second trial: repeat with the thicker freezer bag in the second glass. Keep everything else the same.

7. Make a line graph with time on the horizontal axis and color score on the vertical axis, one line for each bag.
8. Write a conclusion: Which molecule passed through the bag? Which did not? How is the bag like a cell membrane, and how is it different?

Tips

- If the outside water turns blue-black, the bag probably leaked or had starch on the outside. Rinse well and try again.
- A real cell membrane is far more choosy than a bag. It has protein doors and pumps that can let certain molecules in even when they are large, and the cell can open and close them.

Check your work

- I recorded color scores every 5 minutes for both bags.
- I graphed my data and explained which molecules passed through.
- I can explain what “selectively permeable” means and give one way the model is not like a real membrane.

NATURE NOTEBOOK

1. Draw a large animal cell and label nine parts: cell membrane, cytoplasm, nucleus, nucleolus, ribosomes, rough ER, Golgi apparatus, mitochondrion and lysosome. Color each a different color and make a color key.
2. Make a two-column chart called “The Cell Bakery.” In one column list nine organelles; in the other, the bakery job each one matches.
3. Draw a mitochondrion as it looks in an electron microscope photograph: a bean-shaped outer membrane with a folded inner membrane. Write one sentence about what it does.

> **From history.** Golgi’s discovery came in the same year, 1898, that the German scientist Carl Benda named the mitochondrion, from Greek words meaning “thread” and “grain,” because that is how they looked under his microscope. In 1906 Golgi shared the Nobel Prize with the Spanish scientist Santiago Ramón y Cajal, who had used Golgi’s own black stain to show that the brain is made of separate nerve cells. The two men disagreed so strongly about how nerve cells connect that their Nobel lectures argued against each other. Cajal turned out to be right about that, and Golgi was right about his apparatus.

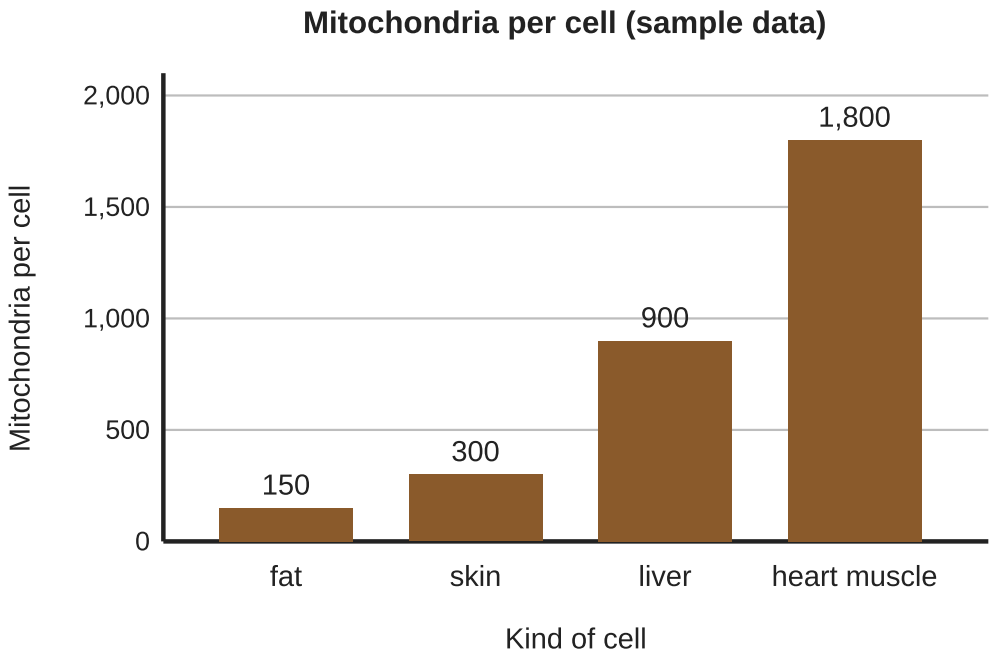
WORKED EXAMPLES

Example 1. A typical animal cell is about 20 µm across, its nucleus about 6 µm, a mitochondrion about 2 µm long and a ribosome about 0.025 µm across. If you built a model of the cell as big as a 20-meter building, how big would each part be?

1. Find the scale factor: 20 m = 20,000,000 µm (because 1 m = 1,000,000 µm). Scale factor = 20,000,000 ÷ 20 = 1,000,000.
2. Nucleus: 6 µm × 1,000,000 = 6,000,000 µm = 6 m, about as tall as a two-story house.
3. Mitochondrion: 2 µm × 1,000,000 = 2 m, about the size of a sofa.
4. Ribosome: 0.025 µm × 1,000,000 = 25,000 µm = 25 mm = 2.5 cm, about the size of a grape.

Answer: Nucleus 6 m, mitochondrion 2 m, ribosome 2.5 cm. Even in a cell the size of a building, a ribosome would be no bigger than a grape.

Example 2. The bar graph shows sample data for practice: the average number of mitochondria a student counted in drawings of four kinds of cells (fat 150, skin 300, liver 900, heart muscle 1,800). (a) How many times more mitochondria does the heart muscle cell have than the skin cell? (b) What pattern does the graph suggest?



Sample practice data: mitochondria per cell in four kinds of cells.

1. Read the bars: heart muscle = 1,800; skin = 300.
2. Divide: 1,800 ÷ 300 = 6.
3. Compare with what each cell does: heart muscle cells contract every second of your life; fat cells mostly store fat.
4. The cells that use the most energy have the most mitochondria.

Answer: (a) 6 times as many. (b) Cells that use more energy have more mitochondria, because mitochondria release the energy stored in food.

Example 3. A good light microscope can show details down to about 0.2 µm. Which of these can it show: a nucleus (6 µm), a mitochondrion (about 1 to 2 µm), a ribosome (0.025 µm)? How many times smaller than the limit is a ribosome?

1. Nucleus: 6 µm is much larger than 0.2 µm, so it is easy to see, especially when stained.
2. Mitochondrion: 1 to 2 µm is 5 to 10 times larger than 0.2 µm, so it shows up only as a tiny dot or rod, with no inside detail.
3. Ribosome: 0.025 µm is smaller than 0.2 µm, so it cannot be seen. Divide: 0.2 ÷ 0.025 = 8.
4. This is why ribosomes were not seen until the electron microscope.

Answer: The nucleus is clearly visible, mitochondria appear only as tiny dots, and ribosomes are invisible; a ribosome is 8 times smaller than the light microscope's limit.

PRACTICE

1. EASY Which organelle holds the cell's DNA?
2. EASY Which structures build proteins, and where are they found?
3. EASY What does it mean that the cell membrane is selectively permeable?
4. MEDIUM In the cell-as-a-bakery comparison, the Golgi apparatus is the wrapping and shipping room. Explain why that is a good match.
5. MEDIUM You build a cell model at a scale of 10,000 times life size. How big should you make a 6 µm nucleus and a 2 µm mitochondrion, in centimeters?
6. MEDIUM A student ran the bag experiment and recorded color scores (0 = no change, 4 = deep blue-black). Inside the bag: 0 min 0, 10 min 1, 20 min 3, 30 min 4. Outside the bag: 0 at every time. Which statement is best supported? (A) Starch moved out of the bag. (B) Iodine moved into the bag, but starch did not move out. (C) Neither substance moved. (D) Water cannot pass through the bag.
7. HARD Use the table to answer. Smallest detail each can show: unaided eye, about 0.1 mm; light microscope, about 0.2 µm; electron microscope, smaller than 0.001 µm. (a) Which of these can show a ribosome (0.025 µm)? (b) Which can show a single 60 µm cheek cell? (c) How many times finer is the light microscope's limit than the unaided eye's limit?
8. HARD Mature human red blood cells lose their nucleus and most organelles before they enter the blood, which leaves more room for the oxygen-carrying protein hemoglobin. Predict one problem this causes for the cell, and explain.
9. CHALLENGE Design an experiment to test whether temperature changes how fast iodine passes through a plastic-bag membrane. Name the independent variable, the dependent variable, three things to keep the same, and how you would show your results.
10. MEDIUM In three or four sentences, explain why scientists argued for about fifty years about whether the Golgi apparatus was real, and how the question was settled.

COMMON MISTAKES

- Thinking cells are flat, like drawings in a book. Real cells are three-dimensional, and the organelles are packed tightly together, not floating far apart.
- Saying mitochondria "make energy." Energy is not created. Mitochondria release energy that is already stored in sugar and put it into a form the cell can use.
- Thinking the cell membrane is a solid wall. It is a thin, flexible, oily double layer, and it is always letting materials in and out.

> **Make it a game · Who Am I? Organelle Edition.** Write each organelle on a slip of paper and tape one slip to a player's back without letting them see it. The player asks yes-or-no questions ("Do I make proteins?" "Am I found near the nucleus?" "Do I use oxygen?") until they can name their organelle. Fewer questions wins. For a harder round, the player must also name the bakery job that matches their organelle.

> **Scripture.** "But now hath God set the members every one of them in the body, as it hath pleased him." (1 Corinthians 12:18, KJV)

Paul was writing about the church as a body with many members, each with its own part to play. A cell is a small picture of the same truth: many different parts, each placed where it belongs, all working for the good of the whole.

Practice answers

1. The nucleus.
2. Ribosomes. They float free in the cytoplasm or are attached to the rough endoplasmic reticulum.
3. It lets some substances pass through (such as water, oxygen and certain food molecules) while keeping others in or out.
4. The Golgi apparatus receives proteins from the ER, sorts and modifies them, packs them into small membrane sacs called vesicles, and sends them to other parts of the cell or out of the cell, just as a shipping room wraps and sends out finished goods.
5. Nucleus: 6 µm × 10,000 = 60,000 µm = 60 mm = 6 cm. Mitochondrion: 2 µm × 10,000 = 20,000 µm = 2 cm.
6. (B). The inside turned blue-black, so iodine entered; the outside never changed, so starch stayed in the bag. The data say nothing direct about water, so D is not supported.
7. (a) Only the electron microscope. (b) The light microscope and the electron microscope; a 60 µm cell (0.06 mm) is smaller than the eye's 0.1 mm limit, so the unaided eye cannot show it clearly. (c) 0.1 mm = 100 µm; 100 ÷ 0.2 = 500 times finer.
8. Without a nucleus (and without ribosomes), the red blood cell cannot make new proteins or repair itself, so it wears out. Human red blood cells last only about 120 days and must be replaced by new ones made in the bone marrow.
9. Independent variable: water temperature (for example, cold 10 °C, room 20 °C, warm 40 °C). Dependent variable: time until the inside of the bag reaches a color score of 3, or the color score after 15 minutes. Keep the same: kind and size of bag, amount of starch mixture, amount of water, number of iodine drops (and repeat each temperature at least twice). Show

results in a table and a bar graph of time versus temperature. Molecules move faster in warmer water, so the prediction is that the warm bag changes color fastest.

10. Golgi saw his “internal reticular apparatus” only with a silver stain, and the net was at the very edge of what a light microscope could show. Many scientists thought the stain itself had made a false pattern. In the 1950s, the electron microscope showed stacks of flattened membrane sacs in the same place in many kinds of cells, without Golgi’s stain. Seeing the same structure by a completely different method settled that it was real.

2.3 Plant versus Animal Cells

OBJECTIVES

- List the parts plant and animal cells share and the three main parts found in plant cells but not animal cells: the cell wall, chloroplasts and a large central vacuole.
- Explain how each plant-cell part fits the life of a plant, and how osmosis and turgor pressure keep a plant firm or let it wilt.
- Measure and graph the percent change in mass of potato strips in salt water, and use the graph to estimate the point where water stops moving in or out.

Warm-up. Take a limp stalk of celery or a wilted lettuce leaf. Bend it and feel how floppy it is. Now trim the end and stand it in a glass of cold water. Predict what it will feel like in a few hours, and write your prediction down. Check it at the end of the lesson or tomorrow morning.

Video. Coming soon. Plant cells versus animal cells under the microscope, Elodea chloroplasts moving, and red onion cells shrinking in salt water (plasmolysis); search Amoeba Sisters, Homeschool Pop (Alternate), SciShow Kids, Science Max, Smithsonian, and real microscope footage channels.

KEY TERMS

cell wall
A stiff layer outside the cell membrane of plant cells (and of fungi and most bacteria). In plants it is made mostly of cellulose and gives the cell support and shape.

cellulose
A tough material made of long chains of sugar molecules that forms plant cell walls. Wood, cotton and paper are mostly cellulose.

chloroplast
A green organelle in many plant cells that captures the energy of sunlight and uses it to make sugar (photosynthesis). Its green color comes from chlorophyll.

central vacuole
A large sac in a plant cell that stores water and dissolved substances and presses outward to keep the cell firm. It can fill most of the cell.

osmosis
The movement of water across a selectively permeable membrane, from the side where there is more water (fewer dissolved particles) to the side where there is less water (more dissolved particles).

turgor pressure
The pressure of water inside a plant cell pushing the membrane against the cell wall. It keeps leaves and stems firm.

CORE IDEAS

The story. In 1831, in London, the Scottish botanist Robert Brown was examining the skin of orchid leaves under his microscope. Brown had spent nearly four years collecting plants in Australia on Matthew Flinders's voyage, and he had a patient eye. In cell after cell he noticed one small, round, darker spot. It was not a chance speck: it showed up in nearly every cell, in orchid after orchid and in other plants too. He called it the *nucleus*, the Latin word for a kernel or little nut, and described it in a paper read to the Linnean Society of London. Brown did not know what the nucleus did, but his name for it stuck, and seven years later it helped Schleiden and Schwann see that plant and animal cells are built on the same plan.

How they figured it out. Put a leaf of the pond plant *Elodea* and a few of your own cheek cells under the same microscope, and the likenesses and differences jump out. The Elodea cells are neat green boxes lined up like bricks, each with a clear outline, packed with small green ovals that slowly stream around the edge. The cheek cells are thin, flat, rounded or ragged, and colorless; they lie loose and folded like dropped pancakes. Both show a nucleus when stained. Scientists over the next century, using stains and then the electron microscope, worked out exactly which parts the two kinds of cells share and which belong to plants alone.

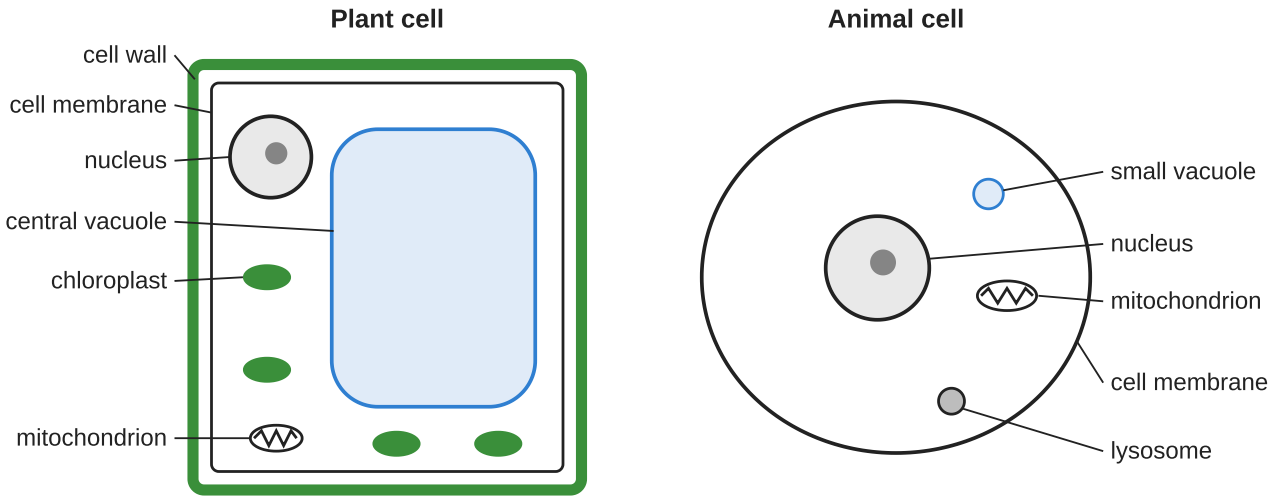
The big idea. Plant and animal cells share most of their parts: the **cell membrane**, **cytoplasm**, **nucleus**, **ribosomes**, **endoplasmic reticulum**, **Golgi apparatus** and **mitochondria**. Yes, plants have mitochondria; they need to release energy from sugar just as you do. Plant cells add three main parts. (1) A **cell wall** of **cellulose** outside the membrane, which gives support. (2) **Chloroplasts**, which use sunlight to make sugar (photosynthesis, Unit 5). (3) A large **central vacuole** that stores water and can take up most of the cell's space. Animal cells have no wall, so they can be many shapes and can bend and move. They have small vacuoles, and they usually have lysosomes and a pair of centrioles, which help when the cell divides.

Structure fits function. A plant cannot walk to water or run from wind. It stands in one place, and it must hold itself up toward the light. The cell walls work like the walls of a building, and the water-filled central vacuole presses outward like air in a tire. That push, called **turgor pressure**, is why a well-watered plant stands firm and a thirsty one droops. Not every plant cell is green: root cells and the cells of an onion bulb grow away from light and have no chloroplasts. Animal cells, by contrast, take many shapes for their work: long, thin nerve cells carry messages, red blood cells are smooth disks that slip through tiny vessels, and muscle cells are long fibers that shorten and pull.

Try it and see. Water moves across a membrane by **osmosis**, from where there is more water to where there is less (where more salt or sugar is dissolved). Put a slice of potato in salty water and water leaves its cells; the potato loses mass and goes floppy. Put it in plain water and water moves in; it gains mass and turns crisp. Under a microscope, red onion cells in salt water shrink away from their walls, a change called plasmolysis. Animal cells have no

wall to stop them: a red blood cell placed in pure water swells until it bursts. A plant cell in pure water simply grows firm, because its wall pushes back.

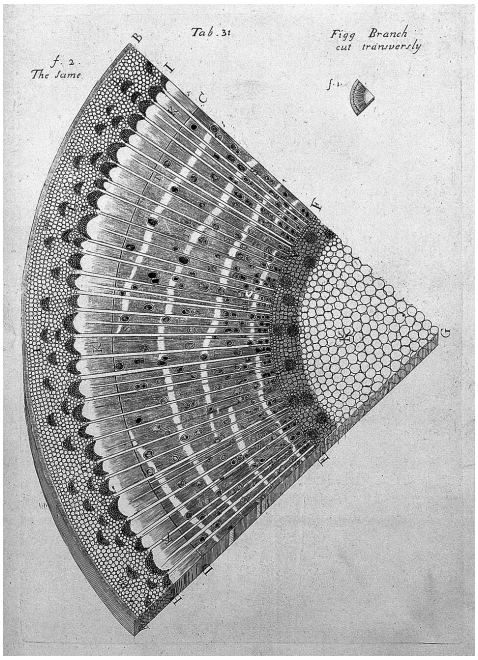
God’s handiwork. On the third day, Genesis says, the earth brought forth grass, herbs and trees, each “after his kind,” and on the following days came the creatures of sea, air and land. Plants and animals live very different lives, and their cells show it: the plant cell is built to stand still, catch light and make food; the animal cell is built to move, take in food and respond. Yet the same basic parts run through both, one plan with many wise variations.



Only the plant cell has a cell wall, chloroplasts and a large central vacuole.

Plant and animal cells share most parts. Only the plant cell has a cell wall, chloroplasts and a large central vacuole.

PICTURE STUDY



A Fig Branch Cut Across, Table 31 from The Anatomy of Plants, Nehemiah Grew, 1682 · Engravings printed in a book · Published in London; copies are kept in many libraries

Photo: Wellcome Collection · CC BY 4.0 (https://creativecommons.org/licenses/by/4.0) · via Wikimedia Commons

Nehemiah Grew was an English physician and a Fellow of the Royal Society, the same society that published Hooke's *Micrographia*. For years he cut thin slices of roots, stems, leaves, flowers, fruits and seeds and studied them with lenses. In 1682 he gathered his work into *The Anatomy of Plants*, filled with large engraved plates. With the Italian Marcello Malpighi, who was doing the same work at the same time, he is remembered as a founder of plant anatomy.

Grew saw that the soft parts of plants were built of countless tiny chambers, with tubes running through the stem to carry sap. He was a devout Christian, and late in life he wrote a book about God's works in creation and in Scripture.

Look closely

- Find the soft tissue drawn as tiny rounded chambers. What do you think each chamber is?
- Find the rings or bundles of tubes in a stem slice. What do you think they carry?
- Compare the size of the chambers in different parts of the slice. Are they all the same?
- Look for anything that tells you whether the slice was cut across the stem or along it.

Talk about it

1. Grew drew plant cells more than 150 years before Schleiden. What did he see correctly, and what could he not yet know?

2. Why do plant tissues show cells so clearly, even with weak lenses, while animal tissues are harder to see?
3. If Grew had drawn a slice of meat, how might it have looked different from his plant slices?

> **Safety first.** An adult should cut the potato and onion with a sharp knife on a cutting board. If you use a microscope, carry glass slides by the edges and let an adult clean up broken glass. Do not eat the potato strips after the experiment. Wash your hands and the work area when you finish.

EXPERIMENT: THE POTATO STRIP OSMOSIS EXPERIMENT

Plant cells gain or lose water by osmosis, and a potato is a convenient bag of millions of plant cells. In this experiment you will soak potato strips in water with different amounts of salt and measure how their mass changes.

Question: How does the amount of salt in the water affect the mass of potato strips? **Prediction:** Write what you expect for plain water and for the saltiest water. **What we change:** the amount of salt (0%, 1%, 3% and 5%). **What we keep the same:** the potato, the size of the strips, the amount of water, the soaking time and the temperature. **What we measure:** the mass of each set of strips before and after soaking, and how firm or floppy they feel.

You will need

- 1 large raw potato
- Kitchen scale that reads to 0.1 g (or 1 g, using bigger strips)
- Table salt and measuring spoons or the scale to weigh it
- 4 cups or jars, each labeled 0%, 1%, 3% and 5%, with 200 mL of water in each
- Ruler, paper towels, a timer, lab notebook and graph paper
- Optional: a red onion, a microscope, slides and cover slips

Steps

1. Make the salt solutions. In the 200 mL cups, dissolve 0 g, 2 g, 6 g and 10 g of salt. (2 g of salt in 200 mL is about 1%, 6 g about 3%, 10 g about 5%.)
2. Have an adult cut 12 potato strips, each about 5 cm long and 1 cm by 1 cm thick. Peel them so the skin does not change the results.
3. Blot the strips dry. Weigh them in groups of three and record each group’s starting mass in a table with columns: Salt %, Mass before (g), Mass after (g), Change (g), Percent change, How it feels.
4. Put one group of three strips into each cup. Make sure they are covered. Leave them for at least 2 hours (overnight in the refrigerator works even better).
5. Take the strips out, blot them gently and the same way each time, and weigh each group again.

6. Calculate the percent change for each group: (mass after − mass before) ÷ mass before × 100. Gains are positive, losses negative.
7. Make a bar graph of percent change against salt concentration. Draw a line at 0% change.
8. Optional: peel a thin layer of red onion skin, mount it in a drop of plain water and look at 100×. Then add a drop of salt water at one edge of the cover slip and draw a paper towel at the other edge to pull it under. Draw what happens to the red cells over five minutes.
9. Write a conclusion: Which way did water move in each cup? About what salt concentration would cause no change?

Tips

- Blotting matters. If one group is dripping wet and another is dry, your masses will not be fair.
- Bend a strip from each cup. The plain-water strips should snap or feel stiff; the saltiest should flop. That is turgor pressure you can feel.

Check your work

- I measured and recorded mass before and after for all four salt concentrations.
- I calculated percent change correctly and made a labeled bar graph.
- I explained my results using the words osmosis, cell membrane, cell wall and turgor pressure.

NATURE NOTEBOOK

1. Draw a plant cell and an animal cell side by side, the same size. Label every shared part in pencil and the three plant-only parts in green. Add a title and a color key.
2. Make a Venn diagram: “Plant cells only,” “Both,” “Animal cells only.” Put at least ten parts in the right places.
3. Sketch three potato strips: one from plain water, one from 1% salt and one from 5% salt. Under each, write its percent change and one sentence about how it felt.

> **From history.** Robert Brown is also remembered for something he saw in 1827. Watching tiny particles that came out of pollen grains floating in water, he noticed they jiggled constantly. At first he wondered if they were alive, so he tested particles of ground glass, rock and soot, and they jiggled too. He concluded the motion was not a sign of life. The jiggling, now called Brownian motion, was explained in 1905 by Albert Einstein as the result of invisible water molecules bumping into the particles.

WORKED EXAMPLES

Example 1. An onion skin cell is about 250 µm long. A cheek cell is about 60 µm across. About how many cheek cells would fit end to end along one onion cell? What is the ratio of their sizes?

1. Divide the larger size by the smaller: 250 µm ÷ 60 µm ≈ 4.2.
2. So about 4 cheek cells would fit along one onion cell.
3. The ratio of onion cell length to cheek cell width is about 4.2 to 1, or roughly 4 to 1.

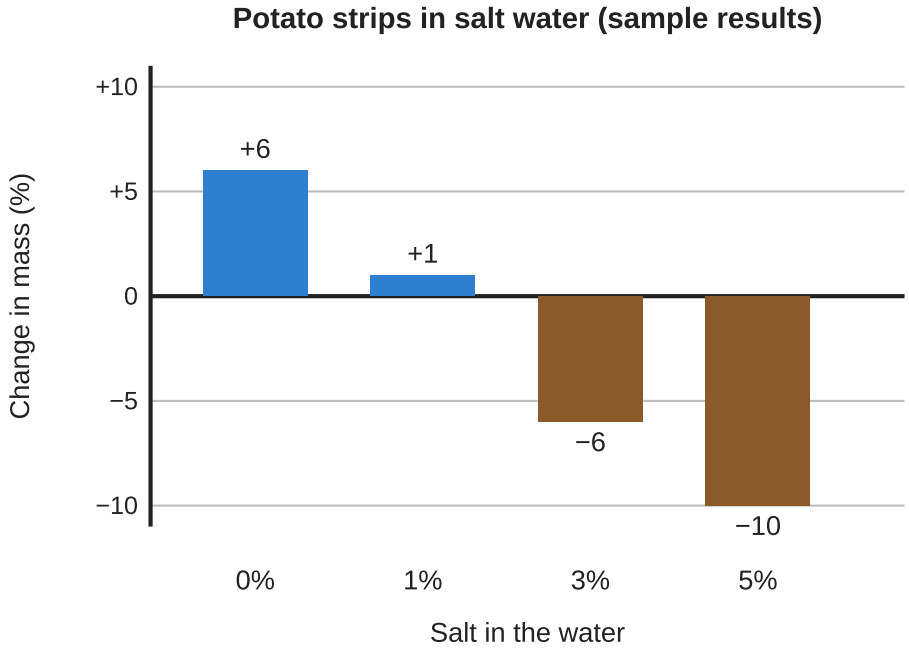
Answer: About 4 cheek cells; the ratio is about 4 to 1.

Example 2. In the potato experiment, a student’s group of strips in plain water went from 10.0 g to 10.6 g. The group in 5% salt water went from 10.0 g to 9.0 g. Find the percent change for each and say which way water moved.

1. Percent change = (mass after − mass before) ÷ mass before × 100.
2. Plain water: (10.6 − 10.0) ÷ 10.0 × 100 = 0.6 ÷ 10.0 × 100 = +6%.
3. 5% salt: (9.0 − 10.0) ÷ 10.0 × 100 = −1.0 ÷ 10.0 × 100 = −10%.
4. A gain means water moved into the potato cells; a loss means water moved out by osmosis into the saltier water.

Answer: Plain water: +6% (water moved in). 5% salt: −10% (water moved out).

Example 3. The graph shows a student's sample results: 0% salt +6%, 1% salt +1%, 3% salt −6%, 5% salt −10%. Estimate the salt concentration at which the potato would neither gain nor lose mass.



Sample results: potato strips gain mass in plain water and lose mass in saltier water.

1. The change crosses from positive to negative between 1% salt (+1%) and 3% salt (−6%).
2. Between those two points the change falls by 7 percentage points (from +1 to −6) over 2% of salt, or 3.5 points for each 1% of salt.
3. To fall from +1 to 0 takes 1 ÷ 3.5 ≈ 0.3% more salt.
4. 1% + 0.3% ≈ 1.3% salt.

Answer: About 1.3% salt. At that concentration the water inside and outside the potato cells is balanced, so there is no net movement of water.

PRACTICE

1. EASY Name the three main parts found in plant cells but not in animal cells.
2. EASY Name four parts that both plant and animal cells have.
3. EASY True or false: plant cells have chloroplasts instead of mitochondria. Explain.
4. MEDIUM Explain why a wilted lettuce leaf becomes crisp again after it sits in cold water.
5. MEDIUM A potato strip went from 8.0 g to 7.2 g in salt water. Find the percent change.
6. MEDIUM A student observed three samples. Sample A: cell wall, chloroplasts, large vacuole. Sample B: no cell wall, no chloroplasts, small vacuoles, irregular shape. Sample C: cell wall, no chloroplasts, large vacuole. The student says C must be an

animal cell because it has no chloroplasts. Is the student right? What could each sample be?

7. HARD Sample data: percent of red blood cells that burst after 10 minutes in salt solutions. 0% salt: 100%. 0.3% salt: 90%. 0.5% salt: 20%. 0.7% salt: 0%. 0.9% salt: 0%. (a) Describe the trend. (b) Why do so many cells burst in pure water? (c) Why would onion cells in pure water not burst?
8. HARD You place an Elodea leaf in strong salt water and watch it under the microscope. Predict what you will see happen to the cell membrane, the chloroplasts and the cell wall, and explain.
9. CHALLENGE Design an experiment to test whether sugar water causes potato strips to lose mass the way salt water does. Give the question, the independent and dependent variables, at least three controlled variables, and a control group.
10. MEDIUM In three or four sentences, explain how the parts of a plant cell fit a plant's way of life, and how an animal cell's lack of a wall fits an animal's way of life.

COMMON MISTAKES

- Thinking plant cells have chloroplasts instead of mitochondria. Plant cells have both; they make sugar in chloroplasts and release its energy in mitochondria.
- Thinking every plant cell is green. Root cells, onion bulb cells and the cells inside a potato have no chloroplasts.
- Thinking the cell wall replaces the cell membrane. A plant cell has both: the membrane controls what goes in and out, and the wall outside it gives support.

> **Make it a game · Plant, Animal or Both?.** Make cards for cell parts: cell wall, chloroplast, central vacuole, nucleus, mitochondrion, ribosome, cell membrane, Golgi apparatus, lysosome, centriole, cytoplasm, cellulose. Draw a card and shout "Plant!", "Animal!" or "Both!" within three seconds. Score a point for each correct answer and a bonus point for naming the part's job. Lysosomes and centrioles count as "Animal" for this game.

> **Scripture.** "And the earth brought forth grass, and herb yielding seed after his kind, and the tree yielding fruit, whose seed was in itself, after his kind: and God saw that it was good." (Genesis 1:12, KJV)

Plants were made to stand rooted in the earth, bear seed and feed the world. Their cell walls, chloroplasts and water-filled vacuoles are exactly what a life like that requires.

Practice answers

1. The cell wall, chloroplasts and a large central vacuole.
2. Any four of: cell membrane, cytoplasm, nucleus, ribosomes, endoplasmic reticulum, Golgi apparatus, mitochondria.
3. False. Plant cells have both. Chloroplasts make sugar using sunlight, and mitochondria release the energy stored in that sugar.

4. Water moves into the leaf cells by osmosis and fills the central vacuoles. The full vacuoles press the cell membranes against the cell walls (turgor pressure), so the cells, and the leaf, become firm.
5. $(7.2 - 8.0) \div 8.0 \times 100 = -0.8 \div 8.0 \times 100 = -10\%$.
6. No. C has a cell wall and a large central vacuole, so it is a plant cell; many plant cells, such as onion bulb or root cells, have no chloroplasts because they grow away from light. A could be a leaf cell (such as Elodea), B an animal cell (such as a cheek cell), and C an onion or root cell.
7. (a) As the salt concentration rises, fewer red blood cells burst; at 0.7% salt and above, none burst. (b) In pure water there is more water outside the cell than inside, so water rushes in by osmosis; with no cell wall, the membrane stretches until it breaks. (c) Onion cells have a cell wall that pushes back as the cell fills, so the cell becomes firm instead of bursting.
8. Water leaves the cells by osmosis. The central vacuole shrinks, and the cell membrane pulls away from the cell wall (plasmolysis), so the cytoplasm and chloroplasts crowd into a smaller clump in the middle. The cell wall keeps its shape because it is stiff.
9. Question: Does the amount of sugar in water affect the mass of potato strips? Independent variable: sugar concentration (for example 0%, 5%, 10%, 20%). Dependent variable: percent change in mass. Controlled variables: same potato, same strip size, same volume of liquid, same soaking time and temperature, same blotting method. Control group: strips in plain water (0% sugar). Use at least three strips per cup, calculate percent change, and graph it. If sugar water causes mass loss as salt does, it shows that osmosis depends on dissolved particles in general, not on salt alone.
10. A plant stays in one place and must hold itself up toward the light, so its cells have stiff walls and water-filled vacuoles that press outward and keep it firm. Chloroplasts let it make its own food from sunlight, since it cannot go and find food. Animals move and must take in food, so their cells have no stiff wall; this lets the cells take many shapes, such as long nerve cells and bending muscle cells, and lets the body move freely.

2.4 Edible Cell Model

OBJECTIVES

- Plan and build a scale model of an animal or plant cell from food, with a key that matches each part to an organelle and its job.
- Calculate a scale factor and use it to find the model size of each organelle, and identify at least two limitations of the model.
- Test whether fresh pineapple keeps gelatin from setting, and explain the result using what you know about proteins and enzymes.

Warm-up. Look around your kitchen and find five things that could stand for parts of a cell: something round and large, something long and folded, something tiny and plentiful, something like a jelly, and something like a bag or box. Write each item next to the cell part you would use it for, and one reason it is a good match.

Video. Coming soon. How to build an edible or 3D cell model, and why fresh pineapple keeps gelatin from setting (the enzyme bromelain); search Amoeba Sisters, Homeschool Pop (Alternate), Science Max, SciShow Kids, and Harvard Museum of Natural History footage of the Blaschka Glass Flowers.

KEY TERMS

model
A simplified copy of a real thing that helps us study or explain it. A model can be a drawing, an object, a set of numbers or a computer program.

scale factor
The number of times bigger (or smaller) a model is than the real thing. A cell model 10,000 times life size has a scale factor of 10,000.

analogy
A comparison that explains something new by showing how it is like something familiar, such as “the nucleus is like an office that keeps the recipe book.”

limitation
A way in which a model is not like the real thing. Every model has limitations.

protein
A large molecule made of long chains of smaller units called amino acids. Proteins build cell parts and do much of the cell’s work. Ribosomes make them.

enzyme
A protein that speeds up a particular chemical change in a living thing, such as cutting other proteins apart. Strong heat can destroy most enzymes.

CORE IDEAS

The story. In 1886 a Harvard botany professor named George Lincoln Goodale had a problem. He wanted his students to study real flowers all year long, but pressed flowers lose their color and shape, and wax models melt or crack. So he traveled to the workshop of Leopold Blaschka and his son Rudolf near Dresden, in Germany. The Blaschkas were glassworkers who had made beautiful glass models of sea creatures for museums. Goodale persuaded them to try plants. For the next fifty years, first together and then Rudolf alone, they made more than 4,000 glass models of plants and flower parts, many of them enlarged so students could see the tiny parts clearly. The Glass Flowers are still on display at the Harvard Museum of Natural History, and they have never wilted.

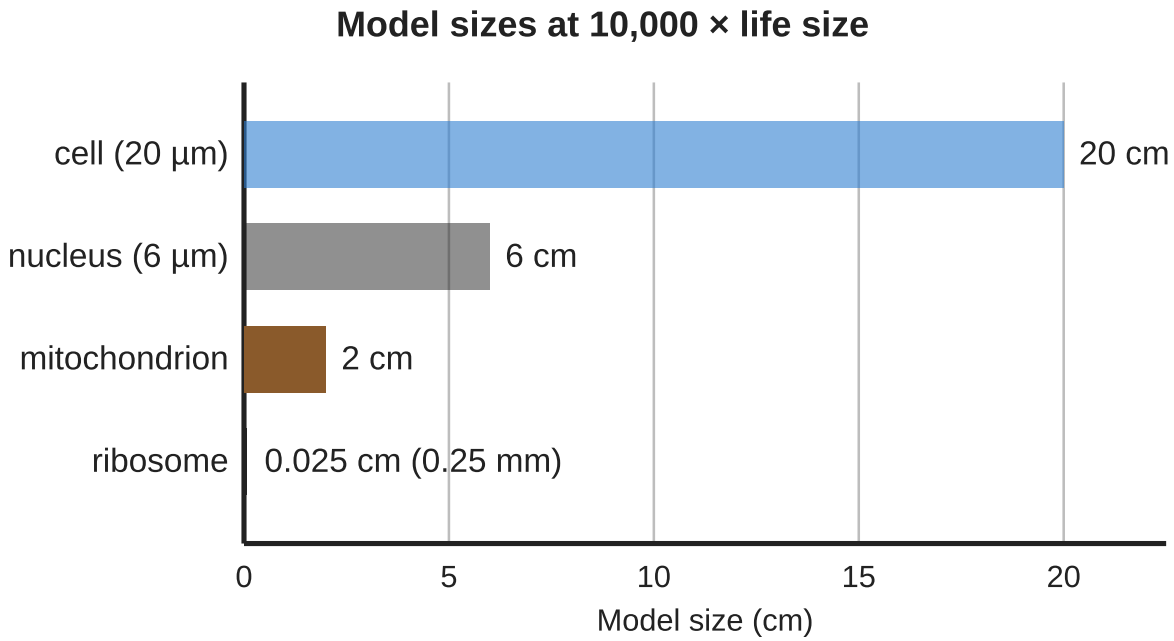
How they figured it out. The Blaschkas were not just artists; they were careful observers. Rudolf studied living plants in gardens and in the field, and he dissected flowers to see their inner parts before shaping them in glass. Every good model maker faces the same three questions. *What will I show?* No model can include everything. *How big will each part be?* That is the question of **scale**. *How will people know what each part stands for?* That is why models come with a key or labels. Your edible cell model must answer the same three questions.

The big idea. A **model** is a simplified copy that helps us understand something too small, too big, too far away or too complicated to see all at once. A cell is about 20 µm across, far too small to handle. If you build it 20 cm across, you have made it 10,000 times bigger, so your **scale factor** is 10,000, and every part must be made 10,000 times bigger too. A 6 µm nucleus becomes 6 cm, about the size of a plum. A 2 µm mitochondrion becomes 2 cm, about the size of a jelly bean. But a ribosome, only 0.025 µm across, becomes 0.25 mm, smaller than a grain of sugar. Even the tiniest sprinkle is too big. That is a **limitation** of the model, a place where it does not match the real thing, and good scientists always say where their models fall short.

Try it and see. Gelatin makes a fine cytoplasm: it is mostly water, it is clear and jelly-like, and it holds things in place. Gelatin is itself made of a **protein**, collagen from animal skin and bones, whose long chains tangle into a net when it cools. That gives you a bonus experiment. Fresh pineapple contains an **enzyme** called bromelain that cuts protein chains apart, so fresh pineapple keeps gelatin from setting. Canned pineapple has been heated, which destroys the enzyme, so it works just fine. Kiwi fruit and papaya have similar enzymes. Your cells are full of enzymes like this; they are proteins built by ribosomes, shipped by the Golgi, and used to speed up thousands of chemical changes every second.

In the world today. Cell biologists still build models, though most are now on computers. Using thousands of electron microscope images taken at different angles, they build three-dimensional pictures of cells and organelles that can be turned and sliced on a screen. Doctors and students use these models the way Goodale’s students used the Glass Flowers: to see clearly what nature hides because it is too small or too delicate to hold.

God's handiwork. The psalmist cried, "O LORD, how manifold are thy works! in wisdom hast thou made them all." Building a model teaches humility. After hours of work, the best edible cell still leaves out most of what a real cell holds: thousands of different proteins, millions of ribosomes, and a constant traffic of molecules in and out. The model helps us see the plan, and it also shows us how much richer the real thing is.



At a scale of 10,000 times, the cell is 20 cm across, but a ribosome is only a quarter of a millimeter, too small to see on the chart.

PICTURE STUDY



Quince, Cabbage, Melon and Cucumber, Juan Sánchez Cotán, about 1602 · Oil on canvas · San Diego Museum of Art, San Diego, California

Juan Sánchez Cotán was a Spanish painter from the region of Toledo. Around 1602 he painted this still life of ordinary foods arranged in a dark stone window frame, a kind of cool pantry where fruits and vegetables were kept fresh. Soon afterward he gave up his career and entered a Carthusian monastery in Granada as a lay brother, where he kept painting for the rest of his life.

His foods are arranged along a gentle curve, from the high hanging quince down to the cucumber poking out over the ledge, and painted with such care that you can almost feel the cabbage leaves and the wet melon flesh. Every one of these foods is built of plant cells.

Look closely

- Find the two foods that hang on strings. Why might people have hung food this way?
- Look at the cut melon. How does the inside look different from the outside rind?
- Follow the curve from the quince down to the cucumber. Where does your eye end up?
- Look at the dark background. What does it do to the colors of the foods?

Talk about it

1. The cabbage leaves look firm and crisp. Using what you learned in Lesson 2.3, what inside the cells keeps them that way?
2. Which of these foods would you expect to have cells with chloroplasts, and which might not? Why?
3. Sánchez Cotán painted plain foods with great care. What do you think he wanted viewers to notice about ordinary things?

> **Safety first.** An adult should boil the water and handle the hot liquid, and an adult should cut the pineapple and other fruit. Ask about food allergies (gelatin, nuts, food dyes) before choosing candies, and keep small, hard candies away from young children, who could choke. Keep the models in the refrigerator, wash your hands before you start, and do not eat any part that has been left out at room temperature for more than two hours.

EXPERIMENT: THE EDIBLE CELL, WITH A PINEAPPLE TEST

You will build a scale model of an animal cell (in a round bowl or a zip-top bag) or a plant cell (in a square or rectangular dish), using gelatin as cytoplasm and foods as organelles. Beside it you will run a small experiment on the protein in the gelatin itself.

Question: Does fresh pineapple keep gelatin from setting, and does heating the pineapple first change the result? **Prediction:** Write yours. **What we change:** what is added to each test cup (nothing, fresh pineapple, canned pineapple, fresh pineapple that was boiled first). **What we keep the same:** amount of gelatin and water, amount of fruit, cup size, time and refrigerator. **What we measure:** whether each cup has set after 4 hours, and how far a marble pressed gently on the surface sinks, in millimeters.

You will need

- Light-colored gelatin (unflavored, lemon or another clear flavor) and the water the package calls for; a clear bowl about 20 cm across or a square dish
- Organelles (choose and adjust to taste): a plum, apricot or large round candy for the nucleus, with a small candy inside for the nucleolus; jelly beans or gummy candies about 2 cm long for mitochondria; ribbon candy, fruit leather or licorice laces folded near the nucleus for the endoplasmic reticulum; a small stack of curved fruit-leather strips or gummy slices for the Golgi apparatus; small round candies for lysosomes; sprinkles for ribosomes
- For a plant cell: green grapes or green candies for chloroplasts, a small clear zip-top bag of water or a large peeled grape for the central vacuole, and the walls of the square dish (or a frame of graham crackers) for the cell wall
- 4 small clear cups for the test, fresh pineapple, canned pineapple, a small pot, a marble and a ruler
- Index card for your key, lab notebook and pencil

Steps

1. Plan first. In your notebook, list each organelle, its real size, its model size at your scale (scale factor 10,000 for a 20 cm animal cell), the food you will use, and one reason that food fits.
2. With an adult, make the gelatin according to the package. Pour a layer about 2 cm deep into the bowl and chill it until it is soft-set (about 1 hour), so the organelles will not sink to the bottom.
3. Meanwhile set up the test cups. Label them A (plain), B (fresh pineapple), C (canned pineapple) and D (fresh pineapple boiled 5 minutes by an adult, then cooled). Put the same amount of fruit, about 2 tablespoons of small pieces, in B, C and D.
4. Pour the same amount of warm gelatin, about 100 mL, into each test cup. Put them in the refrigerator.
5. Arrange the organelles on the soft-set layer of the cell: nucleus near the middle, ER folded beside the nucleus, Golgi near the ER, mitochondria, lysosomes and ribosomes spread through the cytoplasm. For a plant cell, push the chloroplasts and vacuole into place.
6. Pour the rest of the cooled but still liquid gelatin over the organelles and chill until firm (about 3 to 4 hours).
7. After 4 hours, check the test cups. Record whether each has set. Lay the marble gently on the center of each and measure how far it sinks with the ruler.
8. Make the key: an index card listing each food, the organelle it stands for, and the organelle’s job. Write two limitations of your model on the back.
9. Photograph or draw your model before you eat it. Then enjoy your cell, starting with the cytoplasm.

Tips

- The soft-set layer is the secret to a model where the organelles float in place instead of all sinking to the bottom.

- Real organelles are packed close together. Do not be afraid to crowd your cell.
- If cup B stays runny and D sets, you have shown that heat destroys whatever in fresh pineapple stops the gelatin, which is what you would expect of an enzyme.

Check your work

- My model shows at least eight organelles, and my key names each one and its job.
- I calculated the model size of the nucleus and mitochondria from my scale factor.
- I recorded and explained the results of the pineapple test and named two limitations of my model.

NATURE NOTEBOOK

1. Draw your finished cell model from above, as a scientist would, with every part labeled and the scale written in the corner (for example, “10,000 × life size”).
2. Make a table with three columns: Organelle, Real size (µm), Model size at 10,000 × (cm). Fill it in for the cell, nucleus, mitochondrion and ribosome.
3. Draw the four pineapple test cups after 4 hours. Under each, write “set” or “not set” and the marble depth in millimeters.

> **From history.** Gelatin has been made by boiling animal bones and skin for centuries, but it was hard work. In 1845 the American inventor and businessman Peter Cooper received a patent for a powdered gelatin that could be stirred into hot water, and in 1897 the name Jell-O was given to a fruit-flavored gelatin dessert made in Le Roy, New York. Long before either, cooks knew from experience that some fresh fruits ruin a jelly; only later did scientists learn that the cause is an enzyme.

WORKED EXAMPLES

Example 1. You want your animal cell model to be 20 cm across. A real animal cell is about 20 µm across. What is the scale factor? How big should you make a 6 µm nucleus and a 2 µm mitochondrion?

(Use the map or drawing “Model sizes at a scale factor of 10,000.” shown earlier in this lesson.)

1. Put both sizes in the same unit. 20 cm = 200 mm = 200,000 µm.
2. Scale factor = model size ÷ real size = 200,000 µm ÷ 20 µm = 10,000.
3. Nucleus: 6 µm × 10,000 = 60,000 µm = 60 mm = 6 cm.
4. Mitochondrion: 2 µm × 10,000 = 20,000 µm = 20 mm = 2 cm.

Answer: Scale factor 10,000. Nucleus 6 cm (a plum), mitochondrion 2 cm (a jelly bean).

Example 2. A ribosome is about 0.025 µm across. How big would it be at the same scale of 10,000? If your sprinkles are about 1 mm across, how many times too big are they?

1. Model ribosome: 0.025 µm × 10,000 = 250 µm.
2. Convert: 250 µm ÷ 1,000 = 0.25 mm.
3. Compare with a 1 mm sprinkle: 1 mm ÷ 0.25 mm = 4.

Answer: A model ribosome should be 0.25 mm across; a 1 mm sprinkle is 4 times too big. This is a limitation of the model to write on your key.

Example 3. Suppose your gelatin box says one 7 g envelope sets about 480 mL of liquid (check your own box, since brands differ). Your bowl holds 1,200 mL. How many envelopes and how many grams of gelatin do you need?

1. Envelopes needed: 1,200 mL ÷ 480 mL per envelope = 2.5 envelopes.
2. Grams: 2.5 × 7 g = 17.5 g.
3. Since you cannot buy half an envelope, use 3 envelopes and keep the extra for the test cups, or measure 17.5 g on a kitchen scale.

Answer: 2.5 envelopes, or 17.5 g of gelatin.

PRACTICE

1. EASY What is a model, and why do scientists use models of cells?
2. EASY In an edible model, why is gelatin a good choice for cytoplasm?
3. EASY Name two limitations that almost every edible cell model has.
4. MEDIUM At a scale factor of 5,000, how big should you make a 20 µm cell and a 6 µm nucleus, in centimeters?
5. MEDIUM A student's model of a heart muscle cell has 4 jelly-bean mitochondria. A real heart muscle cell can have thousands. Why might the student have used so few, and what should the key say about it?
6. MEDIUM Test cups after 4 hours in the refrigerator. A (plain gelatin): set, marble sank 2 mm. B (fresh pineapple): not set, marble sank to the bottom. C (canned pineapple): set, marble sank 3 mm. D (fresh pineapple boiled first): set, marble sank 3 mm. Which conclusion is best supported? (A) All pineapple stops gelatin from setting. (B) Something in fresh pineapple stops gelatin from setting, and heating destroys it. (C) Pineapple makes gelatin set faster. (D) Gelatin will not set in a refrigerator.
7. HARD At a scale factor of 10,000, a mitochondrion (2 µm long) should be 2 cm. Which food is the best match? Lengths: sprinkle 0.1 cm, small gumdrop 1.2 cm, jelly bean 2.0 cm, gummy worm 6.0 cm. By what percent is the next-closest choice off?
8. HARD Gelatin is made of protein, and bromelain is an enzyme that cuts proteins apart. Explain why fresh pineapple leaves gelatin runny, and why canned pineapple does not.

9. CHALLENGE Design an experiment to test whether kiwi fruit, like fresh pineapple, keeps gelatin from setting. Include the independent and dependent variables, a control, controlled variables and how many cups you will use.
10. MEDIUM In three or four sentences, explain how building an edible cell model is like the work the Blaschkas did on the Glass Flowers.

COMMON MISTAKES

- Thinking a model is an exact copy. Every model leaves things out or changes them, and a good scientist says what the limitations are.
- Making every organelle the same size, or the nucleus tiny. Use the scale factor: the nucleus is one of the largest parts, and ribosomes are by far the smallest.
- Spreading organelles far apart as if they float alone. In a real cell they are crowded, and the endoplasmic reticulum is connected to the envelope around the nucleus.

Make it a game · Model Critic. Trade cell models (or drawings of them) with a sibling or friend. Each critic gets two minutes to find as many limitations as possible: parts that are the wrong size, missing, misplaced or not explained in the key. Score a point for each fair limitation named and a point for each one the builder can answer with a good reason ("I left out ribosomes on the ER because sprinkles would not stick"). Then eat the models.

Scripture. "O LORD, how manifold are thy works! in wisdom hast thou made them all: the earth is full of thy riches." (Psalm 104:24, KJV)

"Manifold" means many and varied. A cell model shows only a few of the riches inside one cell, and there are tens of trillions of cells in your body alone.

Practice answers

1. A model is a simplified copy of a real thing. Scientists use cell models because real cells are too small to see or handle, and a model helps show how the parts are arranged and what they do.
2. Gelatin is mostly water, clear and jelly-like, and it holds the "organelles" in place, much as cytoplasm is a watery gel that holds the organelles.
3. Any two: the ribosomes are far too big; there are far too few ribosomes and mitochondria; the parts do not move, change or work; the model does not show the membrane's double layer or the ER connected to the nuclear envelope; the colors are not real.
4. Cell: 20 µm × 5,000 = 100,000 µm = 100 mm = 10 cm. Nucleus: 6 µm × 5,000 = 30,000 µm = 30 mm = 3 cm.
5. Thousands of jelly beans would not fit and would hide the other parts, so the student chose to show only a few. The key should say that real heart muscle cells have far more mitochondria, because they use a great deal of energy, and that the number in the model is a limitation.
6. (B). Only unheated fresh pineapple stopped the gelatin; canned and boiled pineapple, both heated, did not. This fits the enzyme bromelain, a protein destroyed by heat.
7. The jelly bean (2.0 cm) matches exactly. The next closest is the small gumdrop: (2.0 − 1.2) ÷ 2.0 × 100 = 40% too small.

8. Gelatin sets when its long protein chains tangle into a net as it cools. Bromelain in fresh pineapple cuts those chains into short pieces that cannot form a net, so the gelatin stays liquid. Canned pineapple has been heated during canning; heat changes the shape of the bromelain so it no longer works, and the gelatin sets normally.
9. Independent variable: what is added to the gelatin (nothing, fresh kiwi, kiwi that has been boiled and cooled). Dependent variable: whether the gelatin sets after 4 hours and how far a marble sinks in millimeters. Control: plain gelatin with no fruit. Controlled variables: amount and type of gelatin, amount of water, mass of fruit (for example 30 g), cup size, refrigerator and time. Use three cups for each condition (nine cups in all) so one odd cup does not decide the result.
10. Both had to decide what to show, since no model can include everything. Both had to choose a scale, often enlarging tiny parts so people could see them. Both had to study the real thing carefully first so the model would be accurate, and both made something that helps students learn about living things that are hard to see or keep.

Unit 2 review

Answer each question. Show your work for calculations, include units, and use complete sentences for explanations. The test is worth 60 points.

1. (4 pts) Multiple choice. Which statement is part of the cell theory? (A) Only animals are made of cells. (B) New cells form from a clear fluid. (C) All cells come from cells that already exist. (D) Bigger organisms have bigger cells.
2. (5 pts) What did Matthias Schleiden conclude in 1838, and what did Theodor Schwann conclude in 1839? What structure helped Schwann see the connection?
3. (5 pts) Schleiden and Schwann were wrong about one part of the cell theory. What did they believe, and what did Virchow and others show instead?
4. (5 pts) At 100× the field of view is 1.8 mm across, and 9 cells fit across it end to end. How long is each cell in micrometers? Show your work.
5. (4 pts) Multiple choice. Which organelle builds proteins? (A) mitochondrion (B) ribosome (C) lysosome (D) vacuole
6. (5 pts) Match each organelle to its job: nucleus, mitochondrion, Golgi apparatus, lysosome, cell membrane. Jobs: (a) packages and ships proteins; (b) controls what enters and leaves the cell; (c) holds the DNA; (d) digests worn-out parts; (e) releases energy from sugar using oxygen.
7. (6 pts) A sealed plastic bag of starch mixture was placed in water with iodine. Color scores inside the bag (0 to 4): 0 min 0, 10 min 1, 20 min 3, 30 min 4. Outside the bag: 0 at every time. (a) Which substance crossed the bag? (b) Which did not? (c) What property of a cell membrane does this model?
8. (6 pts) Diagram item. Draw a plant cell and label six parts: cell wall, cell membrane, nucleus, cytoplasm, chloroplast and central vacuole. Then circle the three labels for parts that animal cells do not have.
9. (5 pts) A classmate says, “Plant cells don’t need mitochondria because they have chloroplasts.” Explain why this is wrong.
10. (5 pts) A potato strip had a mass of 12.0 g before soaking in salt water and 10.8 g after. Find the percent change, and explain which way water moved and why.
11. (5 pts) You build a cell model 30 cm across of a cell that is really 20 µm across. What is the scale factor? How big should a 6 µm nucleus be in the model?
12. (5 pts) Gelatin with fresh pineapple did not set, but gelatin with canned pineapple did. Explain the result, and name one limitation of an edible cell model.

Unit project: my cell atlas and model

Make a cell atlas that brings together your work from this unit. Include your microscope survey table and sketches from Lesson 2.1 with a cell size calculated for at least two samples; a large, labeled diagram of an animal cell and a plant cell side by side with a key to each organelle’s job; your potato osmosis data table and bar graph from Lesson 2.3; and a photograph or drawing of your edible cell model with its key, scale-factor calculations, pineapple test results and two limitations. Finish with a one-page essay, “What Schleiden

and Schwann Would Think of My Cell," explaining the cell theory and what scientists have learned about cells since 1839.

- **Accuracy**: Organelles are correctly named, placed and described; plant and animal differences are correct; the cell theory is stated correctly.
- **Data and calculations**: Tables are complete, measurements have units, and cell sizes, percent changes and scale factors are calculated correctly with work shown.
- **Scientific thinking**: Experiments name the variables kept the same and changed, conclusions are supported by the data, and model limitations are honestly stated.
- **Care and presentation**: Drawings are neat, labeled and titled; the atlas is organized and easy to follow; the essay is clear and in complete sentences.

Answer key

1. (C).
2. Schleiden concluded that all parts of plants are made of cells. Schwann concluded that animals are made of cells too, so plants and animals are built on the same plan. The nucleus, which Schleiden described in plant cells and Schwann recognized in tadpole notochord cells, showed the connection.
3. They believed new cells formed out of a clear fluid, something like crystals. Remak, Virchow and others observed that new cells come only from the division of existing cells; Virchow made famous the saying "every cell from a cell" (omnis cellula e cellula) in 1855.
4. 1.8 mm = 1,800 µm. 1,800 µm ÷ 9 = 200 µm per cell.
5. (B) ribosome.
6. Nucleus (c); mitochondrion (e); Golgi apparatus (a); lysosome (d); cell membrane (b).
7. (a) Iodine moved into the bag, turning the starch blue-black. (b) Starch did not move out, since the outside never changed color. (c) Selective permeability: the membrane lets some (small) molecules through and blocks others.
8. A boxy cell with a thick outer wall, a thin membrane just inside it, a nucleus, cytoplasm, several chloroplasts and a large central vacuole, all correctly labeled. The circled labels are cell wall, chloroplast and central vacuole. (Award 1/2 point for each correct label, 3 points in all; 1 point for drawing the wall outside the membrane; and 2 points for circling the correct three.)
9. Plant cells have both. Chloroplasts capture light energy and store it in sugar, but the plant still needs mitochondria to release the energy from that sugar for the cell's work, day and night, including in root cells that have no chloroplasts.
10. (10.8 − 12.0) ÷ 12.0 × 100 = −1.2 ÷ 12.0 × 100 = −10%. Water moved out of the potato cells by osmosis, from where there was more water (inside the cells) to where there was less water and more dissolved salt (the salt water).
11. 30 cm = 300,000 µm. Scale factor = 300,000 ÷ 20 = 15,000. Nucleus: 6 µm × 15,000 = 90,000 µm = 9 cm.
12. Fresh pineapple contains bromelain, an enzyme (a protein) that cuts apart the protein chains in gelatin so they cannot form a net. Canned pineapple has been heated, which destroys the enzyme, so the gelatin sets. Limitation (any one): the ribosomes are far too large and too few; the parts do not move or work; the model is not to scale in every part; it leaves out most of the cell's molecules.

UNIT 3

Single-Celled Life

In Unit 1 you looked through a microscope like Leeuwenhoek, and in Unit 2 you learned that every living thing is made of cells. Now you will meet living things that are only **one cell** and yet do everything a living thing must do: take in food, grow, respond, get rid of wastes and reproduce after their kind. You will watch bacteria turn milk into yogurt, grow protists in a jar of hay water, count the bubbles of yeast that raise a loaf of bread, and culture microbes from your own kitchen counter, safely, the way real microbiologists do.

Along the way you will meet the people who learned to study these tiny lives: Hans Christian Gram with his violet stain in Berlin, Alphonse Laveran and Ronald Ross tracking the malaria parasite from blood to mosquito, Emil Christian Hansen growing pure yeast from a single cell in Copenhagen, and Robert Koch's laboratory, where Fanny Hesse's kitchen jelly and Julius Petri's simple glass dish became the tools of every microbiology lab in the world.

You will work like a scientist the whole way: calculating doubling and growth rates, reading growth curves and bar graphs, using ratios and percentages, and designing fair tests with controls. And you will see that the smallest living things are no accident. As the psalmist said, "O LORD, how manifold are thy works! in wisdom hast thou made them all" (Psalm 104:24).

BEFORE YOU BEGIN

- Can focus a microscope at 100× and 400× and make a wet-mount slide (Unit 1)
- Can name the main organelles and explain osmosis (Unit 2)
- Can multiply, divide and find percents, and convert millimeters to micrometers
- Can read a bar graph and a line graph and name independent and dependent variables

MASTERY CHECKLIST

- I can describe a bacterial cell, explain why bacteria are prokaryotes, and identify the three common bacterial shapes and the two Gram-stain groups.
- I can describe animal-like, plant-like and fungus-like protists and explain how amoebas, paramecia and euglenas move and feed.
- I can explain how yeast ferments sugar and how yeast, sugar and gluten make bread rise.
- I can prepare, seal, incubate and dispose of a culture plate safely and explain the reason for each safety rule.
- I can calculate doubling, rates, percent reductions and dilutions from microbe data and read growth curves.
- I can design a fair experiment with a control and explain what my results do and do not prove.

OPTIONAL BOOKS AND RESOURCES

- **Cells Alive** (https://www.cellsalive.com/): Free. Interactive diagrams of a bacterial cell and other cells you can explore part by part. Useful for review with Lessons 3.1 and 3.2.
- **Carolina Biological Supply** (https://www.carolina.com/): A long-established science supplier that sells ready-made sterile nutrient agar plates (the safest choice for Lesson 3.4) and live cultures of *Paramecium, Amoeba* and *Euglena* if you cannot find them in pond water for Lesson 3.2.
- **King Arthur Baking** (https://www.kingarthurbaking.com/): Free recipes and clear guides to yeast bread and sourdough, with explanations of kneading, rising and baker's percentages. Good for the optional loaf in Lesson 3.3.

3.1 Bacteria ★

OBJECTIVES

- Describe the structure of a bacterial cell (cell wall, cell membrane, cytoplasm, ribosomes, nucleoid DNA, and often flagella) and explain why bacteria are called prokaryotes.
- Tell how Hans Christian Gram's stain (1884) sorted bacteria into two great groups, and identify bacteria by their three common shapes.
- Calculate bacterial growth by doubling, read a growth curve, and name helpful as well as harmful things bacteria do.

Warm-up. Put a spoonful of plain yogurt in a small dish and a spoonful of milk in another. Look, smell (gently waft it toward your nose) and stir each with a spoon. List three differences. The milk became yogurt because of living things far too small to see. Write a guess: how many of them might be in that one spoonful?

Video. Coming soon. Bacteria under the microscope: shapes (cocci, bacilli, spirilla), binary fission time-lapse, and the Gram stain procedure; search Amoeba Sisters, Journey to the Microcosmos, SciShow Kids, Homeschool Pop (Alternate), and university microbiology lab demonstration channels.

KEY TERMS

bacterium (plural: bacteria)
A single-celled living thing with no nucleus. Its DNA lies loose in the cytoplasm in a region called the nucleoid.

prokaryote
A cell without a nucleus or other membrane-wrapped organelles. Bacteria (and the similar-looking archaea) are prokaryotes. Cells with a nucleus, like yours, are eukaryotes.

binary fission
The way bacteria reproduce: one cell copies its DNA, grows and splits into two identical cells.

Gram stain
A staining method invented by Hans Christian Gram in 1884. Gram-positive bacteria, with thick cell walls, stay purple; Gram-negative bacteria, with thin walls and an outer membrane, lose the purple and are counterstained pink.

flagellum (plural: flagella)
A long, whip-like tail that some cells spin or lash to swim.

endospore
A tough, resting form that some bacteria make when conditions turn harsh. It can survive boiling, drying and years of waiting, then grow again.

CORE IDEAS

The story. In 1884 a young Danish doctor named Hans Christian Gram was working in the morgue of the city hospital in Berlin, in the laboratory of Carl Friedländer. He was studying lung tissue from patients who had died of pneumonia, and he had a problem: under the microscope the tiny bacteria and the lung cells around them were all the same faint color. Gram tried soaking his slides in a violet dye, then in an iodine solution, then washing them with alcohol. Something surprising happened. Some bacteria held on to the deep violet color, while others washed out pale. Gram published his method that year, and in his short paper he modestly admitted that it still had faults. It became the most used stain in all of microbiology.

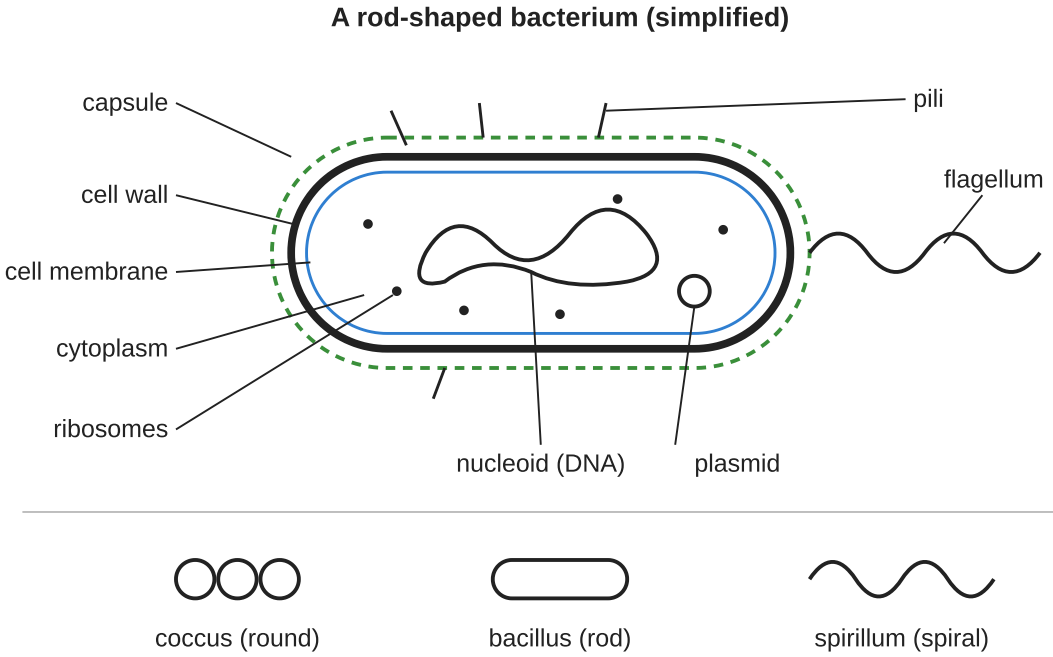
How they figured it out. Later scientists added a pink dye, called a counterstain, at the end, so the pale bacteria show up pink. Gram's method sorts bacteria into two great groups. **Gram-positive** bacteria stay purple because they have a thick cell wall that traps the dye. **Gram-negative** bacteria turn pink because their wall is thin and wrapped in an extra outer membrane, which the alcohol breaks open. Gram did not know why his stain worked; that answer came with the electron microscope many years later. But even without knowing why, doctors could now tell bacteria apart in hours, and that still helps them choose a treatment today.

The big idea. A **bacterium** is one complete living cell, usually only 1 to 5 micrometers (µm) long. It is a **prokaryote**: it has no nucleus. Its DNA is a single loop lying in a region of the cytoplasm called the nucleoid, often with a few tiny extra rings of DNA called plasmids. It has ribosomes to build proteins, a cell membrane, and outside that a stiff cell wall. Many bacteria have a slimy outer capsule, short hair-like pili for sticking to things, or one or more **flagella** for swimming. Bacteria come in three common shapes: round **cocci**, rod-shaped **bacilli** and spiral **spirilla**. When food and warmth are right, a bacterium reproduces by **binary fission**, copying its DNA and splitting in two. The common gut bacterium *E. coli* can divide every 20 minutes in ideal lab conditions. When conditions turn harsh, some bacteria form **endospores** that can wait out boiling and drought.

In the world today. Most bacteria are not germs. Your own body carries roughly as many bacterial cells as human cells, most of them in your large intestine, where they help digest food and make vitamin K. Bacteria in the soil break down dead leaves and animals and return their materials to the earth. Bacteria living in little bumps on the roots of beans, peas and peanuts take nitrogen from the air and turn it into a form plants can use, which is why George Washington Carver urged Southern farmers to plant peanuts and cowpeas to restore worn-out cotton fields. Bacteria turn milk into yogurt and cheese and cabbage into sauerkraut. A small number do cause disease, such as strep throat, tuberculosis and cholera, and you will meet the people who fought them in Unit 4. Scientists today also recognize a second group of prokaryotes, the **archaea**, which look like bacteria but differ in their chemistry and often live in extreme places such as hot springs and salt flats.

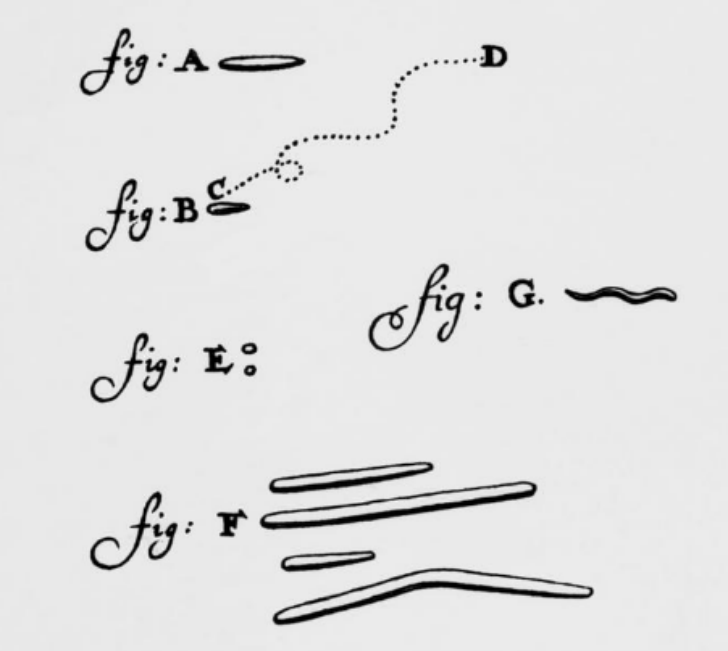
God's handiwork. A bacterium is one of the smallest living things we know, yet it is no simple blob. In a space hundreds of times narrower than a pencil line it takes in food, builds

thousands of kinds of proteins, copies its DNA without error almost every time, and swims with a rotating flagellum that works like a tiny motor. Each kind reproduces after its kind: a rod gives rise to rods and a coccus to cocci. The psalmist was right to say, “O LORD, how manifold are thy works!”



A typical rod-shaped bacterium (simplified) and the three common bacterial shapes. Not every bacterium has a capsule, pili or a flagellum.

PICTURE STUDY



Bacteria from the Human Mouth, Antonie van Leeuwenhoek (drawings), engraved for the Royal Society, 1683 (letter); printed 1684 · Engraving made from Leeuwenhoek's drawings, printed in the *Philosophical Transactions* of the Royal Society · Royal Society, London

Photo: Huydang2910 · CC BY-SA 4.0 (https://creativecommons.org/licenses/by-sa/4.0) · via Wikimedia Commons

On 17 September 1683, Antonie van Leeuwenhoek of Delft sent a letter to the Royal Society in London. He had scraped the white matter from between his teeth, mixed it with clean rainwater and looked at it through one of his tiny single-lens microscopes. He saw "animalcules" of several kinds, some darting about, and he drew them. His drawings were engraved and printed the next year.

Today these pictures are counted among the first drawings of bacteria ever made. Leeuwenhoek had no stain and no names for what he saw, but his shapes are so accurate that microbiologists can still recognize rods, round cells and spirals in them.

Look closely

- Find the rod-shaped creatures. Which word from this lesson names that shape?
- Find the round dots in clusters and the thin spiral. Name each shape.
- Find the dotted line. Leeuwenhoek used it to show the path one creature swam. What does that tell you it might have had?
- Notice how small and simple the figures are. Why do you think Leeuwenhoek did not draw any inside parts?

Talk about it

1. Leeuwenhoek saw these bacteria about 200 years before Gram invented his stain. What could Gram's method tell a scientist that Leeuwenhoek's drawings could not?

2. In a letter of 1692, Leeuwenhoek reported that he found almost no living animalcules on his front teeth, and he blamed the very hot coffee he drank each morning; farther back in his mouth, where the coffee could not reach, he found great numbers. What experiment could test whether heat kills mouth bacteria?
3. Why are careful drawings still valuable to scientists, even with cameras?

> **Safety first.** This project uses a stove and milk heated until it steams (about 85 °C). An adult should heat and pour the milk, and you should use oven mitts. Use only clean jars and spoons. Treat the jars as an experiment: do not eat from the refrigerator jar, the room-temperature jar or the control jar. With a parent's approval you may taste the warm jar only if it has set firmly and smells clean and tangy. Wash your hands before and after.

EXPERIMENT: DOES TEMPERATURE CHANGE HOW FAST BACTERIA MAKE YOGURT?

Yogurt is made by two kinds of bacteria, *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. As they grow they change milk sugar (lactose) into lactic acid, and the acid makes the milk proteins thicken and turn tangy. Because the bacteria are alive, their speed should depend on temperature.

Question: At which temperature do yogurt bacteria thicken milk fastest? **Prediction:** Write which jar you think will set first. **What we change:** the temperature (refrigerator, room, warm). **What we keep the same:** the kind and amount of milk, the amount of starter, the jars, the starting time. **What we measure:** thickness every 2 hours, scored 0 (runny as milk) to 3 (holds its shape when the jar is tipped). **Control:** a warm jar of milk with no starter.

You will need

- 1 liter of whole or 2% milk
- 3 tablespoons of plain yogurt labeled "live and active cultures" (the starter)
- 4 clean glass jars with lids (about 250 mL each), labeled A to D
- Saucepan, kitchen thermometer, measuring cup, spoons, oven mitts
- A warm place near 43 °C: an oven with only the light on, or a small cooler filled with water at about 43 °C
- Lab notebook and timer

Steps

1. With an adult, heat the milk on the stove, stirring, until it steams and reaches about 85 °C. Turn off the heat and let it cool to about 43 °C. Check with the thermometer.
2. Pour 250 mL of warm milk into each jar. Stir 1 tablespoon of starter into jars A, B and C. Put nothing in jar D (the control). Close the lids.
3. Place jar A in the refrigerator (about 4 °C), jar B on the counter (about 20 °C), and jars C and D in the warm place (about 43 °C). Record the starting time and each temperature.
4. Every 2 hours for 8 hours, gently tip each jar and score its thickness from 0 to 3. Do not stir. Record your scores in a table.

5. Check all four jars again at 24 hours and record the scores.
6. Make a line graph with time (hours) on the horizontal axis and thickness score on the vertical axis, with one line for each jar.
7. Write a conclusion. Which jar set first? Why did the control jar not set? Use the words bacteria, lactic acid and temperature.

Tips

- If the warm place cools down, add a little hot tap water to the cooler to bring it back near 43 °C.
- Heating the milk first kills most of the bacteria already in it, so the starter bacteria have the milk to themselves.
- If your warm jar has not set by 8 hours, check whether the starter really said “live and active cultures.”

Check your work

- I kept every variable the same except temperature and used a control jar.
- I recorded scores every 2 hours and graphed them.
- I can explain how living bacteria turned milk into yogurt.

NATURE NOTEBOOK

1. Draw a large rod-shaped bacterium and label eight parts: capsule, cell wall, cell membrane, cytoplasm, ribosomes, nucleoid, plasmid and flagellum. Under it, write one sentence on how it differs from the animal cell you drew in Unit 2.
2. Draw the three bacterial shapes (coccus, bacillus, spirillum) as they would look in a Gram stain: color two of them purple and one pink, and label each Gram-positive or Gram-negative.
3. Draw a four-step comic strip of binary fission: one cell, DNA copied, cell pinching in the middle, two cells. Label the time under each frame for *E. coli* in ideal conditions.

> **From history.** In the 1870s the German botanist Ferdinand Cohn, working in Breslau, published some of the first careful classifications of bacteria, sorting them by shape into round cells, short rods, long threads and spirals. In 1876 he showed that the hay bacillus, *Bacillus subtilis*, forms tough endospores that can survive boiling. That discovery explained why some broths spoiled even after they had been boiled, and it helped settle the long argument over spontaneous generation that you will study with Pasteur in Unit 4.

WORKED EXAMPLES

Example 1. In ideal lab conditions, a single *E. coli* cell divides every 20 minutes. If nothing stops it, how many cells will there be after 3 hours?

1. Find the number of doublings: 3 hours = 180 minutes. 180 ÷ 20 = 9 doublings.
2. Each doubling multiplies the number by 2, so the number of cells is 2 multiplied by itself 9 times: 2^9.
3. Count it out: 2, 4, 8, 16, 32, 64, 128, 256, 512.
4. In real life, food and space run out, so growth slows long before the numbers become enormous.

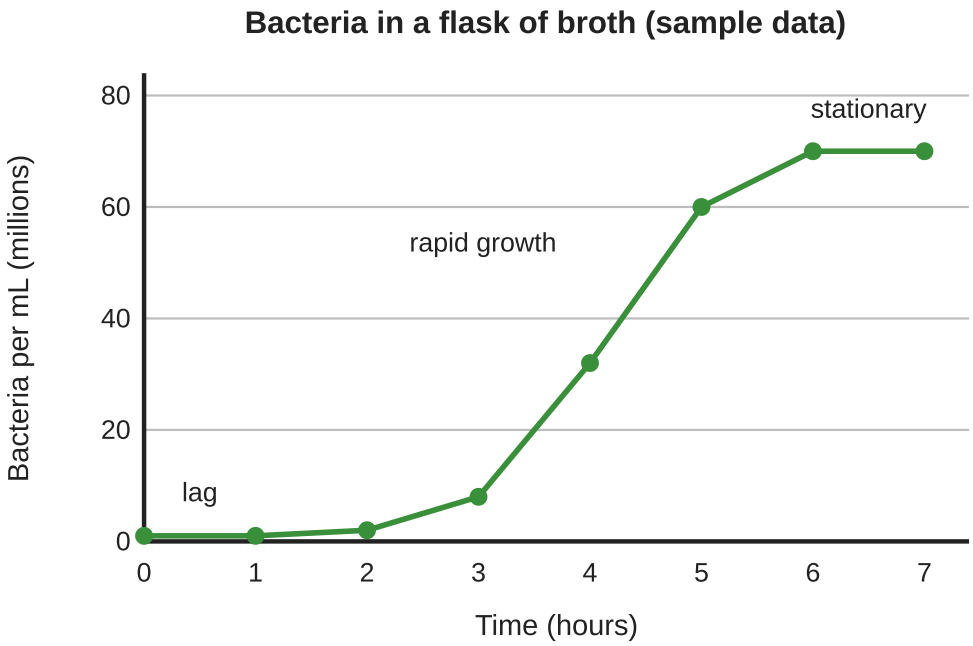
Answer: 9 doublings, giving $2^9 = 512$ cells after 3 hours.

Example 2. A rod-shaped bacterium is 2 µm long. How many of them, laid end to end, would stretch across 1 mm? Across the 0.5 mm width of a pencil line?

1. Convert millimeters to micrometers: 1 mm = 1,000 µm.
2. Divide: 1,000 µm ÷ 2 µm = 500 bacteria per millimeter.
3. A pencil line 0.5 mm wide is 500 µm. 500 µm ÷ 2 µm = 250 bacteria.

Answer: 500 bacteria would stretch across 1 mm, and 250 across a pencil line.

Example 3. The graph shows sample data for practice: a flask of broth was given a few bacteria and the number of bacteria per mL (in millions) was measured each hour. Readings: 0 h, 1; 1 h, 1; 2 h, 2; 3 h, 8; 4 h, 32; 5 h, 60; 6 h, 70; 7 h, 70. (a) What was the doubling time between hours 3 and 4? (b) Why did growth stop after hour 6?



Sample practice data: a bacterial growth curve in a flask of broth.

1. From hour 3 to hour 4 the count went from 8 million to 32 million. 32 ÷ 8 = 4, so the number doubled twice (8 to 16 to 32).
2. Two doublings in 60 minutes means one doubling every 60 ÷ 2 = 30 minutes.
3. At first (hours 0 to 1) the count did not change: the bacteria were adjusting to the broth. This is the lag phase.
4. After hour 6 the count stayed at 70 million. The bacteria had used up much of the food and built up wastes, so new cells formed only as fast as old ones died. This is the stationary phase.

Answer: (a) About 30 minutes. (b) Food was running out and wastes were building up, so the population leveled off (stationary phase).

PRACTICE

1. EASY Why is a bacterium called a prokaryote?
2. EASY Name the three common shapes of bacteria and describe each.
3. EASY In a Gram stain, one kind of bacterium is purple and another is pink. Which is Gram-positive, and what about its cell wall makes it stay purple?
4. MEDIUM Name three ways bacteria help people or the living world.

5. MEDIUM A bacterium divides every 30 minutes. Starting with 1 cell, how many cells will there be after 4 hours?
6. MEDIUM A coccus is 1 µm across. How many would fit side by side across the 0.08 mm width of a human hair?
7. MEDIUM Use the growth curve (l7-3-1-growth-curve). Which statement is best supported by the data? (A) The bacteria grew fastest in the first hour. (B) The population increased most between hours 3 and 5. (C) The population was still doubling every 30 minutes at hour 7. (D) The bacteria died off completely after hour 6.
8. HARD A student tested how long it took milk with yogurt starter to set at different temperatures. Results: 4 °C, did not set in 24 h; 20 °C, set at about 20 h; 32 °C, set at 8 h; 43 °C, set at 5 h; 60 °C, did not set in 24 h. (a) What is the best temperature in this data? (b) Suggest a reason the 60 °C jar did not set, and a reason the 4 °C jar did not set.
9. CHALLENGE Design an experiment to test whether salt slows the growth of yogurt bacteria. Name the independent variable, the dependent variable, three things to keep the same, and the control.
10. HARD In three or four sentences, explain why Gram's stain was so useful even though Gram did not know why it worked.

COMMON MISTAKES

- Thinking all bacteria are germs. Only a small fraction cause disease; most are harmless, and many are necessary for soil, food and our own digestion.
- Thinking bacteria have a nucleus like our cells. Bacteria are prokaryotes: their DNA lies loose in the cytoplasm.
- Confusing bacteria with viruses. A bacterium is a complete living cell that can grow and divide on its own; a virus is far smaller, is not a cell, and can only make copies of itself inside a living cell.

> **Make it a game · Coccus, Bacillus, Spirillum.** One player calls out a shape name and everyone must make that shape with their body: curl into a ball for coccus, stand straight and stiff for bacillus, twist like a corkscrew for spirillum. The caller then says "Gram stain!" and points to a player, who must say whether their bacterium is purple or pink and why. Then call "Fission!" and every player must find a partner and split apart. Anyone who makes the wrong shape becomes the next caller.

> **Scripture.** "O LORD, how manifold are thy works! in wisdom hast thou made them all: the earth is full of thy riches." (Psalm 104:24, KJV)

"Manifold" means many and varied. The earth is full of God's riches down to its smallest living things: the soil, the sea and even our own bodies teem with bacteria doing work that keeps the world alive.

Practice answers

1. Because it has no nucleus (and no other membrane-wrapped organelles). Its DNA lies in the cytoplasm in the nucleoid region.
2. Coccus (round), bacillus (rod-shaped) and spirillum (spiral).
3. The purple one is Gram-positive. It has a thick cell wall that traps the violet dye so the alcohol cannot wash it out.
4. Any three: digesting food and making vitamin K in our intestines; breaking down dead plants and animals in the soil; turning nitrogen from the air into a form plants can use (in root nodules of beans and peanuts); making yogurt, cheese and sauerkraut.
5. 4 hours = 240 minutes; 240 ÷ 30 = 8 doublings; 2^8 = 256 cells.
6. 0.08 mm = 80 µm; 80 µm ÷ 1 µm = 80 cocci.
7. (B). The count rose from 8 million to 60 million between hours 3 and 5, the steepest part of the curve. It was flat in the first hour (A wrong), flat at hour 7 (C wrong), and steady, not zero, after hour 6 (D wrong).
8. (a) 43 °C, which set fastest. (b) At 60 °C the heat probably killed the bacteria. At 4 °C the bacteria were alive but grew too slowly in the cold to make enough acid in 24 hours.
9. Independent variable: amount of salt added to each jar of milk (for example 0 g, 2 g, 5 g, 10 g per 250 mL). Dependent variable: time for the milk to set (or thickness score at 6 hours). Keep the same: kind and amount of milk, amount of starter, temperature (about 43 °C), jars, and timing of checks. Control: the jar with 0 g of salt. Repeat each amount at least twice.
10. Gram’s stain reliably sorted bacteria into two groups, purple Gram-positive and pink Gram-negative, which helped doctors and scientists tell bacteria apart quickly. A method can be useful as long as it gives the same result every time, even before the reason is understood. Later, the electron microscope showed that Gram-positive bacteria have thick walls that trap the dye while Gram-negative bacteria have thin walls and an outer membrane. Knowing the group still helps doctors choose treatments today.

3.2 Protists ★

OBJECTIVES

- Define a protist as a eukaryote that is not a plant, animal or fungus, and describe animal-like, plant-like and fungus-like protists with examples.
- Explain how amoebas, paramecia and euglenas move, feed and get rid of extra water (pseudopods, cilia, flagella, contractile vacuoles).
- Tell how Alphonse Laveran (1880) and Ronald Ross (1897) traced malaria to a protist carried by mosquitoes, and calculate speeds and rates from protist observations.

Warm-up. Fill a clear glass with water and set it in a sunny window beside a second glass kept in a dark cupboard. Drop a pinch of dried grass or a few crumbled dead leaves into each. Predict: in a week, which glass will turn green, and why? Leave them for the project.

Video. Coming soon. Living protists under the microscope: amoeba engulfing food, paramecium cilia and contractile vacuole, euglena swimming, diatoms and slime molds; search Journey to the Microcosmos, Amoeba Sisters, Homeschool Pop (Alternate), SciShow Kids, and pond-water microscopy footage.

KEY TERMS

protist
A eukaryote (a living thing whose cells have a nucleus) that is not a plant, an animal or a fungus. Most are single-celled and live in water or damp places.

protozoa
Animal-like protists that take in food, such as amoebas and paramecia. The word means “first animals,” though they are not animals.

algae
Plant-like protists that make their own food by photosynthesis, from single-celled diatoms to giant seaweeds such as kelp.

pseudopod
A “false foot”: a bulge of cytoplasm that an amoeba pushes out to crawl and to surround food.

cilia
Many short, hair-like structures that beat together like oars, moving a cell or sweeping food toward it.

contractile vacuole
A small sac that collects extra water that has seeped into a freshwater protist and squeezes it out, so the cell does not burst.

CORE IDEAS

The story. On 6 November 1880, in a French military hospital in Constantine, Algeria, an army doctor named Alphonse Laveran was looking at a drop of fresh blood from a soldier sick with malaria. Malaria, with its shaking chills and burning fevers, often killed more soldiers than battle did, and most doctors thought it came from bad air rising from swamps. (The name comes from Italian words meaning “bad air.”) In the red blood cells Laveran saw dark specks of pigment and odd crescent-shaped bodies. Then, as he watched, thin moving threads burst out from one of the bodies and lashed about among the blood cells. These were not bacteria. They were living things with a nucleus, and they were living inside human blood cells.

How they figured it out. Laveran had found the cause, a single-celled parasite now called *Plasmodium*. But how did it get from one person to another? A British army doctor in India, Ronald Ross, suspected mosquitoes. For two years he let mosquitoes feed on malaria patients and then cut the insects open, looking at their stomachs under his microscope. Most were the wrong kind. On 20 August 1897 in Secunderabad, he examined a kind he called “dapple-winged,” now known as *Anopheles*, and found round cells with the same dark pigment in its stomach wall. The parasite was growing inside the mosquito. Ross later showed the full cycle in birds. Laveran received a Nobel Prize in 1907, and Ross in 1902. Draining standing water and screening windows against mosquitoes became the main way to fight the disease.

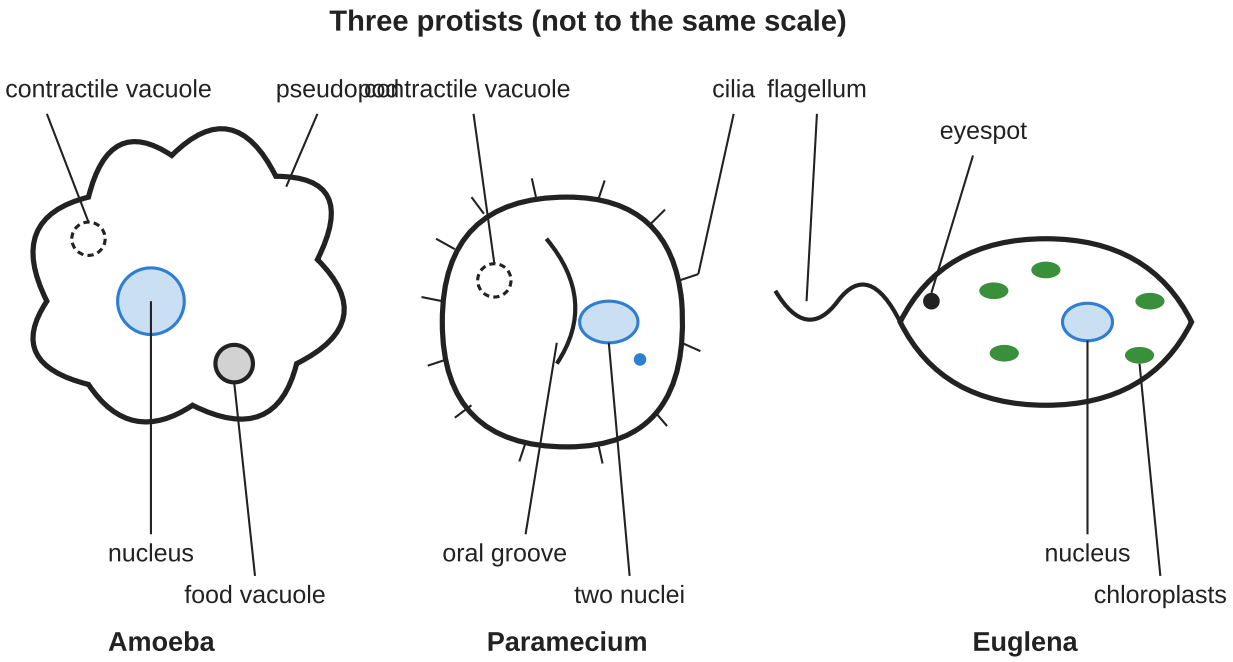
The big idea. *Plasmodium* is a **protist**. Protists are eukaryotes, so their cells have a nucleus and organelles like ours, but they are not plants, animals or fungi. They are so varied that biologists often call this group the “junk drawer” of living things. It helps to sort them by how they live. **Animal-like protists**, or **protozoa**, take in food. An **amoeba** crawls by pushing out **pseudopods** and wraps them around a bit of food, which becomes a food vacuole. A **paramecium** is shaped like a slipper and covered with thousands of **cilia** that row it through the water and sweep food into its oral groove. **Plant-like protists**, the **algae**, make food by photosynthesis. They range from glass-shelled diatoms to giant kelp, a seaweed that can grow more than 30 meters long. **Fungus-like protists**, such as slime molds and water molds, absorb food from dead or living things.

Try it and see. Some protists refuse to fit a single group. **Euglena** swims with a whip-like **flagellum**, steers toward light using a red eyespot, and makes food with its chloroplasts in sunlight, yet in the dark it can absorb food from the water around it. Freshwater protists face a constant danger: water seeps into them by osmosis, as it did into the potato strips in Unit 2. A **contractile vacuole** collects the extra water and squeezes it out, again and again, every few seconds. In this lesson’s project you will grow your own protists in a hay infusion and count them.

In the world today. Protists matter far beyond the pond. Tiny ocean algae, together with photosynthetic bacteria, make up the drifting phytoplankton, which scientists estimate produce about half of the oxygen made on earth and feed the tiny animals that fish and whales eat. The glassy shells of diatoms pile up on the sea floor and are mined as

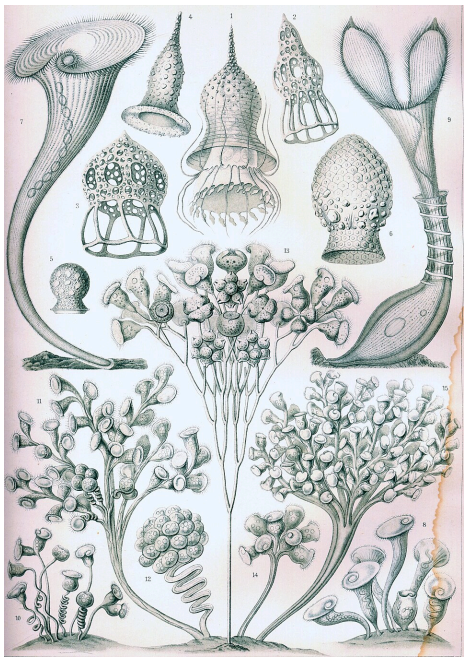
diatomaceous earth, used in filters and gentle scouring powders. A few protists cause disease, including malaria, which is still common in tropical lands, and the “beaver fever” some campers get from drinking unfiltered stream water.

God’s handiwork. A paramecium has no brain, no muscles and no legs, yet it finds food, backs away from obstacles, swims in spirals, pumps out water and divides in two. Job told his friends to ask the beasts and the fishes of the sea, and they would teach them. A single drop of pond water is a classroom of that kind.



Three common protists (not drawn to the same scale). A paramecium is about 0.2 mm long; amoebas and euglenas vary in size.

PICTURE STUDY



Ciliata, Plate 3 from Kunstformen der Natur (Art Forms in Nature), Ernst Haeckel, 1899–1904 · Color lithograph printed from the artist's drawings · Published in Leipzig; copies are kept in many libraries

Ernst Haeckel was a German zoologist who spent years studying tiny sea creatures under the microscope. In *Art Forms in Nature* he arranged them in balanced, almost decorative plates. This plate shows ciliates, the group of protists that includes the paramecium, all of which move or feed with cilia.

You saw Haeckel's diatoms in Unit 1. Haeckel's scientific ideas about how living things arose were often mistaken and some of his drawings of embryos were later shown to be inaccurate, but his drawings of protists are admired for their skill. Remember that he arranged the creatures to make a beautiful page; in real life you would never see so many kinds lined up together.

Look closely

- Find a creature shaped like a bell or a trumpet sitting on a long stalk. Some ciliates attach to plants this way and sweep food toward themselves.
- Find the fine fringe of cilia around the edges of the cells. Where is it thickest?
- Look for round spots inside the cells. Which organelles from this lesson might they be?
- Notice the symmetry of the whole page. How did Haeckel arrange the creatures?

Talk about it

1. Haeckel drew these by looking through a microscope. What would he have had to do to draw a creature that never stopped moving?
2. Why might a scientist and an artist draw the same protist differently?
3. Which protist in this lesson would you most like to draw, and why?

> **Safety first.** Pond water and hay infusions can carry germs. Do not drink or taste them, keep them away from food and small children, and wash your hands well after handling them. Wear gloves if you have a cut on your hands. An adult should be with you near any pond or stream. Handle glass slides and cover slips carefully. When you finish, pour the infusions down an outdoor drain or onto the soil, not the kitchen sink.

EXPERIMENT: LIGHT OR DARK? GROWING PROTISTS IN A HAY INFUSION

A hay infusion is dried grass soaked in water. Bacteria feed on the grass, and protists feed on the bacteria or use sunlight, so in a week or two a clear jar can become a crowded little world.

Question: Does light change which protists grow and how many? **Prediction:** Write which jar you think will have more green protists such as euglena. **What we change:** light (sunny window or dark cupboard). **What we keep the same:** the jars, the amount of hay and water, the pond-water starter, the temperature as nearly as possible. **What we measure:** the number of each kind of protist seen in one drop at 100×, counted every 3 days.

You will need

- 2 clean, identical jars, about 500 mL each, labeled LIGHT and DARK
- A handful of dried grass or hay (no pesticide), cut into short pieces
- Tap water that has stood uncovered for 24 hours, plus 2 tablespoons of pond or puddle water as a starter
- Microscope, slides, cover slips, eyedropper
- Optional: a small dab of clear gel (such as a thickener sold for slowing protists) or a few cotton fibers on the slide to slow fast swimmers
- Lab notebook, ruler and timer

Steps

1. Put equal amounts of hay into both jars and add 400 mL of the standing water to each. Add 1 tablespoon of pond water to each jar.
2. Cover each jar loosely with a paper towel. Put LIGHT in a bright window (out of strong afternoon sun so it does not overheat) and DARK in a cupboard in the same room.
3. On day 0, 3, 6, 9 and 12, take one drop from just under the surface of each jar, make a wet mount and look at 100×.
4. Count every protist you see in the whole drop (or in 5 fields of view, the same number each time). Sort them: round crawlers (amoebas), fast oval swimmers with cilia (paramecia and their relatives), green swimmers (euglenas and other algae).
5. Record your counts in a table for each jar, and sketch any new kind you find.
6. Time one paramecium swimming across the field of view to estimate its speed (see the worked example).
7. Make a double bar graph of green protists in each jar by day, and write a conclusion that answers the question.

Tips

- Protists gather at the surface film and near the hay. Sample from the same place every time so your counts are fair.
- A bad smell after a few days comes from bacteria; it is normal. Keep the jars covered and away from the kitchen.
- Fast swimmers are easier to count if you wait a minute for the slide to settle or add a few cotton fibers to slow them.

Check your work

- I kept everything the same except light and sampled both jars the same way.
- I counted and sorted protists on at least four days and made a graph.
- I can explain my results using the words photosynthesis and food.

NATURE NOTEBOOK

1. Draw an amoeba, a paramecium and a euglena large and side by side. Label how each one moves (pseudopods, cilia, flagellum) and how each one gets food.
2. Draw a paramecium’s contractile vacuole in three stages: filling, full and squeezing out water. Write one sentence explaining why a freshwater protist needs it.
3. Make a two-panel picture of the malaria story: Laveran at his microscope in 1880 and Ross with his mosquitoes in 1897. Under each, write what that person discovered.

> **From history.** Ross’s discovery soon changed history. When the United States began building the Panama Canal in 1904, malaria and yellow fever (spread by a different mosquito) were sickening thousands of workers, as they had during the failed French attempt in the 1880s. The army doctor William Gorgas used what Ross and others had learned: his crews drained swamps, oiled standing water, cut brush and screened buildings. Deaths from disease fell sharply, and the canal opened in 1914.

WORKED EXAMPLES

Example 1. A paramecium 0.2 mm long swims straight across a 3 mm field of view in 3 seconds. (a) What is its speed in mm per second? (b) How many of its own body lengths does it travel each second? (c) How fast would a 1.5 m person have to run to match that in body lengths?

1. Speed = distance ÷ time = 3 mm ÷ 3 s = 1 mm/s.
2. Body lengths per second = 1 mm ÷ 0.2 mm = 5 body lengths each second.
3. A 1.5 m person going 5 body lengths each second would run 5 × 1.5 m = 7.5 m/s.
4. That is about the speed of a good sprinter, so for its size the paramecium is a fast swimmer.

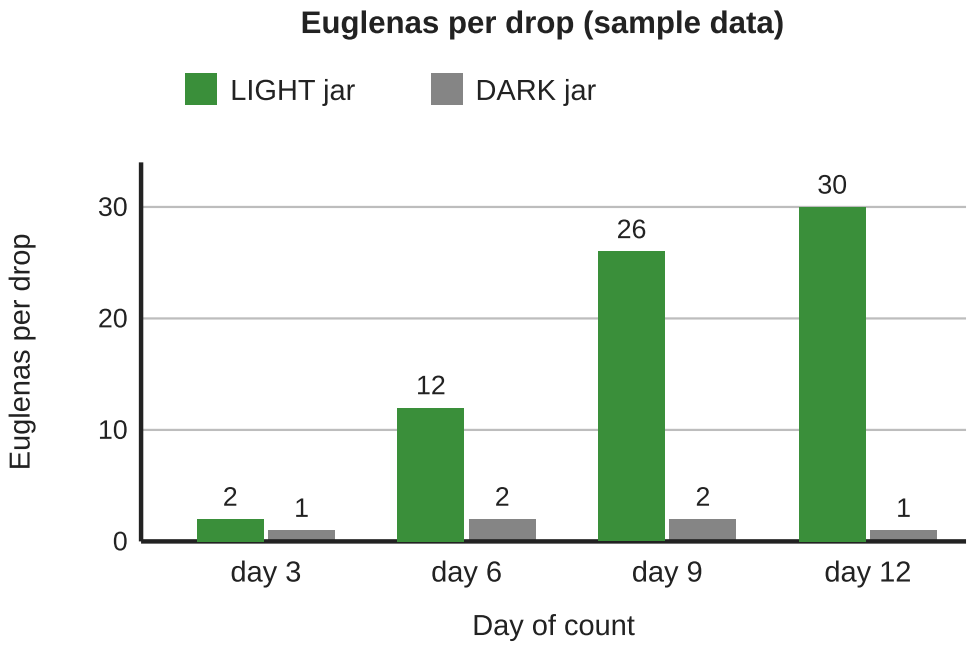
Answer: (a) 1 mm/s. (b) 5 body lengths per second. (c) 7.5 m/s, a sprinter’s speed.

Example 2. Sample practice data: a paramecium in pond water empties its contractile vacuole every 10 seconds. When moved to slightly salty water, it empties every 30 seconds. (a) How many times per hour does it empty in each? (b) Explain the difference.

1. One hour = 3,600 seconds.
2. Pond water: 3,600 ÷ 10 = 360 times per hour.
3. Salty water: 3,600 ÷ 30 = 120 times per hour. 360 ÷ 120 = 3, so it empties three times as often in pond water.
4. Pond water has far fewer dissolved particles than the inside of the cell, so water rushes in by osmosis and must be pumped out often. In salty water, less water flows in, so the vacuole has less to remove.

Answer: (a) 360 times per hour in pond water; 120 times per hour in salty water. (b) Less water enters the cell by osmosis in salty water, so the vacuole works one-third as often.

Example 3. The graph shows sample data for practice: the number of green euglenas counted in one drop from a LIGHT jar and a DARK jar. LIGHT: day 3, 2; day 6, 12; day 9, 26; day 12, 30. DARK: day 3, 1; day 6, 2; day 9, 2; day 12, 1. (a) How many times more euglenas were in the LIGHT jar on day 12? (b) What was the average increase per day in the LIGHT jar from day 3 to day 9? (c) What conclusion do the data support?



Sample practice data: euglenas per drop in a light jar and a dark jar.

1. Day 12: LIGHT 30, DARK 1. 30 ÷ 1 = 30 times as many.
2. From day 3 to day 9 the LIGHT count rose from 2 to 26, an increase of 24 in 6 days. 24 ÷ 6 = 4 euglenas per day.
3. Euglenas make most of their food by photosynthesis, which needs light. In the dark they can only absorb food from the water, so they barely grew.

Answer: (a) 30 times as many. (b) 4 euglenas per day. (c) Light greatly increases the growth of euglenas, which fits the idea that they depend mostly on photosynthesis.

PRACTICE

1. EASY What is a protist?
2. EASY Match each protist to the way it moves: amoeba, paramecium, euglena. Ways: flagellum, cilia, pseudopods.
3. EASY What did Ronald Ross find inside an *Anopheles* mosquito in 1897, and why did it matter?
4. MEDIUM Why is euglena hard to classify as either plant-like or animal-like?

5. MEDIUM A saltwater protist placed in fresh water swells up and bursts. Explain why, using the words osmosis and contractile vacuole.
6. MEDIUM An amoeba crawls 0.06 mm in 1 minute. How far would it travel in 1 hour, in millimeters? How long would it take to cross a 3 mm field of view?
7. MEDIUM Use the euglena graph (l7-3-2-euglena-graph). Which statement is NOT supported by the data? (A) The LIGHT jar had more euglenas on every day measured. (B) The LIGHT jar's growth slowed between day 9 and day 12. (C) The DARK jar's euglenas all died by day 12. (D) The largest difference between the jars was on day 12.
8. HARD A student counted protists in a hay infusion. Day 2: bacteria cloudy, 0 protists. Day 5: 40 small flagellates, 2 paramecia. Day 8: 15 flagellates, 25 paramecia. Day 11: 5 flagellates, 30 paramecia, 3 amoebas. Describe the pattern and suggest an explanation.
9. CHALLENGE Design an experiment to test whether paramecia swim toward or away from vinegar (a weak acid). Describe your setup, your independent and dependent variables, a control, and what you would record.
10. HARD In three or four sentences, explain how the discoveries of Laveran and Ross together changed the way people fought malaria.

COMMON MISTAKES

- Thinking protists are tiny animals or tiny plants. They are their own varied group of eukaryotes; "animal-like" and "plant-like" describe how they get food, not what they are.
- Thinking protists and bacteria are the same because both are small and single-celled. Protists have a nucleus and organelles; bacteria do not.
- Thinking malaria is spread by bad air or by any mosquito. It is caused by the protist *Plasmodium* and is spread by the bite of certain *Anopheles* mosquitoes.

> **Make it a game · Protist Relay.** Set a start line and a finish line about 10 meters apart. Each runner draws a card: AMOEBA (move by stretching one foot forward and pulling the body after it, the pseudopod crawl), PARAMECIUM (row with both arms like many cilia), or EUGLENA (spin one arm overhead like a flagellum and dash). At the finish line the runner must name one fact about their protist (how it eats, what organelle it has, where it lives) before tagging the next teammate. A wrong fact means one more lap.

> **Scripture.** "But ask now the beasts, and they shall teach thee; and the fowls of the air, and they shall tell thee: Or speak to the earth, and it shall teach thee: and the fishes of the sea shall declare unto thee." (Job 12:7–8, KJV)

Job told his friends to learn from God's creatures. Laveran and Ross learned from a drop of blood and the stomach of a mosquito, and what they learned has saved many lives. Even the smallest living things have something to teach a patient observer.

Practice answers

1. A eukaryote (its cells have a nucleus) that is not a plant, animal or fungus. Most protists are single-celled.
2. Amoeba: pseudopods. Paramecium: cilia. Euglena: flagellum.
3. He found the malaria parasite (round cells with dark pigment) growing in the mosquito's stomach wall. It showed that mosquitoes carry malaria from person to person, so controlling mosquitoes could prevent the disease.
4. It has chloroplasts and makes food by photosynthesis like a plant, but it also swims with a flagellum, senses light with an eyespot, and can absorb food from the water in the dark, more like an animal-like protist.
5. Fresh water has fewer dissolved particles than the inside of the cell, so water moves into the cell by osmosis. A saltwater protist has no contractile vacuole (or not one strong enough) to pump the extra water out, so it swells until it bursts.
6. 0.06 mm × 60 = 3.6 mm in 1 hour. 3 mm ÷ 0.06 mm per minute = 50 minutes.
7. (C). The DARK jar still had 1 euglena per drop on day 12, so they had not all died. A, B (an increase of only 4 from day 9 to 12) and D (a difference of 29) are supported.
8. Small flagellates appeared first and then fell in number as paramecia rose; amoebas appeared last. One explanation: bacteria grew first on the hay, small flagellates fed on the bacteria, then paramecia (which eat bacteria and small protists) multiplied and outcompeted or ate the flagellates, and slower-growing amoebas appeared later. The jar went through a succession of different protists.
9. Setup: put a drop of hay-infusion water with paramecia on a slide; at one edge touch a tiny drop of very dilute vinegar, and on a second slide touch a tiny drop of plain water at the edge (the control). Independent variable: vinegar or plain water. Dependent variable: number of paramecia in the half of the drop nearest the added liquid after 2 minutes (or the direction most swim). Keep the same: drop size, size of the added drop, time, magnification. Repeat at least three times and record counts in a table.
10. Before them, people blamed malaria on bad air from swamps. Laveran showed in 1880 that it was caused by a living protist in the blood, and Ross showed in 1897 that the parasite grows inside *Anopheles* mosquitoes and is passed on by their bites. Knowing both the cause and the carrier meant people could fight malaria by draining standing water, screening windows and controlling mosquitoes, as well as by treating the sick.

3.3 Yeast and Bread

OBJECTIVES

- Describe yeast as a single-celled fungus that reproduces by budding, and explain how Emil Christian Hansen grew the first pure yeast culture from a single cell (1883).
- Write the word equation for fermentation, compare it with cellular respiration, and explain how yeast, sugar and gluten make bread rise.
- Measure the gas made by yeast in a controlled experiment, and use ratios (baker's percentages) and graphs to analyze results.

Warm-up. Stir half a teaspoon of dry yeast into a glass of warm water (about as warm as a comfortable bath) with a teaspoon of sugar, and stir half a teaspoon into a glass of cold water with no sugar. Watch both for 5 minutes. Write down every difference you see, hear or smell. Which glass looks alive?

Video. Coming soon. Yeast cells budding under the microscope, yeast balloon experiment, and how bread dough rises (fermentation and gluten); search Journey to the Microcosmos, SciShow Kids, Science Max, Homeschool Pop (Alternate), King Arthur Baking bread science, and university microbiology demonstrations.

KEY TERMS

yeast
A single-celled fungus. Baker's yeast, *Saccharomyces cerevisiae*, feeds on sugar and gives off carbon dioxide gas.

budding
The way yeast reproduces: a small bump grows on the parent cell, receives a copy of the nucleus, and pinches off as a new cell.

fermentation
A way of releasing energy from sugar without oxygen. In yeast it produces alcohol (ethanol) and carbon dioxide.

cellular respiration
Releasing energy from sugar using oxygen, producing carbon dioxide and water. It gives far more energy than fermentation.

gluten
A stretchy network of proteins formed when wheat flour is mixed with water and kneaded. It traps gas bubbles so dough can rise.

leaven
Anything that makes dough rise; in the Bible, a lump of old fermented dough saved and mixed into new dough.

CORE IDEAS

The story. In the early 1880s the brewers of Copenhagen, Denmark, had a costly problem. Batch after batch of their product would suddenly turn sour, bitter or foul-smelling, and no one could say why. At the Carlsberg Laboratory, founded by the brewer J. C. Jacobsen to study the science behind his trade, a young Danish scientist named Emil Christian Hansen studied the yeast under his microscope. He found that the yeast in the vats was not one kind but a mixture of several kinds of yeast cells, and some of these "wild yeasts" were spoiling the batches.

How they figured it out. Hansen asked a bold question: what if a brewer could start with yeast that all came from one single, healthy cell? He diluted yeast in sterile water again and again until a drop held only one cell, which he confirmed with his microscope. He let that one cell grow and bud until it formed a colony, then tested the yeast it produced. In 1883 Carlsberg began using Hansen's pure culture, and the spoiling stopped. Jacobsen refused to patent the method and shared the yeast and the technique freely with other brewers. Bakers, winemakers and yeast factories soon followed, and today the baker's yeast you buy in a packet comes from carefully kept pure cultures.

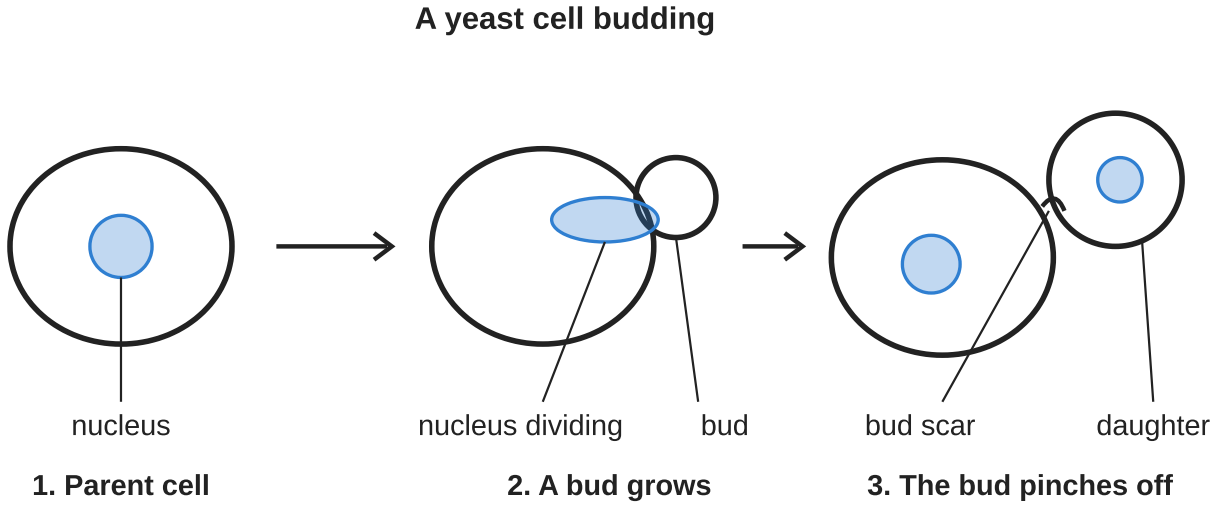
The big idea. Yeast is a single-celled **fungus**, a eukaryote with a nucleus, mitochondria and a cell wall, about 5 to 10 µm across. (You will study its larger cousins, the mushrooms and molds, in Unit 6.) It reproduces by **budding**: a small bump grows on the side of the parent, gets a copy of the nucleus, and pinches off. Yeast feeds on sugar. When oxygen is plentiful it uses **cellular respiration**: sugar + oxygen → carbon dioxide + water + energy. When oxygen runs out, as it soon does inside a ball of dough, it switches to **fermentation**: sugar → alcohol (ethanol) + carbon dioxide + a little energy. Fermentation releases much less energy than respiration, but it keeps the yeast alive and, for the baker, it makes gas.

Try it and see. When you mix flour and water and knead them, two wheat proteins join into a stretchy net called **gluten**. Enzymes in the flour turn some of its starch into sugar, the yeast ferments the sugar, and bubbles of carbon dioxide are caught in the gluten net like air in a thousand tiny balloons. The dough rises. In the oven the gas expands further, the yeast dies once the dough passes about 60 °C, nearly all the alcohol evaporates, and the starch and gluten set firm around the holes. Every hole in a slice of bread was once a bubble of yeast's breath. Temperature matters: yeast is sluggish in the cold, busiest in warm dough around 25 to 35 °C, and killed by water that is too hot.

In the world today. For thousands of years bakers did not buy yeast. They saved a lump of yesterday's fermented dough, the **leaven**, and mixed it into the new batch. That is still how sourdough is made: a starter of wild yeasts and lactic acid bacteria, the same kind of bacteria that make yogurt, which give the bread its tang. Yeast is also one of the most studied living things in the world. Because its cells are so much like ours, scientists use it to learn how cells divide and how genes work.

God's handiwork. Jesus chose the humble work of bread-making to picture the kingdom of heaven: a little leaven, hidden in a large batch of flour, works quietly until the whole lump is

raised. The baker cannot see a single yeast cell, yet billions of them work silently and the change is plain for all to see.



Yeast reproduces by budding. Each bud leaves a small scar on the parent cell.

PICTURE STUDY



The Milkmaid, Johannes Vermeer, about 1657–1658 · Oil on canvas · Rijksmuseum, Amsterdam

Johannes Vermeer lived in Delft, the same Dutch town as Antonie van Leeuwenhoek; Leeuwenhoek was born there in October 1632, the same month Vermeer was baptized. Vermeer painted quiet indoor scenes of ordinary work, filled with soft light from a window.

Here a kitchen maid pours milk slowly into a bowl. On the table lies broken, crusty bread. Many people who study the painting think she is making a bread pudding from stale bread.

In a Dutch home of that time, bread was the center of nearly every meal, and every loaf rose because of yeast.

Look closely

- Find the bread. How many pieces can you see, and what does the crust look like?
- Look closely at the bread's surface. Vermeer used tiny dots of light paint. What do they make the bread look like?
- Follow the stream of milk. How did Vermeer make it seem to be moving?
- Find the window. Where does the light fall brightest?

Talk about it

1. If you could cut a slice of this bread, what would you expect to see inside, and what made it?
2. Vermeer chose to paint a servant doing ordinary kitchen work with great care and dignity. What does that suggest about how he saw ordinary work?
3. Leeuwenhoek, living in the same town, was the first to see yeast cells under a microscope, in 1680. What do you think he would have said about the bread on this table?

> **Safety first.** Use warm tap water (about 40 °C, comfortable to the touch), never boiling water. If you bake the optional loaf, an adult should handle the hot oven and pans, and you should use oven mitts. The yeast mixtures in the bottles are not food; pour them out when you finish and wash your hands.

EXPERIMENT: THE YEAST BALLOON EXPERIMENT

Yeast gives off carbon dioxide as it ferments sugar. If you trap the gas in a balloon, the balloon will grow, and the size of the balloon tells you how much gas was made.

Question: How does the amount of sugar change how much gas yeast makes? **Prediction:** Write which bottle you think will make the biggest balloon. **What we change:** the amount of sugar (0, 1, 2 and 4 teaspoons). **What we keep the same:** the amount of yeast, the amount and temperature of water, the size of bottle and balloon, the timing. **What we measure:** the distance around each balloon (circumference) every 10 minutes for 40 minutes.

You will need

- 4 identical small plastic bottles (about 500 mL), labeled 0, 1, 2 and 4
- 4 identical balloons (stretch each a few times first)
- 4 teaspoons of dry yeast (about 1 teaspoon per bottle)
- Sugar, measuring spoons, a funnel
- Warm tap water at about 40 °C, a kitchen thermometer, a measuring cup
- A soft tape measure or a string and ruler; a timer; lab notebook

- Optional: ingredients for one simple loaf (500 g bread flour, 325 g water, 10 g salt, 7 g yeast)

Steps

1. Use the funnel to put 1 teaspoon of yeast into each bottle. Add 0, 1, 2 or 4 teaspoons of sugar, matching each bottle’s label.
2. Measure 120 mL of 40 °C water for each bottle. Pour it in, quickly stretch a balloon over the mouth of each bottle, and swirl gently to mix. Start the timer.
3. Set the bottles side by side in the same warm spot.
4. Every 10 minutes, measure the circumference of each balloon at its widest part. Record the results in a table.
5. At 40 minutes, compare the bottles. Make a bar graph of circumference at 40 minutes for each amount of sugar.
6. Write a conclusion: Did more sugar always mean more gas? Why might the effect level off?
7. Optional: bake the simple loaf with a parent. Mix, knead 10 minutes, let rise in a covered bowl until doubled, shape, rise again, and bake at about 220 °C for about 30 minutes. Cut a slice and look at the holes with a hand lens.

Tips

- Water that is too hot (above about 55 °C) will kill the yeast, and nothing will happen. Use the thermometer.
- Measuring a balloon is easiest with a string: wrap it around the widest part, mark it and measure the string with a ruler.
- For a fair test, put all four balloons on as fast as you can, or work with a helper.

Check your work

- I changed only the amount of sugar and kept everything else the same.
- I measured every balloon every 10 minutes and graphed my results.
- I can explain where the gas came from, using the word fermentation.

NATURE NOTEBOOK

1. Draw what you see when you look at a drop of yeast mixture under the microscope at 400×. Find and label a cell with a bud, and estimate the size of one cell using your field of view.
2. Draw a cross-section of a slice of bread at actual size and label: gas hole (once a carbon dioxide bubble), gluten wall, crust. Write one sentence explaining how each hole formed.
3. Make a side-by-side chart comparing cellular respiration and fermentation in yeast: what goes in, what comes out, whether oxygen is needed, and how much energy is released.

From history. For centuries people knew how to use leaven without knowing what it was. In 1680 Leeuwenhoek saw tiny round globules in fermenting beer but did not realize they were alive. In 1837 three scientists working separately, Charles Cagniard de la Tour in France and Theodor Schwann (whom you met in Unit 2) and Friedrich Kützing in Germany, reported that yeast is made of living cells that grow by budding. Some famous chemists mocked the idea, but in the 1850s and 1860s Louis Pasteur showed that fermentation happens only when living yeast is present. In 1897 Eduard Buchner discovered that juice squeezed from crushed yeast could still ferment sugar, because of the enzymes inside the cells, and that discovery helped launch the science of biochemistry.

WORKED EXAMPLES

Example 1. Bakers write recipes as **baker's percentages**, where the flour is always 100% and every other ingredient is a percent of the flour's mass. A loaf uses 500 g flour, 325 g water, 10 g salt and 7 g yeast. (a) Find the percentage of each. (b) Scale the recipe for 750 g of flour.

1. Water: 325 ÷ 500 × 100 = 65%. Salt: 10 ÷ 500 × 100 = 2%. Yeast: 7 ÷ 500 × 100 = 1.4%.
2. Scale factor: 750 g ÷ 500 g = 1.5.
3. Water: 325 g × 1.5 = 487.5 g. Salt: 10 g × 1.5 = 15 g. Yeast: 7 g × 1.5 = 10.5 g.
4. Check: 487.5 ÷ 750 = 0.65, still 65%. The proportions have not changed.

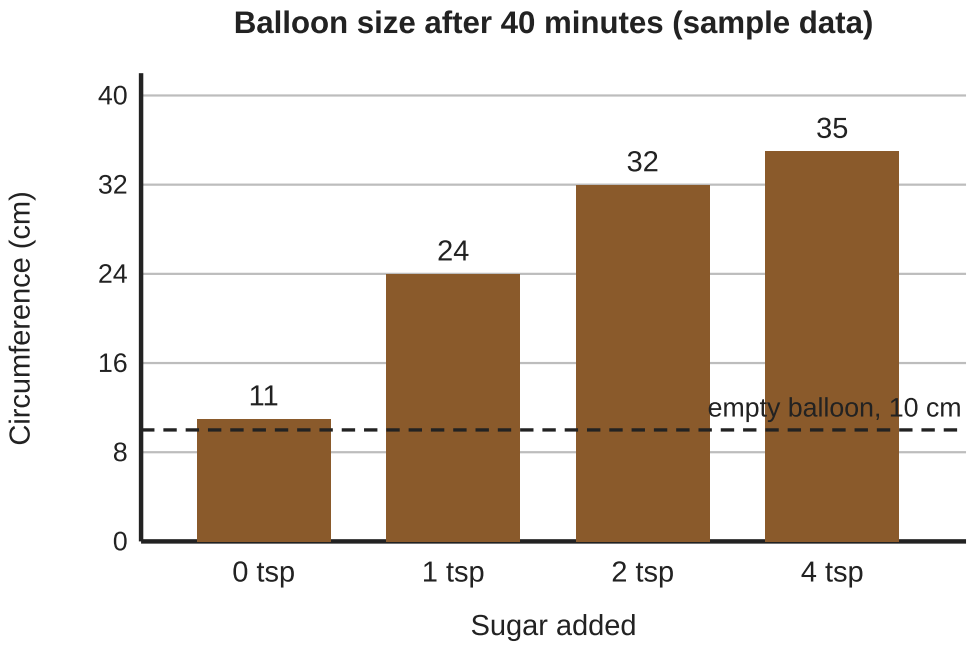
Answer: (a) Flour 100%, water 65%, salt 2%, yeast 1.4%. (b) 750 g flour, 487.5 g water, 15 g salt, 10.5 g yeast.

Example 2. In fermentation, 180 g of glucose sugar is turned into 92 g of ethanol and some carbon dioxide. (a) How much carbon dioxide is made? (b) If the yeast in a loaf ferments 9 g of glucose, how many grams of carbon dioxide does it make?

1. Matter is not created or destroyed, so the mass of the products equals the mass of the sugar used: 180 g = 92 g ethanol + carbon dioxide.
2. Carbon dioxide = 180 g − 92 g = 88 g.
3. 9 g is 9 ÷ 180 = 1/20 of 180 g, so every product is 1/20 as much.
4. Carbon dioxide = 88 g ÷ 20 = 4.4 g. (At room temperature that much gas fills about 2.4 liters, enough to puff up a whole loaf.)

Answer: (a) 88 g of carbon dioxide. (b) 4.4 g of carbon dioxide.

Example 3. The graph shows sample data for practice from a yeast balloon experiment: circumference of each balloon after 40 minutes. 0 tsp sugar, 11 cm; 1 tsp, 24 cm; 2 tsp, 32 cm; 4 tsp, 35 cm. An empty, unstretched balloon measured 10 cm around. (a) How much did each balloon grow? (b) Doubling the sugar from 2 to 4 tsp did not double the growth. Suggest why.



Sample practice data: yeast balloon circumference after 40 minutes.

1. Subtract the empty balloon's 10 cm from each: 0 tsp, 1 cm; 1 tsp, 14 cm; 2 tsp, 22 cm; 4 tsp, 25 cm.
2. From 2 to 4 tsp the sugar doubled, but growth rose only from 22 cm to 25 cm.
3. Each bottle had the same amount of yeast. Once the yeast has more sugar than it can use in 40 minutes, extra sugar does not help: the yeast is working as fast as it can. (Very sugary water can even slow yeast by drawing water out of its cells.)

Answer: (a) 1 cm, 14 cm, 22 cm and 25 cm. (b) The amount of yeast, not sugar, became the limit; the yeast could not ferment sugar any faster.

PRACTICE

1. EASY Is yeast a bacterium, a protist, a plant or a fungus? How does it reproduce?
2. EASY Write the word equation for fermentation in yeast.
3. EASY What did Emil Christian Hansen do in 1883, and why did it help brewers and bakers?
4. MEDIUM Explain why kneading matters in making yeast bread.

5. MEDIUM A ball of dough has a volume of 500 mL. After 50 minutes it has risen to 1,000 mL. What was its average rate of rise in mL per minute?
6. MEDIUM In warm sugar water, a yeast cell buds about every 90 minutes. Starting from 1 cell, how many cells could there be after 6 hours?
7. MEDIUM A pizza dough uses 400 g flour at 60% hydration and 2% salt. How many grams of water and of salt does it need?
8. HARD A student measured balloon circumference after 30 minutes for yeast in water of different temperatures (same yeast and sugar): 10 °C, 12 cm; 25 °C, 20 cm; 40 °C, 30 cm; 55 °C, 14 cm; 70 °C, 10 cm. The empty balloon was 10 cm. (a) At which temperature was the yeast most active? (b) Explain the results at 10 °C and at 70 °C.
9. CHALLENGE Design an experiment to test whether salt slows down yeast. Name the independent variable, the dependent variable, at least three things to keep the same, and the control.
10. HARD A friend says, “Bread has holes because air gets stirred in when you knead it.” In three or four sentences, explain what really makes most of the holes.

COMMON MISTAKES

- Thinking yeast is a chemical powder like baking soda. Yeast is a living single-celled fungus; the granules in a packet are dried, resting cells that wake up in warm water.
- Thinking hotter is always better for yeast. Warmth speeds it up only to a point; water above about 55 °C begins killing yeast, and by about 60 °C it dies.
- Thinking fermentation and respiration are the same. Respiration uses oxygen and releases much more energy; fermentation works without oxygen and produces alcohol and carbon dioxide.

> **Make it a game · Rise or Flop?.** A parent reads out a bread-making situation, and players stand up tall (“Rise!”) or crouch down (“Flop!”) to show what the dough will do, then explain why. Examples: yeast mixed with boiling water (flop: the yeast is killed); dough left in a warm kitchen for an hour (rise); dough with no kneading (flops or rises poorly: weak gluten); dough in the refrigerator overnight (rises slowly); flour, water and salt with no yeast (flop: no gas). A correct answer with a correct reason earns a point.

> **Scripture.** “Another parable spake he unto them; The kingdom of heaven is like unto leaven, which a woman took, and hid in three measures of meal, till the whole was leavened.” (Matthew 13:33, KJV)

Three measures of meal was a large batch of flour, enough to feed a crowd. A small, hidden lump of living leaven worked through it all. Jesus used a sight every household knew to show how God’s kingdom grows quietly from small beginnings.

Practice answers

1. A single-celled fungus. It reproduces by budding.
2. Sugar → alcohol (ethanol) + carbon dioxide (+ a little energy).

3. He grew a pure yeast culture starting from a single cell. Using yeast of one known, healthy kind kept wild yeasts from spoiling batches, so results were reliable.
4. Kneading builds the gluten network. Gluten is stretchy and traps the carbon dioxide made by the yeast, so the dough can rise instead of letting the gas escape.
5. 1,000 − 500 = 500 mL increase. 500 mL ÷ 50 min = 10 mL per minute.
6. 6 hours = 360 minutes; 360 ÷ 90 = 4 doublings; 2^4 = 16 cells.
7. Water: 400 × 0.60 = 240 g. Salt: 400 × 0.02 = 8 g.
8. (a) 40 °C, which made the most gas (20 cm of growth). (b) At 10 °C the yeast was alive but slow in the cold, so it made little gas (2 cm). At 70 °C the hot water killed the yeast, so no gas was made and the balloon stayed at 10 cm.
9. Independent variable: amount of salt (for example 0, 1/2, 1 and 2 teaspoons per bottle). Dependent variable: balloon circumference after 40 minutes (or height of rise of small dough balls in measuring cups). Keep the same: amount of yeast, amount of sugar, water volume and temperature, bottle and balloon size, timing. Control: the bottle with no salt. Repeat each trial and average the results.
10. Kneading does trap a few tiny air pockets, but most of the gas in bread comes from yeast. Yeast ferments sugar in the dough and gives off carbon dioxide, which collects in those small pockets and makes them grow, held in by the stretchy gluten. In the oven the gas expands even more before the bread sets. Bread made without yeast (or another leaven) stays flat and dense, which shows the yeast’s gas is what raises it.

3.4 Culturing Bacteria Safely

OBJECTIVES

- Tell how Robert Koch's laboratory developed solid culture media in the early 1880s, including Fanny Hesse's suggestion of agar (1881) and Julius Petri's dish (1887).
- Follow safe, sterile (aseptic) technique to prepare, inoculate, seal, incubate and dispose of culture plates, and explain the reason for each safety rule.
- Count and describe colonies, calculate percent reductions and estimate bacteria per milliliter from a dilution.

Warm-up. Make two cups of unflavored gelatin (or fruit gelatin) and let them set in the refrigerator. Leave one on the counter in a warm room for an hour and keep the other cold. Compare them. Then ask: if you wanted to grow bacteria at body temperature, about 37 °C, why would gelatin be a poor thing to grow them on?

Video. Coming soon. How to pour and streak an agar plate, aseptic technique, and the history of Robert Koch, Fanny Hesse and the petri dish; search university microbiology lab channels, Smithsonian, SciShow, Science Max, Homeschool Pop (Alternate), and the American Society for Microbiology.

KEY TERMS

culture
A population of microbes grown on purpose in a laboratory, in or on a food source made for them.

agar
A jelly made from red seaweed (a protist). It stays solid at body temperature and very few bacteria can digest it, so it makes an ideal surface for growing microbes.

colony
A visible spot of microbes, often millions of cells, that grew from a single cell (or a small clump of cells) on a solid medium.

sterile
Completely free of living microbes and their spores.

aseptic technique
Careful habits that keep unwanted microbes out of a culture and keep the culture from escaping: clean hands and surfaces, sterile tools, lids kept closed.

incubate
To keep a culture at a steady temperature for a period of time so the microbes can grow.

CORE IDEAS

The story. Around 1880, in Germany, the former country doctor Robert Koch noticed something about a boiled potato that had been cut open and left out. Its flat surface was dotted with little spots of different colors. Each spot, he realized, was a pile of microbes that had grown from a single cell that landed there, and every cell in that spot was of the same kind. In a flask of broth, all the kinds of bacteria swim together in a muddle; on a solid surface they stay apart. Koch, now leading a laboratory at the Imperial Health Office in Berlin, set out to find the best solid food for growing one kind of bacteria at a time.

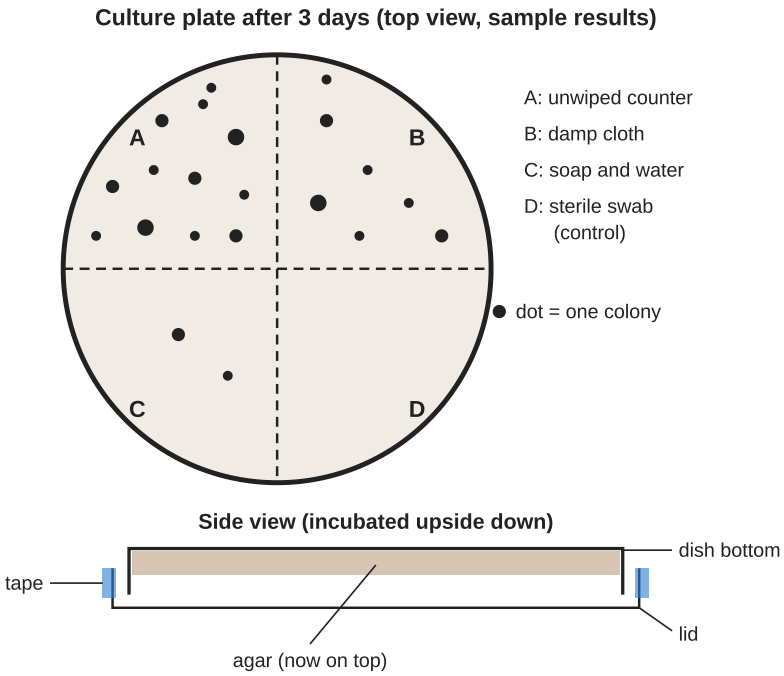
How they figured it out. Koch tried slices of potato, then broth stiffened with gelatin. But gelatin melts at body temperature, which is just where disease bacteria grow best, and some bacteria digest it into a puddle. In 1881 one of his workers, Walther Hesse, mentioned the problem at home. His wife, Fanny Hesse, made jellies and puddings that stayed firm even in hot summer weather, because she used agar, a seaweed jelly she had learned about from a Dutch friend who had lived in Java. Agar stays solid up to about 85 °C, and almost no bacteria can eat it. Koch's laboratory adopted it at once. Then, in 1887, Julius Richard Petri, who had worked as an assistant in Koch's laboratory, described a shallow glass dish covered by a slightly larger lid that kept dust out but let scientists stack and examine their cultures. The **Petri dish** filled with agar is still the basic tool of every microbiology lab in the world.

The big idea. A **culture** grows microbes on purpose. The scientist makes a **culture medium**, usually nutrient agar with broth for food, and makes it **sterile** by heating it and the dishes. A sample is then touched to the surface. Each living cell that lands there divides again and again (remember binary fission from Lesson 3.1), and after a day or two the pile of millions of cells becomes a visible **colony**. Colonies can be described by size, color, shape, edge and texture: bacterial colonies are usually smooth, shiny or dull spots, while mold colonies are fuzzy. Counting colonies lets a scientist estimate how many living microbes were in the sample. One important caution: a plate shows only the microbes that can grow on that food, at that temperature, in that time, so a plate with no colonies does not prove a surface had no microbes at all.

Safety first. When you culture microbes, you are growing millions of cells from an unknown sample. Most will be harmless, but you cannot tell by looking, so microbiologists treat every unknown culture as if it might be harmful. These rules are the same ones that protect students in a high school or college lab: tape every plate shut after you add the sample and **never open it again**; grow plates at room temperature, not in a warm place near body temperature, which would favor germs that live in people; never sample body fluids, toilets, or anyone who is sick; label plates on the bottom; incubate them upside down so drops of water do not fall on the colonies; never smell or touch the colonies; and have an adult destroy the plates at the end with bleach or seal them in a bag for the trash. Wash your hands before and after.

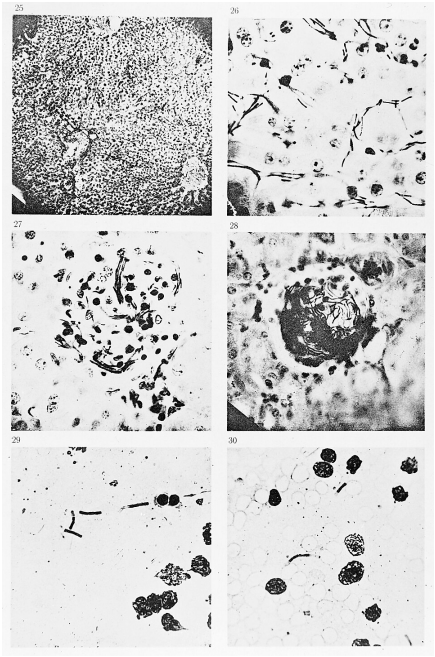
In the world today. Hospitals culture samples to find out which bacteria are making a patient sick. Food companies culture samples of milk, meat and water to make sure they are safe, and city water plants test drinking water every day. Dairies use pure cultures to make

the same yogurt and cheese every time. All of this rests on the potato slice, the jelly in Fanny Hesse's kitchen and the simple glass dish of Julius Petri.



A culture plate divided into four quadrants (sample results). Below: the plate is taped shut and incubated upside down.

PICTURE STUDY



Photographs of Anthrax Bacteria, Robert Koch, 1881 · Photomicrographs (photographs taken through a microscope), printed as a plate in Koch's paper *Zur Untersuchung von pathogenen Organismen* (On the Study of Disease-Causing Organisms) · Published in Berlin by the Imperial Health Office; copies are kept in many libraries

Photo: Wellcome Collection · CC BY 4.0 (https://creativecommons.org/licenses/by/4.0) · via Wikimedia Commons

Before Koch, scientists had to draw what they saw under a microscope, and two people looking at the same slide often drew it differently. In 1877 Koch published a paper on how to dry, stain and photograph bacteria, with some of the first photographs of bacteria ever printed. The photographs on this plate come from his longer 1881 paper on ways to study disease germs, published by the Imperial Health Office in Berlin.

Koch believed a photograph was more honest than a drawing, because the camera could not see what it hoped to see. He wrote that a photograph of a microscope image could sometimes be more important than the specimen itself, since anyone could study it. His photographs were small and grainy by today's standards, but they helped make bacteriology an exact science.

Look closely

- Find the rod-shaped bacteria. Are they single, or joined end to end in chains?
- Look for round dots. Are they bacteria, or could they be something else, such as spores?
- Look at the edges of each photograph. Why does a photograph taken through a microscope show only a small patch of the slide?
- Compare these photographs with Leeuwenhoek's drawings from Lesson 3.1. What can each show that the other cannot?

Talk about it

1. Why might a photograph be more trustworthy evidence than a drawing? Can you think of a way a photograph could still mislead?
2. Koch needed his bacteria to hold still to photograph them. How did drying and staining them help?
3. How did growing pure cultures make Koch's photographs more useful?

> **Safety first.** Grow microbes only with an adult's permission and supervision. Do not sample body fluids, toilets, pets' mouths, or anyone who is sick. After adding a sample, tape each plate shut and never open it again; observe through the lid only. Incubate at room temperature (about 20 to 25 °C), never in a warm oven or near body temperature. Do not smell or touch colonies. If a plate cracks or opens, have an adult wipe the area with a bleach solution. At the end, an adult should either soak the closed plates in a solution of 1 part household bleach to 9 parts water for at least an hour before throwing them away, or seal them in a zip bag inside a second bag for the trash. Never mix bleach with other cleaners. People with weakened immune systems should not handle the plates. Hot agar can burn; an adult should heat and pour it. Wash your hands before and after every step.

EXPERIMENT: DOES WIPING REALLY CLEAN THE COUNTER?

Kitchen counters look clean after a quick wipe, but are they? You will culture samples from three parts of the same counter after three different treatments and count the colonies.

Question: Which cleaning method leaves the fewest living microbes on a kitchen counter? **Prediction:** Rank the three methods. **What we change:** the cleaning method (none, damp cloth only, soap and water then a clean towel). **What we keep the same:** the counter, the size of each sampled area (10 cm by 10 cm), the swabbing method, the plates, the incubation time and temperature. **What we measure:** the number of colonies in each quadrant after 3 days. **Control:** a sterile swab touched to nothing.

You will need

- 2 sterile nutrient agar plates (ready-made from a science supplier; or homemade: 250 mL water, 1 beef bouillon cube, 1 teaspoon sugar and about 4 g agar powder, boiled 2 minutes and poured by an adult into clean dishes that were boiled or microwaved first)
- Sterile cotton swabs, still in their wrapper
- Clear tape and a permanent marker
- A clean dish cloth, dish soap, a fresh paper towel, a ruler
- Masking tape to mark three 10 cm by 10 cm squares on the counter
- Hand lens, lab notebook, and a zip bag for disposal

Steps

1. Wash your hands. On the bottom of each plate, draw a cross with the marker to divide it into four quadrants. Label them A (none), B (damp cloth), C (soap and water) and D (control), and write the date and your initials.
2. Mark three 10 cm squares next to each other on the same counter. Leave square A as it is. Wipe square B with a damp cloth. Wash square C with soapy water, rinse and dry it with a fresh paper towel.
3. Take a fresh sterile swab, moisten it with a drop of boiled and cooled water, and rub it over square A, rolling it as you go. Lift the plate lid just enough, gently zigzag the swab across quadrant A without breaking the agar, and close the lid at once.
4. Repeat with a new swab for squares B and C. For quadrant D, use a new swab that touches only the boiled water.
5. Tape the plate shut all the way around. Make a second plate the same way as a repeat trial.
6. Place both plates upside down in a dark place at room temperature. Do not open them again.
7. On day 1, 2 and 3, count the colonies in each quadrant through the lid and describe them (color, size, shape, fuzzy or smooth). Record in a table.
8. Average the two plates, calculate the percent reduction for methods B and C compared with A, and make a bar graph. Then have an adult destroy the plates as described in the safety box.

Tips

- Count colonies by marking each one with a dot on the bottom of the plate as you count, so you do not count any twice.

- If quadrant D grows colonies, something was not sterile; your control has warned you that the other counts may be too high.
- Homemade plates often grow mold, which looks fuzzy. Record it, but count it separately from the shiny bacterial colonies.

Check your work

- I followed every safety rule, kept my plates sealed and gave them to an adult for disposal.
- I used a control and a repeat trial, and counted colonies for three days.
- I calculated percent reduction and explained what my results do and do not prove.

NATURE NOTEBOOK

1. Draw your plate at actual size on day 3, through the lid, showing every colony in the right quadrant. Add a key for colony color and texture.
2. Draw three colonies through a hand lens and describe each using four words: size, color, edge (smooth or wavy) and surface (shiny, dull or fuzzy).
3. Make a safety poster for a culture lab with the six most important rules and a one-sentence reason for each.

> **From history.** Agar itself comes from red seaweeds, which are protists like those in Lesson 3.2. According to a Japanese tradition, about the year 1658 an innkeeper threw leftover seaweed jelly out into the winter cold, and found days later that it had frozen and dried into a light, papery jelly that could be boiled and set again. The Japanese called it *kanten*, and it was used in sweets across East Asia. Dutch traders and settlers in Java learned of it, and that is how it came to Fanny Hesse's kitchen and then to Koch's laboratory.

WORKED EXAMPLES

Example 1. A student's counter plates showed these averages after 3 days: unwiped (A) 120 colonies, damp cloth (B) 80 colonies, soap and water (C) 12 colonies, control (D) 0. Find the percent reduction for B and C compared with A.

1. Percent reduction = (starting count − new count) ÷ starting count × 100.
2. Damp cloth: (120 − 80) ÷ 120 × 100 = 40 ÷ 120 × 100 ≈ 33%.
3. Soap and water: (120 − 12) ÷ 120 × 100 = 108 ÷ 120 × 100 = 90%.
4. The control had 0 colonies, so the swabs and plates were sterile and the counts can be trusted.

Answer: Damp cloth reduced colonies by about 33%; soap and water by 90%.

Example 2. A food lab tests milk. It mixes 1 mL of milk into 9 mL of sterile water (a 1:10 dilution), then repeats this twice more, making a 1:1,000 dilution. It spreads 1 mL of the 1:1,000 dilution on a plate and counts 38 colonies. About how many living bacteria were in 1 mL of the original milk?

1. Each step dilutes by 10. Three steps: 10 × 10 × 10 = 1,000, so the final tube is 1:1,000.
2. Each colony grew from one living cell (or tiny clump) in the 1 mL spread on the plate, so the 1:1,000 dilution had about 38 cells per mL.
3. Multiply by the dilution to get back to the original: 38 × 1,000 = 38,000.
4. Scientists call these colony-forming units (CFU), since one colony might start from a small clump rather than one cell.

Answer: About 38,000 bacteria (colony-forming units) per mL of milk.

Example 3. Sample practice data: the diameter of one colony was measured each day: day 1, 1 mm; day 2, 3 mm; day 3, 5 mm; day 4, 6 mm; day 5, 6 mm. (a) What was the average growth rate from day 1 to day 3? (b) Describe what happened after day 3, and suggest a reason.

1. From day 1 to day 3 the diameter grew from 1 mm to 5 mm, an increase of 4 mm in 2 days.
2. Rate = 4 mm ÷ 2 days = 2 mm per day.
3. From day 3 to day 4 it grew only 1 mm, and from day 4 to day 5 not at all.
4. As the colony grows, the cells use up the food in the agar near them and their wastes build up, so growth slows and stops, like the stationary phase of the growth curve in Lesson 3.1.

Answer: (a) 2 mm per day. (b) Growth slowed and then stopped, probably because nearby food ran out and wastes built up.

PRACTICE

1. EASY Why did Koch’s laboratory switch from gelatin to agar? Give two reasons.
2. EASY What is a colony, and where did it come from?
3. EASY Give the reason for each rule: (a) tape the plate shut and never open it; (b) incubate at room temperature; (c) incubate the plate upside down.
4. MEDIUM Use the plate figure (l7-3-4-plate). Count the colonies in each quadrant, and find the percent reduction from A to C.
5. MEDIUM Why does the experiment include quadrant D, a swab that touched only sterile water?

6. MEDIUM A 1:100 dilution of pond water was made, and 1 mL of it was spread on a plate. 65 colonies grew. Estimate the bacteria per mL in the pond water.
7. MEDIUM Data reading. A student incubated identical plates (same sample) at different temperatures and counted colonies after 2 days: 5 °C, 0; 15 °C, 14; 25 °C, 52; 37 °C, 95. (a) Describe the trend. (b) Using the safety rules, explain why the student's home experiments should still use about 25 °C, not 37 °C.
8. HARD Data reading. Colony descriptions from a plate: Colony 1, 2 mm, cream, round, smooth edge, shiny. Colony 2, 1 mm, yellow, round, shiny. Colony 3, 15 mm, white then green, fuzzy, spreading. Colony 4, 3 mm, white, wavy edge, dull and wrinkled. Which colony is most likely a mold rather than bacteria? How many different kinds of microbe are on the plate, at the least?
9. CHALLENGE Design an experiment to test whether a cutting board made of wood or one made of plastic holds more bacteria after the same washing. Give the independent and dependent variables, three controlled variables, a control, and two safety rules you would follow.
10. HARD A student says, "My plate from the doorknob had no colonies, so the doorknob had no germs on it." In three or four sentences, explain why this conclusion goes too far.

COMMON MISTAKES

- Thinking each colony is a single bacterium. A colony you can see is a pile of millions of cells that grew from one.
- Thinking a plate with no colonies proves a surface is germ-free. Many microbes cannot grow on a given medium or temperature, and viruses never grow on agar at all.
- Thinking that more colonies means more danger. Most colonies from a home are harmless; the number tells how many microbes could grow, not which ones cause disease.

> **Make it a game · Safe or Sorry?.** A parent describes a lab action, and players hold up a thumbs-up for safe or a thumbs-down for unsafe, then give the reason. Examples: peeking under the lid to smell a colony (unsafe); labeling the plate on the bottom (safe); putting plates on a warm radiator to grow faster (unsafe); swabbing a sick sibling's throat (unsafe); taping the lid all around (safe); throwing open plates straight in the kitchen trash (unsafe); washing hands before and after (safe). One point for the right thumb and one more for the right reason.

> **Scripture.** "The works of the LORD are great, sought out of all them that have pleasure therein." (Psalm 111:2, KJV)

The physicist James Clerk Maxwell had this verse carved in Latin over the door of the Cavendish Laboratory in Cambridge, which opened in 1874. A culture plate is one small way of seeking out God's works carefully, and safety rules are part of doing that work with wisdom and love for our neighbors.

Practice answers

1. Gelatin melts at body temperature (where many disease bacteria grow best), and some bacteria digest gelatin into liquid. Agar stays solid up to about 85 °C, and almost no bacteria can digest it.
2. A visible spot of millions of microbes on a solid medium that grew by repeated division from a single cell (or a small clump) that landed on that spot.
3. (a) To keep the millions of grown microbes, which might include harmful ones, from escaping. (b) Warm temperatures near body heat favor microbes that grow in people, which are more likely to cause disease. (c) So drops of water that condense on the lid do not fall on the agar and smear the colonies together.
4. A 12, B 7, C 2, D 0. Percent reduction from A to C: $(12 - 2) \div 12 \times 100 = 10 \div 12 \times 100 \approx 83\%$.
5. It is the control. If no colonies grow in D, the swabs, water and plate were sterile, so any colonies in A, B and C came from the counter. If D grows colonies, the results are contaminated and cannot be trusted.
6. $65 \times 100 = 6{,}500$ bacteria (colony-forming units) per mL.
7. (a) More colonies grew as temperature rose, from 0 at 5 °C to 95 at 37 °C. (b) Body temperature favors bacteria that live in and on people, which includes most disease-causing bacteria. Room temperature grows enough colonies to count while making harmful ones less likely to thrive.
8. Colony 3 is most likely a mold, because it is large, fuzzy and spreading and changes color as it makes spores. Since all four colonies look different, there are at least four different kinds of microbe (colonies that look the same could still be different kinds, but different-looking ones cannot be the same kind).
9. Independent variable: the material of the cutting board (wood or plastic). Dependent variable: number of colonies after 3 days at room temperature. Controlled variables (any three): same food cut on each board, same washing method and time, same size of area swabbed, same swabbing technique, same kind of plates, same incubation time and temperature. Control: a sterile swab touched only to sterile water. Repeat with at least two plates per board. Safety rules (any two): tape plates shut and never open them; incubate at room temperature; adult disposes with bleach; wash hands; do not cut raw meat for the test without an adult.
10. A plate grows only the microbes that can live on that particular food, at that temperature, in that time, so many kinds of bacteria (and all viruses) will never show up on it. Some bacteria may have been on the doorknob but been too few, too dry or not picked up by the swab. The careful conclusion is that no colonies grew under these conditions, not that the doorknob was free of microbes. Repeating the test, with a control and more plates, would make the result more trustworthy.

Unit 3 review

Answer each question. Show your work for calculations, include units, and use complete sentences for explanations. The test is worth 60 points.

1. (4 pts) Multiple choice. Which statement about bacteria is correct? (A) Bacteria have a nucleus wrapped in a membrane. (B) Bacteria are prokaryotes; their DNA lies in the cytoplasm. (C) All bacteria cause disease. (D) Bacteria reproduce by budding.
2. (5 pts) Describe the Gram stain. What color are Gram-positive and Gram-negative bacteria at the end, and what difference in their cell walls causes it?
3. (5 pts) A bacterium divides every 20 minutes. Starting from 1 cell, how many cells are there after 2 hours? Name one reason real populations stop growing this way.
4. (4 pts) Multiple choice. A slipper-shaped protist covered with short, hair-like structures that beat like oars is (A) an amoeba (B) a euglena (C) a paramecium (D) a diatom.
5. (5 pts) What did Alphonse Laveran discover in 1880 and Ronald Ross in 1897? How did their discoveries change the way people fought malaria?
6. (6 pts) Diagram item. Draw an amoeba, a paramecium and a euglena. On each, label the structure it uses to move and one other structure (such as nucleus, contractile vacuole, food vacuole, oral groove, eyespot or chloroplast).
7. (5 pts) Why does a paramecium living in fresh pond water need a contractile vacuole? What would happen without it?
8. (4 pts) Multiple choice. When yeast ferments sugar in bread dough, it produces (A) oxygen and water (B) alcohol and carbon dioxide (C) starch and oxygen (D) gluten and water.
9. (6 pts) Small dough balls with the same recipe were placed at different temperatures and their rise was measured after 45 minutes: 5 °C, 2 mL; 20 °C, 40 mL; 35 °C, 110 mL; 50 °C, 30 mL; 65 °C, 0 mL. (a) At which temperature was the yeast most active? (b) Explain the results at 5 °C and 65 °C. (c) Name the independent and dependent variables.
10. (5 pts) A bread recipe uses 600 g of flour at 70% hydration with 2% salt and 1.5% yeast (baker’s percentages). How many grams of water, salt and yeast does it need?
11. (5 pts) A plate from an unwashed cutting board grew 150 colonies; after scrubbing with hot soapy water, a plate from the same board grew 15. The control plate grew 0. (a) Find the percent reduction. (b) Why does the control matter?
12. (6 pts) Give three safety rules for culturing microbes at home and explain the reason for each. Then explain why Koch’s laboratory replaced gelatin with agar.

Unit project: my microbe field guide and lab notebook

Make a field guide to the single-celled life you studied in this unit. Give one page each to a bacterium, an amoeba, a paramecium, a euglena and a yeast cell: a large labeled drawing, its size in micrometers, how it moves, how it feeds, how it reproduces, and one way it helps or harms people. Then add your lab notebook from the four projects: the yogurt temperature graph, the hay infusion counts and graph, the yeast balloon data and graph, and your sealed-

plate colony counts with percent reductions. Finish with a one-page essay, “A World in a Drop,” explaining what single-celled life does for the world and what the scientists in this unit teach us about patient, careful and safe observation.

- **Accuracy**: Drawings and labels are correct; prokaryotes and eukaryotes are distinguished; movement, feeding and reproduction are described correctly for each organism.
- **Data and calculations**: All four experiments have complete tables, labeled graphs with units, and correct calculations (rates, doublings, percentages) with work shown.
- **Scientific thinking**: Each experiment names its independent variable, dependent variable, controlled variables and control; conclusions match the data and note their limits.
- **Safety and care**: Safety rules were followed and recorded; the guide is neat, organized and titled; the essay is clear and in complete sentences.

Answer key

1. (B).
2. Bacteria on a slide are stained with violet dye, treated with iodine, washed with alcohol and counterstained pink. Gram-positive bacteria stay purple because their thick cell wall traps the violet dye. Gram-negative bacteria turn pink because their thin wall, wrapped in an outer membrane, lets the alcohol wash the violet out, and they take up the pink counterstain.
3. 2 hours = 120 minutes; 120 ÷ 20 = 6 doublings; $2^6 = 64$ cells. Real populations level off because food runs out, wastes build up, or space is limited.
4. (C) a paramecium; the hair-like structures are cilia.
5. Laveran found that malaria is caused by a single-celled protist (*Plasmodium*) living in red blood cells. Ross found the parasite growing in the stomach wall of *Anopheles* mosquitoes, showing that mosquitoes carry it. People could now fight malaria by controlling mosquitoes (draining standing water, screening windows) instead of blaming bad air.
6. Amoeba with pseudopods labeled; paramecium with cilia labeled; euglena with a flagellum labeled; plus one other correct label on each, for example nucleus (amoeba), oral groove or contractile vacuole (paramecium), eyespot or chloroplasts (euglena). (1 point for each correctly drawn and labeled movement structure, 1 point for each correct second label.)
7. Pond water has fewer dissolved particles than the inside of the cell, so water keeps moving into the paramecium by osmosis. The contractile vacuole collects the extra water and squeezes it out. Without it, the cell would swell and could burst.
8. (B) alcohol and carbon dioxide.
9. (a) 35 °C. (b) At 5 °C the yeast was alive but very slow in the cold. At 65 °C the heat killed the yeast, so no gas was made. (c) Independent: temperature. Dependent: volume of rise after 45 minutes.
10. Water: 600 × 0.70 = 420 g. Salt: 600 × 0.02 = 12 g. Yeast: 600 × 0.015 = 9 g.
11. (a) (150 − 15) ÷ 150 × 100 = 135 ÷ 150 × 100 = 90%. (b) The control shows that the swabs and plates were sterile, so the colonies on the other plates really came from the cutting board.
12. Any three, with reasons: tape plates shut and never open them (keeps grown microbes, which might be harmful, from escaping); incubate at room temperature, not body temperature (avoids favoring germs that grow in people); never sample body fluids, toilets or sick people

(avoids growing disease germs); have an adult destroy plates with bleach or double-bag them (kills or contains the microbes); wash hands before and after (keeps microbes off you and out of the plate); incubate upside down (keeps water drops off the colonies). Agar replaced gelatin because gelatin melts at body temperature and some bacteria digest it, while agar stays solid up to about 85 °C and almost no bacteria can eat it. (1 point per rule with reason, 3 points for the agar explanation.)

UNIT 4

Germs and Disease

In Unit 3 you met bacteria, protists and yeast and learned to culture microbes safely. Now you will ask how some of those tiny living things cause disease, and how people learned to stop them. You will walk the wards of the General Hospital of Vienna in 1847 with Ignaz Semmelweis, who saved mothers' lives by making doctors wash their hands. You will watch Louis Pasteur bend the necks of glass flasks to prove that microbes come only from other microbes, ending the old idea of spontaneous generation and opening the way to the **germ theory of disease**.

Then you will look over Alexander Fleming's shoulder at a moldy culture dish in London in 1928, and follow the Oxford team who turned his mold into penicillin, the first great antibiotic. Finally you will turn to the defenses God built into your own body: skin and tears, the "eating cells" Élie Metchnikoff saw gathering around a rose thorn, antibodies and memory cells, and the vaccine Edward Jenner tested in 1796.

Throughout the unit you will work like the germ hunters did: calculating death rates and percent changes, following microbes as they double and medicines as they halve, measuring zones of inhibition in millimeters, reading graphs of real and typical data, and designing fair tests with controls. You will also see how these discoveries came from careful, humble observation of the world God made, and how they have been used to love our neighbors by protecting their health.

BEFORE YOU BEGIN

- Knows the parts of a cell and the difference between plant cells, animal cells and bacteria (Units 2 and 3)
- Can follow the safety rules for growing microbes from Lesson 3.4
- Can find a percent, an average and a ratio, and work with powers of 2
- Can read a bar graph and a line graph and name the variables in an experiment

MASTERY CHECKLIST

- I can tell how Semmelweis used two clinics as a natural experiment and calculate death rates from his data.
- I can describe Pasteur's swan-neck flask experiment and explain why it disproved spontaneous generation.
- I can explain how Fleming discovered penicillin, how it kills bacteria, and why antibiotics do not work on viruses.
- I can name the body's three lines of defense and explain how memory cells and vaccines protect us.
- I can calculate percents, averages, doubling and halving steps, and read graphs of microbe and immune data.
- I can design a fair test with one changed variable, a control and a measured result.

OPTIONAL BOOKS AND RESOURCES

- **René Vallery-Radot, *The Life of Pasteur* (free at Project Gutenberg)** (https://www.gutenberg.org/ebooks/search/?query=life+of+pasteur): Free. A biography written by Pasteur's son-in-law. Read aloud the chapters on the swan-neck flasks and the Alps experiments with Lesson 4.2. The book is long and old-fashioned, so a parent may choose the passages.
- **The Nobel Prize in Physiology or Medicine 1945 (Fleming, Chain and Florey)** (https://www.nobelprize.org/prizes/medicine/1945/summary/): Free. The official Nobel site has short biographies of Fleming, Chain and Florey and the text of Fleming's Nobel lecture, including his warning about using too little penicillin (Lesson 4.3).
- **The Nobel Prize in Physiology or Medicine 1908 (Metchnikoff and Ehrlich)** (https://www.nobelprize.org/prizes/medicine/1908/summary/): Free. Biographies of Élie Metchnikoff and Paul Ehrlich, the two sides of the great debate about how the body fights germs (Lesson 4.4).

4.1 Semmelweis and Handwashing (1847) ★

OBJECTIVES

- Tell how Ignaz Semmelweis compared two hospital clinics in Vienna and traced childbed fever to something carried on doctors' hands.
- Calculate and compare death rates as percentages and per 1,000 births, and read a bar graph of before-and-after data.
- Design a fair test of handwashing with a control, one changed variable and a measurable result.

Warm-up. Rub a few drops of cooking oil on your palms, then sprinkle on a pinch of cinnamon. Shake hands with a family member, open a door, and pick up a pencil. Now look closely: how many places did the "germs" travel in one minute? Try rinsing with plain cold water for five seconds. How much cinnamon is left?

Video. Coming soon. Ignaz Semmelweis, childbed fever and the history of handwashing, plus a demonstration of germs spreading on hands (glitter or ultraviolet lotion tests); search Homeschool Pop (Alternate), SciShow Kids, Science Max, Smithsonian and medical-history channels.

KEY TERMS

childbed fever
A deadly infection of mothers soon after giving birth, also called puerperal fever. Today we know it is usually caused by *Streptococcus* bacteria.

mortality rate
The fraction of people in a group who die in a given time, often written as a percent or as deaths per 1,000.

contamination
Getting harmful material, such as germs, onto something that was clean.

antiseptic
A substance that kills or slows germs on skin or surfaces, such as chlorine solution, alcohol or iodine.

control group
The part of an experiment that does not get the change being tested, so the results can be compared with it.

variable
Anything in an experiment that can change. A fair test changes only one variable at a time.

CORE IDEAS

The story. In 1846 a young Hungarian doctor named Ignaz Semmelweis became an assistant in the First Obstetrical Clinic of the great General Hospital of Vienna, Austria. Poor mothers came there to give birth free of charge, and far too many of them died a few days later of **childbed fever**. Next door was the Second Clinic, where the mothers were cared for by midwives instead of doctors. In the Second Clinic, mothers died much less often. Everyone in Vienna knew it. Some mothers begged to be sent to the midwives' clinic instead, and Semmelweis could not stop asking why two clinics under the same roof should be so different.

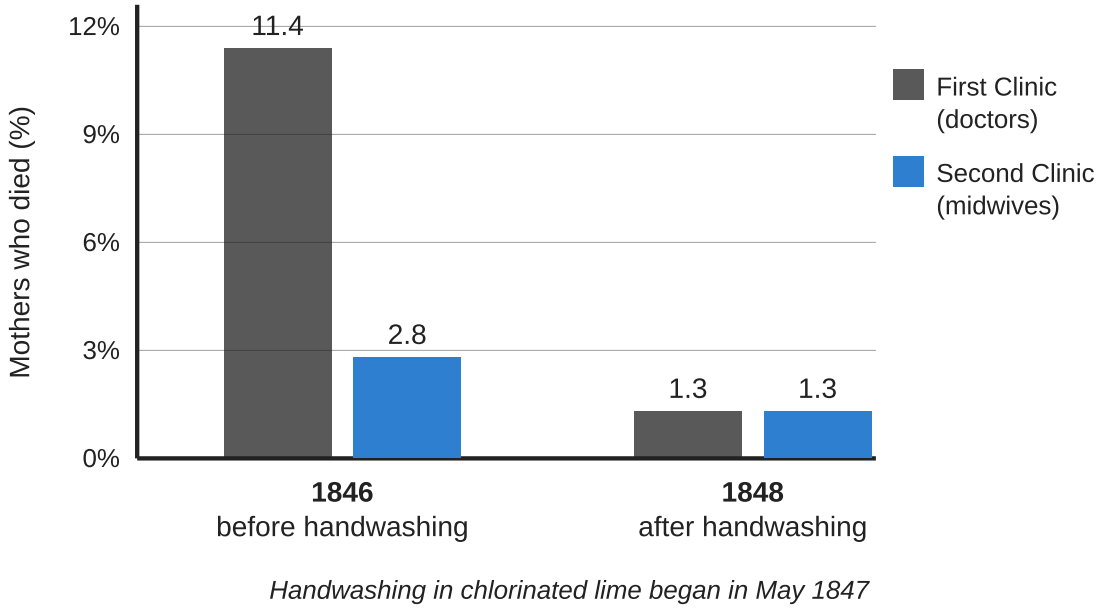
How he figured it out. Semmelweis worked like a detective, crossing out one explanation at a time. Both clinics had the same building, the same air and the same kind of mothers, so "bad air" could not explain it. He changed how mothers lay during birth and even the route the priest walked through the wards; nothing changed the death rate. Then, in March 1847, his friend Jakob Kolletschka, a professor who taught by examining bodies of the dead, was cut by a student's knife during an autopsy. Kolletschka fell sick and died, and his illness looked just like childbed fever. Semmelweis saw the pattern at once. The doctors and medical students began their mornings in the autopsy room and then walked straight to the birthing ward. The midwives never did autopsies. He concluded that "cadaverous particles," something from the dead bodies, were carried on the doctors' hands to the mothers.

The experiment. In May 1847 Semmelweis ordered every doctor and student to scrub their hands in a solution of **chlorinated lime**, a chlorine bleach, before examining a mother. He chose it because it removed the smell of the autopsy room from the hands better than soap. The results were dramatic. In April 1847, before the rule, about 18 out of every 100 mothers in his clinic died. In June, after it, about 2 out of every 100 died. For the whole year of 1848, the First Clinic's **mortality rate** was about 1.3 percent, the same as the midwives' clinic.

The big idea. Semmelweis did not know about bacteria; no one had yet shown that tiny living germs cause disease. But his reasoning was sound science. He compared two groups that differed in one important way, made a change, and measured what happened. The midwives' clinic acted as a natural **control group**. Today we know that childbed fever is usually caused by *Streptococcus* bacteria, and that **contamination** on hands is one of the main ways germs spread. Scrubbing with soap and water for about 20 seconds lifts germs off the skin, and an **antiseptic** such as chlorine or alcohol kills many of the rest.

Why it was hard to believe. Many senior doctors refused to accept Semmelweis's idea. It seemed to blame them, as gentlemen and healers, for the deaths of their own patients. He also waited many years to publish his full evidence, and when he did, in 1861, he wrote angry letters to those who disagreed. He left Vienna and worked at hospitals in Pest, Hungary, where he again lowered deaths with handwashing. He died in 1865, before most doctors accepted his work. Only after Louis Pasteur and Joseph Lister showed that germs cause infection did the world see that Semmelweis had been right.

God’s handiwork. Long before microscopes, the law given through Moses taught Israel that touching a dead body made a person unclean and required washing (Numbers 19). Semmelweis was a Catholic Christian who could not rest while mothers were dying. Caring for the weak and the sick, and doing it with careful measuring and honest records, is a good way to love our neighbor.



Death rates from all causes among mothers in the two clinics, before (1846) and after (1848) Semmelweis’s handwashing rule. Data from Semmelweis’s 1861 book.

PICTURE STUDY



The Gross Clinic (Portrait of Dr. Samuel D. Gross), Thomas Eakins, 1875 · Oil on canvas · Philadelphia Museum of Art and the Pennsylvania Academy of the Fine Arts, Philadelphia

Thomas Eakins of Philadelphia studied anatomy alongside medical students so that he could paint the human body truthfully. In 1875 he painted the famous surgeon Samuel D. Gross teaching students during an operation on a young man's leg bone at Jefferson Medical College. (Parents may wish to preview it: blood is shown on the surgeon's hand and scalpel and at the open wound.)

This painting was made 28 years after Semmelweis's handwashing rule and 8 years after Joseph Lister began using antiseptics in surgery, yet look at how the surgeons are dressed. Many American doctors were still slow to accept that invisible germs cause infection. The painting is a window into a world just before germ theory changed medicine.

Look closely

- Find the surgeon's hands. Is he wearing gloves? What is he holding?
- Look at the clothing of the men around the table. Are they dressed for an operating room or for the street?
- Find the woman on the left side. How does Eakins show her feelings?
- Follow the light. Where does Eakins want your eye to go first, and why?

Talk about it

1. List three things in this painting that a modern surgeon would do differently to prevent infection.
2. Why do you think many doctors in 1875 still did not wash and sterilize as Semmelweis and Lister urged?

3. Eakins painted Dr. Gross as a calm, wise teacher. How can someone be a great doctor and still be wrong about one important thing?

> **Safety first.** Never open the sealed bread bags once mold appears; some people are allergic to mold spores, and anyone with asthma or mold allergies should only look through the plastic. Do not eat the bread. At the end, an adult throws the bags away still sealed. Wash your hands with soap after handling the bags.

EXPERIMENT: THE SEMMELWEIS BREAD TEST

Semmelweis could not see germs, but he could count what they did. You will do the same by letting slices of bread record what your hands carry. Over one to two weeks, the microbes left on each slice will grow into spots of mold you can count and measure.

Question: Does the way we clean our hands change how many microbes they leave on bread? **Prediction:** Write which slice you think will grow the most mold and which the least. **What we change:** how the hands are cleaned. **What we keep the same:** the kind of bread, the size of the slice, how long and how firmly the bread is touched, the bags, the place and the temperature. **What we measure:** the number of mold spots and the percent of the slice covered.

You will need

- 5 slices of the same bread (bakery bread without preservatives grows mold fastest)
- 5 zipper-top plastic bags and a permanent marker
- Clean kitchen tongs
- Soap, warm water, a clean towel and hand sanitizer (at least 60 percent alcohol)
- A transparent sheet or clear plastic with a 1 cm grid drawn on it (or graph paper to trace on)
- A timer and your lab notebook

Steps

1. Label the bags: A Control (tongs only), B Unwashed hands, C Water rinse 5 seconds, D Soap and water 20 seconds, E Hand sanitizer.
2. Use clean tongs to put slice A straight into its bag without touching it. Seal it. This is your control.
3. Before washing, after you have been playing or working for a while, press your fingertips and palm onto slice B for 10 seconds. Bag and seal it.
4. Rinse your hands under cold water for 5 seconds only, dry them on the clean towel, and touch slice C the same way.
5. Get your hands dirty again (touch doorknobs, a phone, the floor of a shoe closet). Wash with soap and warm water for 20 seconds, dry, and touch slice D the same way.
6. Get your hands dirty again, rub in hand sanitizer until dry, and touch slice E the same way.

7. Store all five bags side by side in a dark cupboard at room temperature. Do not open them.
8. Every two days for two weeks, lay the grid over each bag and count the mold spots and the number of grid squares that are at least half covered. Record the date and both numbers in a data table.
9. On the last day, find the percent covered for each slice: squares covered ÷ total squares on the slice × 100. Make a bar graph and write a conclusion.

Tips

- Touch every slice for the same time with the same pressure. If you press harder on one slice, you have changed a second variable.
- If the control slice grows mold, that is useful information: it tells you how much mold came from the air and the bread itself.
- A family can run two sets of slices with two different people, which shows whether the result repeats.

Check your work

- I kept every variable the same except the way hands were cleaned, and I included a control.
- I recorded counts and percent covered with dates in a data table and made a bar graph.
- I wrote a conclusion that compares my results with Semmelweis's and names one weakness in my test.

NATURE NOTEBOOK

1. Draw a floor plan of the Vienna hospital as Semmelweis knew it: the autopsy room, the First Clinic (doctors) and the Second Clinic (midwives). Use arrows to show who walked from where to where each morning.
2. Draw your five bread slices on the last day of the test, shading the mold to scale, and label each with its percent covered.
3. Make a two-column chart: "What Semmelweis observed" and "What he concluded." Write at least four rows.

> **From history.** Semmelweis was not the only one to suspect that doctors carried childbed fever. In 1843 the Boston physician Oliver Wendell Holmes Sr. published an essay called "The Contagiousness of Puerperal Fever," urging doctors who had attended a case to wash and change their clothes before seeing other mothers. Today the medical school in Budapest is named Semmelweis University in his honor.

WORKED EXAMPLES

Example 1. In 1846 the First Clinic (doctors) had 4,010 births and 459 mothers died. The Second Clinic (midwives) had 3,754 births and 105 mothers died. Find each mortality rate as a percent, and how many times higher the First Clinic's rate was.

(Use the map or drawing "Check your answers against the 1846 bars." shown earlier in this lesson.)

1. Mortality rate = deaths ÷ births × 100.
2. First Clinic: 459 ÷ 4,010 = 0.1145, and 0.1145 × 100 = 11.4%.
3. Second Clinic: 105 ÷ 3,754 = 0.0280, and 0.0280 × 100 = 2.8%.
4. Compare: 11.4 ÷ 2.8 = 4.1.

Answer: First Clinic 11.4%, Second Clinic 2.8%. The doctors' clinic was about 4 times deadlier.

Example 2. In 1848, after a full year of handwashing, the First Clinic had 3,556 births and 45 deaths. Find the new mortality rate, the percent decrease from 1846 (11.4%), and the number of deaths per 1,000 births before and after.

1. New rate: 45 ÷ 3,556 = 0.0127, which is 1.3%.
2. Decrease: 11.4% − 1.3% = 10.1 percentage points.
3. Percent decrease: 10.1 ÷ 11.4 × 100 = 88.6%, about 89%.
4. Per 1,000 births: 11.4% means 114 per 1,000 before; 1.3% means 13 per 1,000 after.

Answer: The rate fell to about 1.3%, a decrease of about 89%: from about 114 deaths per 1,000 births to about 13.

Example 3. In April 1847 the First Clinic had 312 births and 57 deaths. In June 1847, after the handwashing rule began in May, it had 268 births and 6 deaths. If 300 mothers were cared for at each rate, about how many lives would be saved?

1. April rate: 57 ÷ 312 = 0.183, or 18.3%.
2. June rate: 6 ÷ 268 = 0.022, or 2.2%.
3. At the April rate, 300 × 0.183 = about 55 deaths.
4. At the June rate, 300 × 0.022 = about 7 deaths.
5. Difference: 55 − 7 = 48.

Answer: About 48 of every 300 mothers would be saved.

PRACTICE

1. EASY Why were the two clinics at the Vienna General Hospital a good natural experiment?
2. EASY What event in March 1847 gave Semmelweis his key clue?
3. EASY What did Semmelweis order doctors and students to do in May 1847, and why did he choose chlorinated lime?
4. MEDIUM A clinic has 2,500 births and 30 deaths in a year. Find the mortality rate as a percent and as deaths per 1,000 births.
5. MEDIUM Data reading. Use the graph in this lesson. Death rates: 1846, First Clinic 11.4% and Second Clinic 2.8%; 1848, First Clinic 1.3% and Second Clinic 1.3%. Which statement is best supported? (A) The midwives' clinic got much worse after 1847. (B) The First Clinic's rate fell to about the same as the Second Clinic's after handwashing began. (C) Handwashing had no effect. (D) Doctors were less skilled than midwives at delivering babies.
6. MEDIUM Semmelweis first wondered whether "bad air" (miasma) caused childbed fever. Use the two clinics to explain why that idea did not fit the evidence.
7. HARD Data reading. A class tested bread slices touched after different hand cleanings. Mold coverage after 10 days: no washing 42%; water rinse 5 s 35%; soap 20 s 8%; sanitizer 6%; tongs only (control) 3%. (a) Which method left the most microbes besides no washing? (b) About how many times more mold grew after a water rinse than after soap? (c) Why is the control important?
8. HARD Semmelweis did not know about bacteria. Was his conclusion still scientific? Explain in two or three sentences.
9. CHALLENGE Experiment design. Design a test to find out whether washing hands for 20 seconds removes more germs than washing for 5 seconds. Name the variable you change, three variables you keep the same, what you measure, and your control.
10. CHALLENGE Give two reasons why many doctors rejected Semmelweis's idea, and explain what finally convinced the medical world.

COMMON MISTAKES

- Thinking a quick rinse with water cleans hands. Water alone removes little; soap and about 20 seconds of scrubbing lift oils and germs from the skin.
- Thinking Semmelweis knew about bacteria. He reasoned from patterns and numbers; germ theory came about twenty years later through Pasteur and Lister.
- Thinking a bigger number of deaths always means a worse hospital. You must compare rates (deaths per births), because the clinics delivered different numbers of babies.

> **Make it a game · Germ Detective.** One player secretly dusts a fingertip with flour or cinnamon and then touches three things in a room. The other players have two minutes to find all three "contaminated" objects and trace the path the germs traveled. Then switch roles. For a bonus point, the detective must name one way the spread could have been stopped, such as washing hands or wiping a surface. Play four rounds.

> **Scripture.** “He that toucheth the dead body of any man shall be unclean seven days.” (Numbers 19:11, KJV)

The law God gave Israel set the dead apart and required washing (Numbers 19:12–19). Semmelweis found, more than three thousand years later, that carrying something from the dead room to the birthing room was killing mothers. Cleanliness that protects others is a way of loving our neighbor.

Practice answers

1. They were in the same building with the same air and the same kind of mothers, but one was run by doctors and students (who did autopsies) and the other by midwives (who did not). The main difference could be compared.
2. His friend Jakob Kolletschka was cut by a student’s knife during an autopsy and died of an illness that looked just like childbed fever.
3. Scrub their hands in a chlorinated lime (chlorine) solution before examining mothers. He chose it because it removed the smell of the autopsy room from the hands better than soap, so he reasoned it also removed the harmful particles.
4. 30 ÷ 2,500 = 0.012 = 1.2%, which is 12 deaths per 1,000 births.
5. (B). The First Clinic fell from 11.4% to 1.3%, matching the Second Clinic. A and C are contradicted by the data, and D does not follow, since the gap disappeared once doctors washed their hands.
6. Both clinics shared the same building and the same city air, yet one had about four times the death rate of the other. If bad air were the cause, both clinics should have suffered about equally.
7. (a) The 5-second water rinse (35%). (b) 35 ÷ 8 = about 4.4 times more. (c) The control shows how much mold grows from the bread and air alone (3%), so we know the extra mold on the other slices came from hands.
8. Yes. He made careful observations, ruled out explanations that did not fit, tested his idea with a clear change (handwashing), and measured the result with numbers. He was wrong about the exact cause (“cadaverous particles” rather than bacteria), but his tested conclusion, that something on the hands caused the disease and washing prevented it, was correct.
9. Sample answer: Change the washing time (5 s or 20 s). Keep the same soap, water temperature, amount of dirt on the hands before washing, the person, the drying towel, the bread or agar used, the touching time and pressure, and the storage place. Measure mold spots or percent covered after 10 days. The control is a slice touched only with clean tongs (and a slice touched by unwashed hands for comparison). Repeat the test at least twice.
10. Reasons (any two): it seemed to blame doctors for their patients’ deaths; no one had shown that invisible germs cause disease, so his “particles” sounded vague; he waited years to publish his full data; his angry letters turned people against him. The medical world was finally convinced when Pasteur showed that microbes cause decay and disease and Lister showed that antiseptics prevent infection in surgery.

4.2 Pasteur and Spontaneous Generation

OBJECTIVES

- Explain the old idea of spontaneous generation and describe how Redi, Spallanzani and Pasteur tested it.
- Describe Pasteur’s swan-neck flask experiment, name its variables and control, and explain why it convinced scientists that microbes come only from other microbes.
- Calculate percents from experimental data and use doubling steps to show how fast one microbe can multiply.

Warm-up. Leave a slice of apple on a plate in the kitchen and put a second slice in a clean jar with the lid screwed on tight. Ask: in a week, which one do you think will be covered with mold or tiny flies? Where would the mold and flies come from? Write your guess in your notebook before you read the lesson.

Video. Coming soon. Louis Pasteur’s swan-neck flask experiment and the end of spontaneous generation, with Redi’s meat-and-gauze jars; search Amoeba Sisters, Homeschool Pop (Alternate), SciShow Kids, Smithsonian, Moody Science classics and microbiology channels such as Journey to the Microcosmos.

KEY TERMS

spontaneous generation
The old idea that living things can arise on their own from nonliving matter, such as maggots from meat or microbes from broth.

biogenesis
The principle that living things come only from other living things.

microbe
A living thing too small to see without a microscope, such as a bacterium, a yeast or a protist. Also called a microorganism.

sterilize
To kill or remove every living microbe from something, often by heating it.

germ theory of disease
The scientific theory that many diseases are caused by microbes (germs) that pass from one living thing to another.

pasteurization
Heating a food or drink to a set temperature for a set time to kill most harmful microbes without boiling it.

CORE IDEAS

The story. For thousands of years, people believed that some living things simply appeared out of nonliving stuff. Mice seemed to come from piles of grain and rags, maggots from rotting meat, and, after Leeuwenhoek's microscope, tiny "animalcules" from a bowl of broth left on the table. This idea was called **spontaneous generation**. In 1859 the French Academy of Sciences offered a prize to anyone who could settle the question with good experiments. A chemist named Louis Pasteur, then working in Paris, took up the challenge. He had already shown that tiny yeast cells cause the fermentation of beer and wine, and he suspected that the microbes in spoiled broth came from somewhere too.

How they figured it out. Pasteur was not the first to test the idea. In 1668 the Italian doctor Francesco Redi put meat in jars. Flies laid eggs on the meat in the open jars, and maggots appeared; in the jars covered with fine gauze, the meat rotted but no maggots grew on it. In the 1760s Lazzaro Spallanzani boiled broth in flasks and sealed them shut, and the broth stayed clear. But defenders of spontaneous generation answered that sealing the flask kept out the fresh air that life needed. Pasteur's answer, suggested by his fellow chemist Antoine Balard, was brilliant. He put broth in a flask, heated the glass neck and bent it into a long S shape like a swan's neck, then boiled the broth to **sterilize** it. Air could flow freely in and out, but dust and microbes settled in the low bend of the neck and never reached the broth. The broth stayed clear for weeks and months. When Pasteur snapped off the neck, or tipped the flask so the broth washed into the dusty bend, the broth turned cloudy with microbes within days.

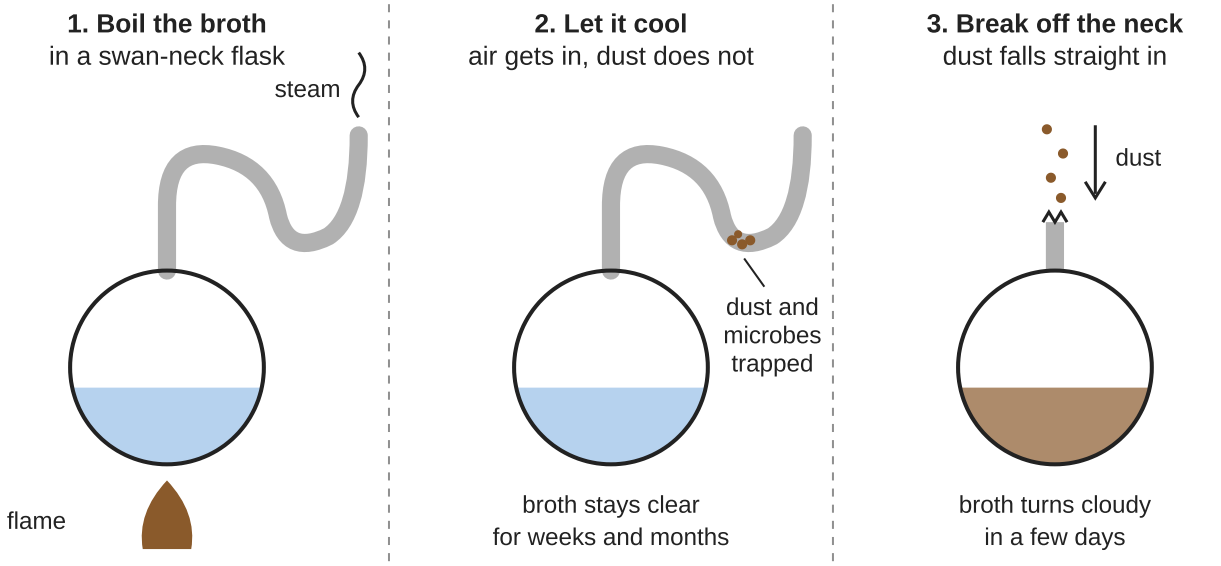
More evidence. Pasteur also pulled air through a plug of cotton and found, under his microscope, that the cotton had trapped tiny round bodies that looked like spores and microbes. In 1860 he carried sterile flasks of broth into the countryside and up into the mountains of France and opened each one briefly to the air. Of 20 flasks opened near the foot of the Jura Mountains, 8 grew microbes. Of 20 opened on the Jura at about 850 meters, 5 grew. Of 20 opened high on the Montanvert, near a glacier in the Alps at about 2,000 meters, only 1 grew. Clean mountain air carried fewer microbes. Life in the broth came from living things already floating in the air.

The big idea. Pasteur's experiments established **biogenesis**: living things come from living things. In a famous lecture at the Sorbonne in Paris in April 1864, he showed his flasks and told the audience that spontaneous generation would never recover from the blow of this simple experiment. Once scientists knew that **microbes** do not spring from nowhere, a new question opened: if microbes travel through air, water and touch, could they cause disease? Pasteur, the German doctor Robert Koch and others soon showed that they do. This became the **germ theory of disease**, and it finally explained why Semmelweis's handwashing worked.

In the world today. Every time you drink milk, open a can of soup or have a cut cleaned at the doctor's office, you are using Pasteur's discovery. In the 1860s he found that gently heating wine killed the microbes that soured it. This process, **pasteurization**, is now used for milk and juice: heating milk to 72 °C for 15 seconds kills most harmful bacteria without

boiling it. Canning, sterile bandages and the steam sterilizers in hospitals all depend on the same truth: kill the microbes and keep new ones out, and nothing will grow.

God's handiwork. Pasteur was a Catholic Christian who believed that careful experiments honor the Creator by revealing how His world really works. His flasks showed that life does not come from broth, dust or air by itself. Scripture says that God "giveth to all life, and breath, and all things" (Acts 17:25), and that He made living things to reproduce "after their kind" (Genesis 1). Every microbe in a cloudy flask came from a parent microbe, just as every oak comes from an acorn.



Pasteur's swan-neck flask experiment. Air reaches the broth in every flask; only the dust and microbes are kept out.

PICTURE STUDY



Louis Pasteur, Albert Edelfelt, 1885 · Oil on canvas · Musée d'Orsay, Paris

Albert Edelfelt was a Finnish painter who lived in Paris and became Pasteur's friend. He painted the great scientist in his laboratory in 1885, the year Pasteur first used his rabies treatment on a boy named Joseph Meister. In his hand Pasteur holds a jar containing a piece of spinal cord from a rabbit that had rabies, hung up to dry and weaken the germ. Pasteur was 62. He had suffered a stroke in 1868 that left one side of his body partly paralyzed, yet he kept working.

Edelfelt shows the scientist not as a magician but as a careful worker, absorbed in observing. There is no grand background, only the tools of the work and the man doing it.

Look closely

- Find the jar in Pasteur's hand. How is he holding it, and where is he looking?
- Look at the objects on the table. Which ones would a scientist in a laboratory use, and which are for writing records?
- Notice the colors. What is bright, and what is dark? How does Edelfelt use light to show what matters?
- Look at Pasteur's face and posture. What words would you use to describe his mood?

Talk about it

1. Why do you think Edelfelt chose to paint Pasteur working instead of posing in fine clothes?
2. Pasteur's notebooks recorded every experiment. Why are careful records important when you are trying to overturn an old idea?
3. If you were painted at work on your favorite project, what would you be holding?

> **Safety first.** An adult should handle boiling broth and hot jars; steam and hot glass burn. Use canning jars or heat-safe glass only. Once the jars are sealed, do not open any cloudy jar or smell it; some microbes that grow in broth can make you sick. At the end, an adult pours the broth down the toilet and washes the jars in very hot soapy water. Wash your hands after handling the jars.

EXPERIMENT: PASTEUR'S FLASKS IN THE KITCHEN

You will repeat the heart of Pasteur's experiment with jars of broth. One jar is open to the air, one is sealed, and one has a bent "swan neck" made from a bendable straw, so air can reach the broth but falling dust cannot.

Question: Does broth turn cloudy because of the air itself, or because of microbes carried by dust in the air? **Prediction:** Write which jars you think will turn cloudy. **What we change:** how each jar is covered. **What we keep the same:** the broth, the amount, the boiling time, the kind of jar, the place and the temperature. **What we measure:** cloudiness on a 0 to 4 scale every day for 10 days.

You will need

- One can of clear chicken or beef broth (or 2 bouillon cubes in 500 mL of water)
- 4 small heat-safe glass jars of the same size
- Aluminum foil, plastic wrap and one lid
- 2 bendable drinking straws, modeling clay and a rubber band
- A saucepan, an oven mitt and an adult helper
- A sheet of white paper with a black line drawn on it (for judging cloudiness), a marker and your lab notebook

Steps

1. Label the jars: A Not boiled, open; B Boiled, open; C Boiled, sealed with a lid; D Boiled, swan neck.
2. Pour about 100 mL of broth into jar A and leave it uncovered.
3. With an adult, boil the rest of the broth for 10 minutes. The adult pours about 100 mL into each of jars B, C and D.
4. Leave jar B uncovered. Screw the lid on jar C right away.
5. For jar D, the adult quickly covers the mouth with foil, pokes a straw through it and seals around the straw with clay. Bend the straw so it goes up, then curves down below the level of the jar's mouth, then turns up again at the end, like a swan's neck. Hold the bends with a rubber band or tape.
6. Place all four jars side by side away from sunlight and heat.
7. Each day, hold the white paper with the black line behind each jar. Score cloudiness: 0 = crystal clear, 1 = slightly hazy, 2 = hazy but the line is sharp, 3 = cloudy and the line is blurry, 4 = cannot see the line. Also note any film, mold or smell you notice without opening the jar.

8. Make a line graph of cloudiness score against days for all four jars on one set of axes.
9. Write a conclusion: Which jars clouded first? Did jar D stay clear longer than jar B, even though air could reach it? What does that tell you about where the microbes came from?

Tips

- A straw is not as good as Pasteur's glass neck, and the seal may leak. If jar D eventually clouds, check whether it clouded later than jar B. A delay is still evidence.
- Jar A (not boiled) shows how fast broth spoils when microbes are already in it. It is a useful comparison.
- Do not shake or tip jar D. Broth that washes into the bend picks up the trapped dust, just as in Pasteur's tipped flasks.

Check your work

- I named the variable I changed, the variables I kept the same and what I measured.
- I scored cloudiness every day and made a line graph for all four jars.
- I explained what my results show about spontaneous generation and where microbes come from.

NATURE NOTEBOOK

1. Draw Redi's experiment as two jars of meat, one open and one covered with gauze. Show where the flies laid their eggs and where maggots appeared.
2. Draw a swan-neck flask full size in your notebook. Label the broth, the bend, the trapped dust and the path of air.
3. Make a timeline card: Redi 1668, Spallanzani 1760s, Pasteur's flasks 1859–1862, Pasteur's Sorbonne lecture 1864. Write one sentence under each.

> **From history.** In 1885 nine-year-old Joseph Meister was badly bitten by a dog with rabies, a disease that was almost always fatal. Pasteur, who was not a medical doctor, treated him with a series of injections made from dried rabbit spinal cord, like the one in Edelfelt's painting. Joseph lived. As an adult, Joseph Meister worked as the caretaker at the Pasteur Institute in Paris, the research center founded in Pasteur's honor in 1888.

WORKED EXAMPLES

Example 1. In 1860 Pasteur opened sterile flasks of broth in three places. Near the foot of the Jura Mountains, 8 of 20 flasks grew microbes. On the Jura at about 850 m, 5 of 20 grew. On the Montanvert in the Alps at about 2,000 m, 1 of 20 grew. Find each percent, and describe the pattern.

1. Percent = flasks that grew ÷ flasks opened × 100.
2. Lowland: 8 ÷ 20 = 0.40, which is 40%.
3. Jura (850 m): 5 ÷ 20 = 0.25, which is 25%.
4. Montanvert (2,000 m): 1 ÷ 20 = 0.05, which is 5%.
5. Pattern: as the height goes up, the percent of flasks that grew microbes goes down.

Answer: 40%, 25% and 5%. Higher, cleaner air carried fewer microbes, so fewer flasks became contaminated.

Example 2. Under ideal conditions some bacteria divide every 20 minutes. If one bacterium falls into a flask of warm broth, how many bacteria could there be after 4 hours? After 8 hours?

1. Number of divisions in 4 hours: 4 × 60 = 240 minutes, and 240 ÷ 20 = 12 divisions.
2. Each division doubles the number: 1 → 2 → 4 → 8 → … After 12 doublings there are 2^{12} = 4,096 bacteria.
3. In 8 hours there are 24 divisions: 2^{24} = 16,777,216 bacteria.
4. That is about 17 million from one cell, which is why a clear broth can turn cloudy overnight.

Answer: About 4,096 after 4 hours and about 17 million (16,777,216) after 8 hours.

Example 3. One method of pasteurizing milk heats it to 63 °C for 30 minutes. A faster method heats it to 72 °C for 15 seconds. How many times longer is the slow method? How many degrees hotter is the fast method?

1. Change minutes to seconds: 30 × 60 = 1,800 seconds.
2. Compare times: 1,800 ÷ 15 = 120.
3. Compare temperatures: 72 °C − 63 °C = 9 °C.

Answer: The slow method takes 120 times longer; the fast method is 9 °C hotter. A higher temperature kills microbes much faster.

PRACTICE

1. EASY What is spontaneous generation? Give one example people once believed.
2. EASY In Redi's 1668 experiment, why did no maggots appear on the meat in the gauze-covered jars?
3. EASY Why did Pasteur bend the necks of his flasks instead of sealing them shut as Spallanzani had?
4. MEDIUM In the swan-neck experiment, name the variable Pasteur changed, one variable he kept the same, and what he observed.
5. MEDIUM A bacterium divides every 30 minutes. Starting with 1 cell, how many cells are there after 3 hours?
6. MEDIUM Data reading. A student's broth jars were scored for cloudiness (0 = clear, 4 = very cloudy). Day 2: A not boiled, open 3; B boiled, open 0; C boiled, sealed 0; D swan neck 0. Day 4: A 4; B 3; C 0; D 0. Day 8: A 4; B 4; C 0; D 1. Which conclusion is best supported? (A) Boiling makes broth cloudy. (B) Air alone causes broth to turn cloudy. (C) Broth turned cloudy fastest when microbes could fall straight in, and boiling delayed cloudiness. (D) Sealed jars become cloudy fastest.
7. HARD Data reading. Use Pasteur's 1860 results: lowland 8 of 20 flasks grew microbes, Jura (850 m) 5 of 20, Montanvert (2,000 m) 1 of 20. (a) Predict whether a flask opened at 1,400 m would be more or less likely to grow microbes than one opened at 850 m. (b) Name one other factor besides height that could explain the pattern.
8. HARD Explain how Pasteur's experiments on spontaneous generation led to the germ theory of disease.
9. CHALLENGE Experiment design. Design an experiment to test whether boiling broth for 1 minute sterilizes it as well as boiling it for 10 minutes. Name the variable you change, the variables you keep the same, your control and what you measure.
10. CHALLENGE Some of Pasteur's swan-neck flasks are said to have stayed clear for over a century. Explain why the broth stays clear even though the flask is open to the air.

COMMON MISTAKES

- Thinking Pasteur's flasks were sealed. The swan neck was open to the air; that was the whole point, since it answered the claim that life needed fresh air.
- Thinking boiling removes all microbes forever. Boiling sterilizes the broth only until new microbes get in; that is why the neck design mattered.
- Thinking maggots, mold or microbes are made by the food they grow on. They come from eggs, spores or parent cells that land on the food.

> **Make it a game · Where Did It Come From?.** A parent names something that seems to appear from nowhere: mold on bread, fruit flies around bananas, cloudiness in old soup, mushrooms after rain, mosquitoes near a puddle, green slime in a birdbath. You have ten seconds to name the living source it really came from (spores, eggs, microbes in the air or water). Score one point for each correct source and a bonus point if you can name how to keep it out. Play to ten points.

> **Scripture.** “Neither is worshipped with men’s hands, as though he needed any thing, seeing he giveth to all life, and breath, and all things;” (Acts 17:25, KJV)

Paul told the philosophers of Athens that life is God’s gift. Pasteur’s flasks showed that, in the world as it runs today, life comes only from life. Every living cell has a living parent.

Practice answers

1. The idea that living things arise on their own from nonliving matter. Examples: maggots from rotting meat, mice from grain and rags, or microbes from broth.
2. The gauze kept flies from reaching the meat to lay their eggs. Maggots come from fly eggs, not from the meat.
3. Critics said sealing kept out the fresh air needed for life. The bent neck let air in freely but trapped dust and microbes in the low bend, so it tested whether air alone could make life appear.
4. Changed: whether dust from the air could reach the broth (intact neck or broken neck). Kept the same (any one): the broth, the boiling, the flask, the air supply, the temperature. Observed: whether the broth turned cloudy with microbes.
5. 3 hours = 180 minutes; 180 ÷ 30 = 6 divisions; $2^6 = 64$ cells.
6. (C). The unboiled jar clouded first, the boiled open jar clouded next, and the swan-neck jar, which had air but little falling dust, stayed nearly clear. B is wrong because jar D had air but stayed mostly clear.
7. (a) Less likely; the trend shows fewer contaminated flasks at greater heights (somewhere between 25% and 5%). (b) Any reasonable factor: fewer people, farms and animals stirring up dust at high places; colder air; cleaner air over snow and ice; less soil and plant matter nearby.
8. Pasteur showed that microbes do not appear on their own but come from other microbes carried in air, dust, water and on surfaces. If microbes travel from place to place, they can also travel from person to person, so scientists asked whether specific microbes cause specific diseases. Pasteur, Koch and others then proved that they do.
9. Sample answer: Change the boiling time (1 minute or 10 minutes, with 3 jars each). Keep the same broth, amount, jar type, sealing method, cooling time, storage place and temperature. Control: a jar of unboiled broth, sealed the same way. Measure cloudiness on a 0–4 scale each day for 10 days. If the 1-minute jars cloud and the 10-minute jars do not, 1 minute is not enough.
10. The broth was sterilized by boiling, so no living microbes remained. Air can move in and out through the neck, but dust and microbes settle in the low bend and stick to the moist glass there. Because no living microbe ever reaches the broth, no new microbes can be produced, since living things come only from living things.

4.3 Fleming's Penicillin (1928)

OBJECTIVES

- Tell how Alexander Fleming noticed penicillin in 1928 and how Florey, Chain and Heatley turned it into a medicine that saved millions of lives.
- Explain how penicillin harms bacteria but not human cells, why antibiotics do not work against viruses, and how antibiotic resistance arises.
- Measure and compare zones of inhibition, average repeated trials, and use halving steps to follow a medicine in the blood.

Warm-up. Look in your refrigerator or bread box for an old orange, lemon or slice of bread with blue-green mold (look only; do not sniff it or touch it). Notice the color, the fuzzy texture and the white edge where it is spreading. Draw it in your notebook. Then ask: could something growing on spoiled food ever be useful?

Video. Coming soon. Alexander Fleming and the discovery of penicillin, Florey and Chain at Oxford, and how antibiotics kill bacteria (zone of inhibition demonstrations); search Homeschool Pop (Alternate), SciShow Kids, Amoeba Sisters, the Nobel Prize channel, Smithsonian and microbiology lab channels.

KEY TERMS

antibiotic
A medicine that kills bacteria or stops them from growing inside the body. Antibiotics do not work against viruses.

penicillin
The first widely used antibiotic, made by the mold *Penicillium*. It stops bacteria from building their cell walls.

Penicillium
A common blue-green mold, a kind of fungus, that grows on bread, fruit and damp places.

colony
A visible spot on a culture plate made of millions of microbes that all grew from one original cell.

zone of inhibition
The clear ring around a substance on a culture plate where microbes could not grow. A wider zone means a stronger effect.

antibiotic resistance
The ability of some bacteria to survive an antibiotic. When the others die, the resistant ones multiply.

CORE IDEAS

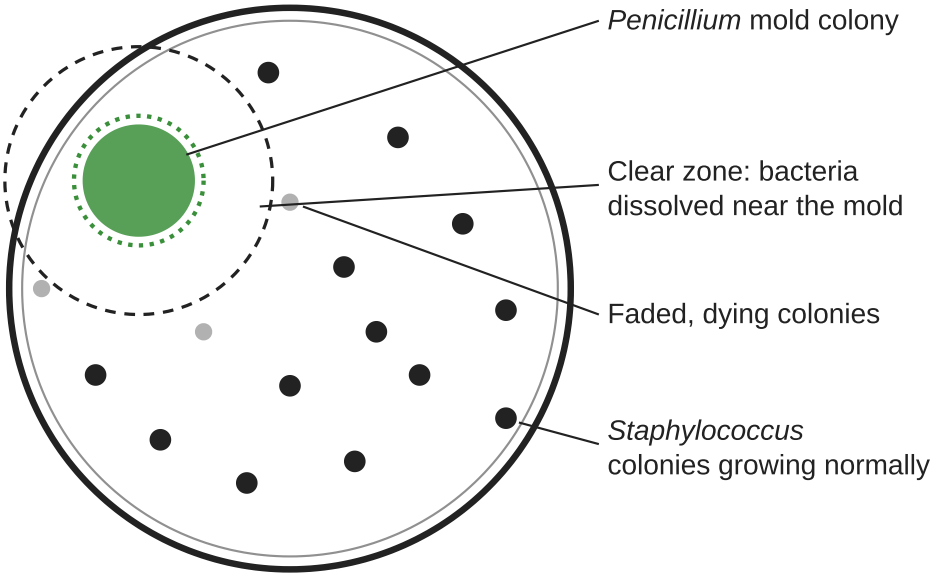
The story. Alexander Fleming was a Scottish farm boy who became a doctor and a researcher at St Mary's Hospital in Paddington, London. He was famous among his co-workers for a cluttered laboratory bench. In the summer of 1928 he went on holiday, leaving stacks of glass culture dishes of *Staphylococcus*, a bacterium that causes boils and dangerous wound infections. When he returned on September 3, 1928, he began sorting through the dishes. One was spoiled by a spot of fuzzy mold, probably blown in from a laboratory on the floor below, where another scientist was studying molds. Many workers would have thrown it away. Fleming looked closer. All around the mold, the bacterial **colonies** had faded and dissolved, leaving a clear ring. Farther away, the colonies grew normally.

How they figured it out. Fleming grew the mold, which was a kind of ***Penicillium***, in broth. He found that the liquid, even when diluted many times, killed many disease-causing bacteria, including *Staphylococcus* and *Streptococcus*, yet it did not harm white blood cells. He named the active substance **penicillin** and published his results in 1929. But penicillin was hard to purify and fell apart easily, and Fleming could not make enough to treat patients. For ten years it was mostly a laboratory curiosity. Then, in 1939, a team at Oxford University led by Howard Florey and Ernst Chain took it up again, and the inventive Norman Heatley worked out how to extract it. On May 25, 1940, they infected eight mice with deadly bacteria and gave penicillin to four. By the next morning all four untreated mice were dead, and the treated mice were alive.

The big idea. Bacteria are surrounded by a tough cell wall that they must keep building as they grow and divide. Penicillin blocks the building of that wall, so growing bacteria weaken and burst. Human and animal cells have no cell wall, so penicillin can kill bacteria inside the body while leaving our own cells unharmed. That is what makes it an **antibiotic** instead of an antiseptic like the chlorine Semmelweis used, which is too harsh to swallow or inject. On a culture dish, the clear ring around a substance is called the **zone of inhibition**, and measuring it is still one way scientists compare antibiotics. Antibiotics do nothing against viruses, such as those that cause colds and flu, because viruses are not cells and have no walls to attack.

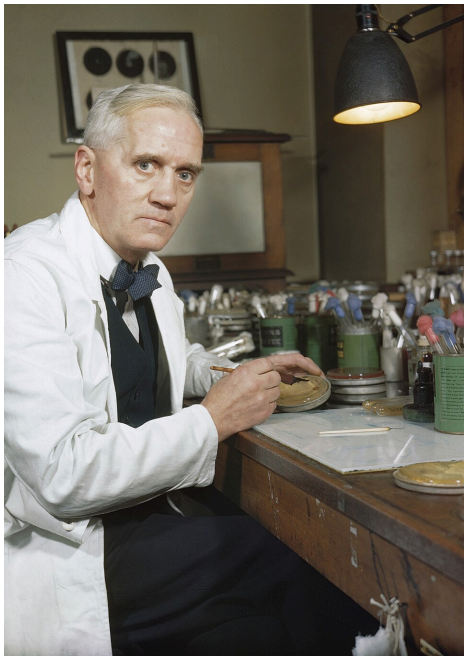
In the world today. The first patient in Oxford, a police constable with a terrible infection, began to recover in February 1941, but the tiny supply ran out and he died. The team even recovered penicillin from his urine to reuse it. American factories then learned to grow the mold in huge tanks, and by the D-Day landings in June 1944 there was enough to treat wounded Allied soldiers. Fleming, Florey and Chain shared the Nobel Prize in 1945. Since then, people have discovered many more antibiotics, often from soil microbes. But bacteria vary, and a few in a population may survive an antibiotic. If they multiply, they pass on that **antibiotic resistance**. In his Nobel lecture Fleming warned that taking too little penicillin could do exactly this. That is why antibiotics should be used only when a doctor says they are needed, and taken exactly as directed.

God’s handiwork. The same mold that spoils an orange carries a substance that saved millions of lives. Fleming noticed it because he looked carefully at a “ruined” experiment instead of throwing it away. The Psalmist wrote that God’s works are “sought out of all them that have pleasure therein” (Psalm 111:2). Some of the most useful things in creation are hidden in the most ordinary places, waiting for a patient and curious person to find them.



A sketch of what Fleming saw in 1928: Staphylococcus colonies dissolving near a Penicillium mold colony.

PICTURE STUDY



Alexander Fleming in His Laboratory, Official photographer, British Ministry of Information, about 1943 · Photograph · Imperial War Museum, London

During the Second World War the British government sent photographers to record the work being done to win the war, including the race to make penicillin for wounded soldiers. This photograph shows Alexander Fleming at work in his laboratory at St Mary's Hospital, London, about fifteen years after his discovery. The photographs of that project are kept at the Imperial War Museum.

A photograph is a different kind of picture from a painting. The photographer still chose where to stand, what to include and how to light the scene, but nothing is invented. Look at it as a record of a real working place.

Look closely

- Find all the pieces of glassware you can. What do you think each was used for?
- Look at Fleming's hands. What is he doing?
- Look at the bench around him. Is it tidy or crowded? How might that have helped or hurt his famous discovery?
- Compare this laboratory with Pasteur's room in Edelfelt's painting from Lesson 4.2. What has changed in sixty years, and what is the same?

Talk about it

1. Why would a government in wartime want photographs of a scientist and his laboratory?
2. Fleming noticed something many people would have thrown away. What habits help a scientist notice the unexpected?
3. Fleming always said that others deserved credit for turning penicillin into a medicine. Who were they, and what did they do?

> **Safety first.** Use only baker's yeast for this experiment; never swab skin, mouths, bathrooms or other places, since those can grow harmful bacteria. An adult heats the gelatin or agar mixture. Keep test substances away from your eyes, and do not taste them. Keep the dishes covered with plastic wrap. When you finish, an adult throws the sealed dishes away or soaks them in hot soapy water. Wash your hands.

EXPERIMENT: THE ZONE OF INHIBITION TEST

Fleming's clue was a clear ring. You will make clear rings of your own, using harmless baker's yeast as your test microbe and paper disks soaked in kitchen substances. (Penicillin itself does not harm yeast, because yeast cells build their walls from different materials than bacteria do, but the method is the same one microbiologists use.)

Question: Which household substances stop yeast from growing, and which work best? **Prediction:** Rank the substances before you start. **What we change:** the substance on the disk. **What we keep the same:** the growth dish, the amount of yeast, the disk size, soaking time, temperature and number of days. **What we measure:** the diameter of the clear zone in millimeters, in three trials.

You will need

- 250 mL water, 1 tablespoon of sugar, and either 1 teaspoon of agar powder or 1 packet of unflavored gelatin
- 3 clean shallow dishes with lids, or small plates covered with plastic wrap
- Active dry baker’s yeast and a cup of lukewarm water
- Clean cotton swabs, tweezers and a paper hole punch (most make disks about 6 mm across)
- White coffee filters or paper towels to punch into disks
- Test substances in small cups: plain water (control), salt water, white vinegar, crushed garlic mixed with a little water, mouthwash, dish soap mixed with water
- A ruler with millimeter marks, a marker and your lab notebook

Steps

1. With an adult, stir the agar or gelatin and sugar into the water and heat until it dissolves. Pour a layer about 5 mm deep into each dish, cover, and let it set in the refrigerator.
2. Stir a pinch of yeast and a pinch of sugar into half a cup of lukewarm water and wait 10 minutes until it is foamy.
3. Dip a clean swab in the yeast and spread it evenly over the whole surface of each dish, going back and forth in three directions.
4. On the bottom of each dish, draw six evenly spaced dots and label them W (water), S, V, G, M and D.
5. Punch 18 paper disks. With tweezers, soak each disk in its substance for 10 seconds, let the extra drip off, and lay it on the dish over its label. Use one disk of each substance on each of the three dishes.
6. Cover the dishes and keep them at room temperature for 2 to 4 days, until the yeast has made a creamy haze over the surface.
7. Measure the diameter of each clear zone across its widest point, in millimeters, including the disk. If there is no clear zone, record 6 mm, the size of the disk alone.
8. Find the average zone for each substance, make a bar graph and write a conclusion.

Tips

- Gelatin melts in warm rooms; keep the dishes below about 25 °C, or use agar, which stays firm.
- Space the disks at least 3 cm apart so the zones do not run together.
- If the water disk has a clear zone, something went wrong. Check whether the yeast was spread evenly.

Check your work

- I used a control disk and ran three trials of each substance.
- I measured every zone in millimeters, found averages and made a bar graph.

- I explained which substance worked best and how this method is like Fleming’s plate.

NATURE NOTEBOOK

1. Draw Fleming’s culture dish as it may have looked on September 3, 1928. Label the mold colony, the clear zone, the dissolving colonies and the normal colonies.
2. Make a relay-race timeline of penicillin: Fleming 1928, Florey and Chain’s team 1939, the mouse test 1940, the first patient 1941, mass production by 1944, the Nobel Prize 1945.
3. Draw a bacterium with its cell wall and a human cell with only a membrane. Under each, write what penicillin does to it and why.

> **From history.** When American scientists at a government laboratory in Peoria, Illinois, searched for a mold that made more penicillin, they asked people to bring in moldy food. In 1943 a moldy cantaloupe from a Peoria market carried a strain of *Penicillium* that produced far more penicillin than Fleming’s. Improved descendants of that cantaloupe mold were used to make much of the world’s penicillin.

WORKED EXAMPLES

Example 1. A paper disk 6 mm across is soaked in an antibiotic and placed on a plate of harmless bacteria. After two days, the clear zone (including the disk) measures 28 mm across. How wide is the clear ring of killed bacteria on each side of the disk?

(Use the map or drawing “A zone of inhibition is measured straight across its widest point.” shown earlier in this lesson.)

1. The zone diameter includes the disk, so subtract the disk: 28 mm − 6 mm = 22 mm.
2. That 22 mm is split between the two sides of the disk: 22 mm ÷ 2 = 11 mm.
3. In centimeters: 11 mm ÷ 10 = 1.1 cm.

Answer: The clear ring is 11 mm (1.1 cm) wide on each side of the disk.

Example 2. A lab tests three substances on 6 mm disks, three trials each. Zone diameters: Substance X 18, 20, 19 mm; Substance Y 12, 10, 11 mm; distilled water 6, 6, 6 mm. Find each average and rank them.

1. X: (18 + 20 + 19) ÷ 3 = 57 ÷ 3 = 19 mm.
2. Y: (12 + 10 + 11) ÷ 3 = 33 ÷ 3 = 11 mm.
3. Water: (6 + 6 + 6) ÷ 3 = 6 mm, the size of the disk alone, so there was no clear zone at all.
4. Rank from strongest to weakest: X, Y, water.

Answer: X averages 19 mm, Y 11 mm and water 6 mm (no zone). X stopped the bacteria best; water, the control, had no effect.

Example 3. Penicillin leaves the blood quickly; in a healthy adult about half of it is removed by the kidneys in roughly 30 minutes. Suppose a dose puts 4,000 units of penicillin in the blood. How much is left after 2 hours?

1. Number of halvings in 2 hours: 120 minutes ÷ 30 minutes = 4 halvings.
2. Start: 4,000 units.
3. After 30 min: 4,000 ÷ 2 = 2,000. After 60 min: 1,000. After 90 min: 500. After 120 min: 250.
4. Check: 4,000 ÷ 2^4 = 4,000 ÷ 16 = 250.

Answer: About 250 units, one-sixteenth of the dose. This is why early penicillin had to be given every few hours, and why the Oxford team collected it from patients' urine to reuse.

PRACTICE

1. EASY What did Fleming notice on his culture dish on September 3, 1928?
2. EASY Why does penicillin kill bacteria but not human cells?
3. EASY Why will an antibiotic not cure a cold?
4. MEDIUM What is the difference between an antiseptic and an antibiotic? Give one example of each.
5. MEDIUM A clear zone measures 34 mm across, and the disk is 6 mm. How wide is the clear ring on each side of the disk?
6. MEDIUM Data reading. In the Oxford experiment of May 25, 1940, eight mice were infected with deadly *Streptococcus* bacteria. Four received penicillin and four did not. The next morning, all four untreated mice were dead and the treated mice were alive. (a) Which group was the control? (b) What percent of untreated mice died? (c) Why did the experiment use mice that were all infected the same way?
7. HARD Data reading. A lab tests penicillin disks on four strains of bacteria (disk = 6 mm). Zone diameters: Strain A 30 mm, Strain B 24 mm, Strain C 6 mm, Strain D 18 mm. (a)

Which strain is most sensitive to penicillin? (b) Which strain appears resistant? (c) A patient is infected with Strain C. Would penicillin be a wise choice? Explain.

8. HARD A medicine's amount in the blood halves every 45 minutes. A dose gives 1,600 units. How much remains after 3 hours?
9. CHALLENGE Experiment design. A student wonders whether garlic juice kills bacteria as well as a penicillin disk does. Describe how a microbiology lab could test this fairly. Name the variable changed, three variables kept the same, the control and what is measured.
10. CHALLENGE Explain in three or four sentences how antibiotic resistance can develop when a few bacteria in a population can survive an antibiotic.

COMMON MISTAKES

- Thinking antibiotics kill viruses. They work only on bacteria; colds and flu are caused by viruses.
- Thinking Fleming alone made penicillin into a medicine. He discovered it, but Florey, Chain, Heatley and many others purified it, tested it and learned to make it in large amounts.
- Thinking a person's body becomes resistant to antibiotics. It is the bacteria that become resistant, not the person.

Make it a game · Antibiotic or Not?. A parent reads an illness or a situation: strep throat, a common cold, the flu, an infected cut, a bacterial ear infection, chicken pox, a scraped knee to clean. You answer "antibiotic," "not an antibiotic" or "antiseptic," and say whether the cause is a bacterium, a virus or just dirt. One point for each correct answer and one bonus point for explaining why. Play to ten points.

Scripture. "The works of the LORD are great, sought out of all them that have pleasure therein." (Psalm 111:2, KJV)

Fleming had pleasure in looking, and so he found what others might have thrown away. God's works are worth seeking out, even on a spoiled dish of bacteria.

Practice answers

1. A mold colony had grown on a dish of *Staphylococcus*, and the bacterial colonies around the mold had faded and dissolved, leaving a clear zone, while colonies farther away grew normally.
2. Penicillin stops bacteria from building their cell walls, so growing bacteria burst. Human cells have no cell wall, so penicillin does not harm them.
3. Colds are caused by viruses. Viruses are not cells and have no cell walls or cell machinery for antibiotics to attack.
4. An antiseptic kills germs on skin or surfaces but is too harsh to put inside the body (chlorine solution, alcohol, iodine). An antibiotic kills bacteria inside the body without harming the body's cells (penicillin).
5. $(34 - 6) \div 2 = 28 \div 2 = 14$ mm on each side.

6. (a) The four untreated mice. (b) 4 of 4 = 100%. (c) So that the only difference between the groups was the penicillin; then the difference in survival could be credited to the penicillin.
7. (a) Strain A, with the widest zone. (b) Strain C; a 6 mm zone means nothing was killed beyond the disk itself. (c) No; Strain C was not stopped by penicillin, so a doctor would need a different antibiotic that the test shows it is sensitive to.
8. 3 hours = 180 minutes; 180 ÷ 45 = 4 halvings. 1,600 → 800 → 400 → 200 → 100 units.
9. Sample answer: Spread the same amount of the same harmless bacterium evenly on identical agar plates. Change only the substance on the disks: garlic juice, penicillin, and plain sterile water as the control. Keep the same disk size, soaking time, plate type, temperature and incubation time. Run at least three plates. Measure the zone diameter in millimeters, average the trials and compare.
10. Bacteria in a population vary, and a few may have a trait that lets them survive the antibiotic. When the antibiotic kills the others, the survivors have food and space to themselves. They multiply quickly, and their offspring inherit the same survival trait. Soon most of the population is resistant, so the antibiotic no longer works well. This is variation and selection within one kind of living thing.

4.4 How the Body Fights

OBJECTIVES

- Describe the body's three lines of defense: barriers, general defenders such as phagocytes and fever, and specific defenders such as antibodies and memory cells.
- Tell how Élie Metchnikoff discovered phagocytes in 1882 and how Edward Jenner's cowpox experiment of 1796 led to vaccines.
- Read a graph of antibody levels after a first and second exposure, and calculate percents and ratios from blood-cell counts.

Warm-up. Look closely at the skin on the back of your hand with a magnifying glass. Notice how it overlaps like shingles on a roof. Then think of every opening in the body where germs could get in (eyes, nose, mouth, cuts). For each one, write one thing the body already has there that might guard the door.

Video. Coming soon. How the immune system works for middle school: skin and mucus barriers, white blood cells engulfing bacteria (real microscope footage of a neutrophil chasing a bacterium), antibodies and memory cells, and the history of Metchnikoff and Jenner; search Amoeba Sisters, Homeschool Pop (Alternate), SciShow Kids, Smithsonian and microscopy channels.

KEY TERMS

immune system
The cells, tissues and organs that work together to defend the body against germs.

pathogen
A germ that causes disease, such as certain bacteria, viruses, fungi or protists.

phagocyte
A white blood cell that surrounds and swallows germs and debris. The name means "eating cell."

inflammation
The body's general response to injury or infection: redness, warmth, swelling and pain as blood and defender cells rush to the spot.

antibody
A Y-shaped protein made by white blood cells called B cells. Each kind fits one particular germ and marks it for destruction.

vaccine
A weakened, killed or harmless form or part of a germ that trains the immune system to recognize it, so the body can respond quickly if the real germ comes.

CORE IDEAS

The story. In 1882 a Russian zoologist named Élie Metchnikoff was living in Messina, on the island of Sicily, studying sea creatures. One day, while his family had gone to the circus, he sat alone with his microscope watching the larvae of starfish. They were tiny and as clear as glass, so he could see the cells moving about inside them. Some of those cells wandered freely through the body. A sudden thought struck him: what if these wandering cells guard the body against invaders? He went into the garden, took a few thorns from a rose bush, and pricked them into the starfish larvae. The next morning he looked again. The thorns were surrounded by a crowd of the wandering cells, which had gathered to attack the intruder.

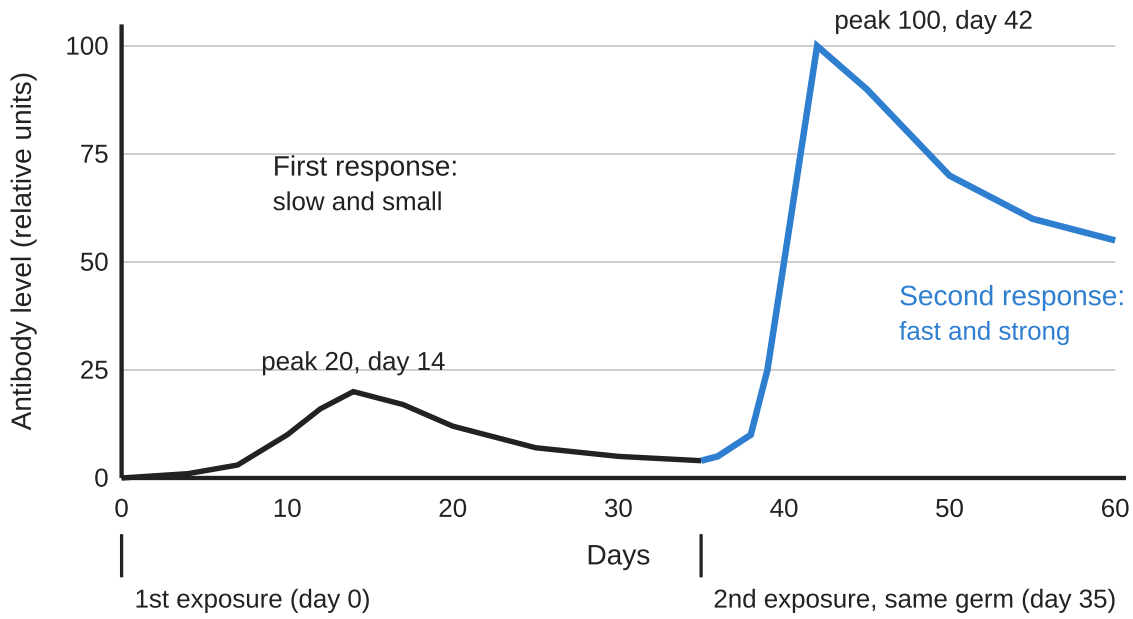
How they figured it out. Metchnikoff went on to watch similar cells in water fleas and in animal blood swallow bacteria whole. He called them **phagocytes**, from Greek words meaning “eating cells.” Other scientists, led by Germans such as Paul Ehrlich, studied a different defense: substances in the blood, later called **antibodies**, that attach to particular germs. For years the two sides argued about which was the true defense. In 1908 Metchnikoff and Ehrlich shared the Nobel Prize, because both were right. The body uses cells and chemicals together.

The big idea. Your **immune system** defends you against **pathogens** in three lines. *The first line is the barriers*: unbroken skin; sticky mucus and tiny beating hairs called cilia in your nose and airways; tears and saliva, which contain lysozyme, a germ-dissolving substance that Fleming discovered in 1921, seven years before penicillin; and strong stomach acid. *The second line attacks any invader that gets through.* When you cut a finger, **inflammation** brings extra blood, so the spot turns red, warm and swollen, and phagocytes squeeze out of the blood vessels to swallow the germs. A fever, a body temperature of about 38 °C or higher instead of the usual 37 °C, can slow some germs and speed up your defenders. *The third line is specific.* White blood cells called lymphocytes learn the exact shape of one germ. B cells make antibodies that lock onto it; T cells destroy infected cells; and memory cells remain for years, so the second time the same germ appears, the body responds faster and stronger, often before you feel sick at all.

Vaccines. That memory is the secret of vaccines. In May 1796 the English country doctor Edward Jenner tested a story he had heard from dairy workers: milkmaids who caught cowpox, a mild disease from cows, did not catch deadly smallpox. He took material from a cowpox sore on the hand of a milkmaid named Sarah Nelmes and put it into small scratches on the arm of eight-year-old James Phipps. Weeks later he exposed the boy to smallpox, and James did not fall ill. The Latin word for cow is *vacca*, so the new method was called vaccination. Pasteur later used the word **vaccine** for all such preparations in Jenner’s honor. Smallpox, one of history’s worst killers, was declared wiped out worldwide in 1980.

God’s handiwork. Every day your body meets millions of microbes, and almost every one is stopped before you ever notice. Skin, tears, mucus, wandering eating cells and molecules that fit their targets like keys in locks all work together without your thinking about them. Paul wrote that the body’s members care for one another: “whether one member suffer, all

the members suffer with it” (1 Corinthians 12:26). Medicine helps, but much of the healing God designed is already built in.



Antibody levels after a first and a second exposure to the same germ (a typical pattern, in relative units). Memory cells make the second response faster and stronger.

PICTURE STUDY



The Doctor, Sir Luke Fildes, 1891 · Oil on canvas · Tate Britain, London

Luke Fildes was an English painter who knew sorrow firsthand: his own young son Philip died on Christmas morning in 1877. He never forgot how the family doctor watched over the

boy. Years later Henry Tate, the sugar merchant who founded the Tate Gallery, asked Fildes for a painting, and Fildes chose this subject. He built a cottage room in his studio to paint it.

In 1891 there were no antibiotics and few medicines that could cure an infection. A doctor could keep a patient warm and comfortable, give water, and watch. After that, the child's own immune system had to win the fight. The light at the window suggests that the long night is ending.

Look closely

- Find the doctor's face. What is he doing with his eyes and hands?
- Look at the child's bed. What has the family used to make it?
- Find the parents in the shadows. How does Fildes show their worry and hope?
- Find the two sources of light. What do the lamp and the window each tell you about the time of night?

Talk about it

1. Before antibiotics and vaccines, what parts of the body's defenses was this child depending on?
2. Why do you think this painting became one of the best-loved pictures of a doctor ever made?
3. What can a person do to help someone who is sick, even if he or she cannot cure them?

> **Safety first.** An adult should handle the toothpick pricks and the rubbing alcohol. Rubbing alcohol is flammable and poisonous to drink; keep it away from flames and wash it off skin. Do not eat any of the test apples. Throw the rotten apple and test apples away in a sealed bag at the end, and wash your hands with soap after every check.

EXPERIMENT: THE SKIN BARRIER APPLE TEST

An apple's skin protects it the way your skin protects you. In this experiment you will break the barrier on purpose, expose apples to the microbes on a rotting apple, and see whether an antiseptic helps, just as Lister's and Semmelweis's methods did.

Question: How well does an unbroken skin keep out rot, and can an antiseptic help a wound? **Prediction:** Write which apple you think will rot fastest. **What we change:** whether the skin is broken and whether it is cleaned. **What we keep the same:** the kind and ripeness of the apples, the rotting apple used, the number of pricks, the storage place and temperature. **What we measure:** the width of the brown, soft area in millimeters each day for 7 days.

You will need

- 4 fresh apples of the same kind and size, washed and dried
- 1 rotting apple (or a piece of one) with soft brown spots
- Clean toothpicks and cotton swabs

- Rubbing alcohol (70 percent) and a cotton ball
- 4 zipper bags, a marker, a ruler with millimeter marks and your lab notebook

Steps

1. Label the apples and bags: A Unbroken skin, not touched by rot (control); B Unbroken skin, rubbed with rot; C Pricked, rubbed with rot; D Pricked, rubbed with rot, then cleaned with alcohol.
2. Put apple A straight into its bag.
3. Rub a swab on the rotting apple, then rub it on one spot on the skin of apple B, about 2 cm across. Bag it.
4. On apple C, have an adult prick 5 small holes with a clean toothpick inside a 2 cm circle. Rub a swab from the rotting apple over the holes. Bag it.
5. Do the same to apple D, then wait 5 minutes and dab the spot well with a cotton ball soaked in rubbing alcohol. Bag it.
6. Keep all four bags side by side at room temperature.
7. Every day for 7 days, measure the widest brown or soft area on each apple in millimeters, without opening bags that smell rotten. Record the numbers in a data table.
8. Make a line graph of rot width against days for all four apples, and write a conclusion comparing them to the body’s first line of defense.

Tips

- Use the same pressure and the same number of pricks on apples C and D, or you have changed two variables.
- If apple A develops rot, note it: the apple may have had a bruise or tiny break you did not see.
- Alcohol works on the surface. If rot gets deep into the holes before you clean them, it may still spread. Try a second apple cleaned after 1 minute instead of 5.

Check your work

- I changed only one thing between each pair of apples and kept a control.
- I measured rot in millimeters every day and graphed all four apples.
- I explained how my results match the body’s first line of defense and the use of antiseptics on wounds.

NATURE NOTEBOOK

1. Draw a castle with three walls and label it “The Body’s Defenses.” On the outer wall write the barriers, on the inner wall the general defenders, and in the keep the specific defenders. Add a germ trying to get in.
2. Draw Metchnikoff’s experiment: a clear starfish larva with a rose thorn inside it, before and after a night. Label the wandering phagocytes.

3. Copy the antibody graph from this lesson and label the first exposure, the second exposure, the two peaks and the number of days to each peak.

From history. Edward Jenner was a country doctor in Berkeley, Gloucestershire, and also a careful naturalist. Before his work on vaccination, he studied the cuckoo and showed that it is the newly hatched cuckoo chick itself that pushes the eggs and chicks of its foster parents out of the nest. His paper on the cuckoo helped him become a Fellow of the Royal Society in 1789, seven years before his famous cowpox experiment.

WORKED EXAMPLES

Example 1. A healthy person's blood has about 7,500 white blood cells and about 5,000,000 red blood cells in each microliter (µL). About how many red blood cells are there for every white blood cell? What percent of these blood cells are white?

1. Ratio: 5,000,000 ÷ 7,500 = 666.7, about 667.
2. So there are about 667 red blood cells for every white blood cell.
3. Total cells: 5,000,000 + 7,500 = 5,007,500.
4. Percent white: 7,500 ÷ 5,007,500 × 100 = 0.15%.

Answer: About 667 red cells for each white cell; white cells are only about 0.15% of these blood cells, yet they defend the whole body.

Example 2. A healthy person has 8,000 white blood cells per µL, and 60% of them are neutrophils (the most common phagocyte). During a bacterial infection the count rises to 15,000 per µL, and 80% are neutrophils. How many neutrophils per µL are there before and during the infection, and how many times as many?

1. Before: 8,000 × 0.60 = 4,800 neutrophils per µL.
2. During: 15,000 × 0.80 = 12,000 neutrophils per µL.
3. Compare: 12,000 ÷ 4,800 = 2.5.

Answer: 4,800 before and 12,000 during the infection, 2.5 times as many. A high neutrophil count is one clue a doctor uses to recognize a bacterial infection.

Example 3. Use the antibody graph. How many days after each exposure does the antibody level reach its peak, and how many times higher is the second peak than the first?

(Use the map or drawing “Read the peaks and the days carefully from the graph.” shown earlier in this lesson.)

1. First exposure at day 0; first peak of 20 units at day 14. Time to peak: 14 − 0 = 14 days.
2. Second exposure at day 35; second peak of 100 units at day 42. Time to peak: 42 − 35 = 7 days.
3. Compare the peaks: 100 ÷ 20 = 5.
4. Compare the times: 14 ÷ 7 = 2.

Answer: The first response peaks after 14 days, the second after only 7 days. The second peak is 5 times higher and comes twice as fast, because memory cells recognized the germ.

PRACTICE

1. EASY Name four parts of the body’s first line of defense.
2. EASY What did Metchnikoff see the morning after he pricked rose thorns into starfish larvae, and what did he name the cells?
3. EASY List the four signs of inflammation and explain why the area gets red and warm.
4. MEDIUM What did Edward Jenner do in 1796, and why does a vaccine protect a person?
5. MEDIUM A blood sample has 6,000 white blood cells per µL, and 30% are lymphocytes. How many lymphocytes per µL is that?
6. MEDIUM Data reading. Use the antibody graph. (a) What is the antibody level on day 10? (b) About when does the antibody level start to rise quickly after the second exposure? (c) Which is higher on day 60, or on day 14?
7. HARD Data reading. A student recorded body temperature during an illness: Day 1 37.0 °C, Day 2 38.6 °C, Day 3 39.1 °C, Day 4 38.2 °C, Day 5 37.1 °C. (a) On which days did the student have a fever (38 °C or above)? (b) What was the largest rise in one day? (c) What does the pattern suggest about how the immune system was doing by Day 5?
8. HARD Explain why a person who had chicken pox as a child usually does not catch it again, using the words memory cells and antibodies.
9. CHALLENGE Experiment design. In the apple test, a student wants to know whether cleaning a wound sooner works better than cleaning it later. Design the experiment: what changes, what stays the same, the control and the measurement.
10. CHALLENGE Metchnikoff thought cells defend the body; Ehrlich’s side thought substances in the blood do. Explain why they both received the Nobel Prize in 1908, and give an example of how the two defenses work together.

COMMON MISTAKES

- Thinking all microbes are harmful. Most microbes are harmless or helpful; the immune system must stop pathogens without attacking the helpful ones or the body's own cells.
- Thinking a fever is always the enemy. A moderate fever is part of the body's defense, though a very high or long fever needs a doctor's care.
- Thinking antibodies kill every kind of germ. Each kind of antibody fits only one particular germ, like a key fits one lock, which is why the body needs time to make the right one the first time.

Make it a game · Defense Line-Up. Make three cards labeled First Line, Second Line and Third Line and lay them on the table. A parent calls out a defender: skin, tears, phagocyte, antibody, stomach acid, fever, memory cell, mucus, inflammation, T cell, cilia, lysozyme. Slap the right card within five seconds and say in one sentence what that defender does. One point for each correct card and a bonus point for a correct sentence. Play to fifteen points.

Scripture. "And whether one member suffer, all the members suffer with it; or one member be honoured, all the members rejoice with it." (1 Corinthians 12:26, KJV)

When one finger is cut, the whole body responds: blood flow, defender cells and fever all come to help. Paul used the body to teach the church to care for each member, and the immune system is a living picture of that care.

Practice answers

1. Any four: unbroken skin, mucus, cilia in the airways, tears, saliva (with lysozyme), stomach acid.
2. The thorns were surrounded by a crowd of wandering cells that had gathered to attack them. He named such cells phagocytes, "eating cells."
3. Redness, warmth, swelling and pain. Blood vessels near the injury widen and bring extra blood (and defender cells) to the spot, which makes it red and warm.
4. He put material from a cowpox sore on milkmaid Sarah Nelmes's hand into scratches on James Phipps's arm; later the boy did not get smallpox. A vaccine trains the immune system with a harmless form or part of a germ, so memory cells can respond quickly and strongly if the real germ comes.
5. 6,000 × 0.30 = 1,800 lymphocytes per µL.
6. (a) About 10 units. (b) Within about 3 to 5 days after the second exposure (between about day 38 and day 40). (c) Day 60 (about 55 units) is higher than the first peak on day 14 (20 units).
7. (a) Days 2, 3 and 4. (b) From Day 1 to Day 2: 38.6 − 37.0 = 1.6 °C. (c) The temperature returned to normal, suggesting the immune system was winning and the infection was ending.
8. During the first illness, B cells learned the shape of the chicken pox virus and made antibodies against it, and memory cells were left behind. If the virus enters again, memory cells recognize it at once and quickly make large amounts of antibodies, stopping the virus before it can make the person sick.

9. Sample answer: Prick and expose several apples the same way, then clean with the same amount of rubbing alcohol after 1 minute, 5 minutes, 30 minutes and 2 hours (at least two apples each). Keep the same apple type and ripeness, number and depth of pricks, rot source, storage place and temperature. Control: an apple pricked and exposed but never cleaned (and one never pricked). Measure the width of the rotten area in millimeters each day for 7 days and compare averages.
10. Both were right: the body uses defender cells such as phagocytes and also chemicals such as antibodies. They work together; for example, antibodies made by B cells attach to bacteria and mark them, which helps phagocytes find and swallow them.

Unit 4 review

Answer each question. Show your work for calculations, include units, and use complete sentences for explanations. The test is worth 60 points.

1. (5 pts) Multiple choice. Semmelweis concluded that the doctors' clinic had far more deaths from childbed fever than the midwives' clinic because (A) the air in the doctors' ward was worse; (B) doctors and students came from autopsies and examined mothers without cleaning their hands; (C) midwives were better trained than doctors; (D) the mothers in the doctors' clinic were sicker when they arrived.
2. (5 pts) A clinic had 2,000 births and 220 deaths in the year before a handwashing rule, and 2,000 births and 26 deaths in the year after. Find the death rate as a percent each year and the percent decrease.
3. (6 pts) Give two reasons many doctors rejected Semmelweis's idea, and explain what later discoveries showed he had been right.
4. (5 pts) Multiple choice. Why did Pasteur use a flask with a long, bent "swan neck"? (A) To keep all air away from the broth. (B) To let air reach the broth while trapping dust and microbes in the bend. (C) To make the broth boil faster. (D) To let flies lay eggs in the broth.
5. (6 pts) Diagram item. Draw two flasks of boiled broth. Flask 1 has an unbroken swan neck; Flask 2 has its neck broken off short. Label the broth, the neck, the trapped dust and the path of the air in Flask 1, and show dust falling into Flask 2. Under each flask write whether the broth stays clear or turns cloudy, and why.
6. (5 pts) A bacterium divides every 20 minutes. Starting with one cell in sterile broth, how many cells will there be after 3 hours? Show your work.
7. (6 pts) What did Alexander Fleming observe in September 1928, and what did Howard Florey, Ernst Chain and Norman Heatley add between 1939 and 1941?
8. (5 pts) Penicillin disks 6 mm across were placed on three strains of bacteria. Zone diameters: Strain A 26 mm, Strain B 6 mm, Strain C 14 mm. (a) Which strain is most sensitive? (b) Which appears resistant? (c) How wide is Strain A's clear ring on each side of the disk?
9. (6 pts) Multiple choice, then explain. An antibiotic would help with which illness? (A) a common cold; (B) the flu; (C) strep throat; (D) chicken pox. Explain your choice and explain how penicillin kills bacteria without harming human cells.
10. (5 pts) Sort these defenders into the first, second or third line of defense: skin, phagocytes, antibodies, stomach acid, fever, memory cells, mucus, inflammation.
11. (6 pts) A graph shows antibody levels. After a first exposure on day 0, the level peaks at 20 units on day 14. After a second exposure to the same germ on day 35, it peaks at 100 units on day 42. (a) How many days after each exposure is the peak? (b) How many times higher is the second peak? (c) Explain the difference, and explain how Jenner's 1796 experiment used this same principle.

UNIT PROJECT: MY GERM HUNTERS CASEBOOK

Make a casebook, like a detective's file, with one chapter for each germ hunter in this unit: Semmelweis, Pasteur, Fleming (with Florey, Chain and Heatley), and Metchnikoff and Jenner. Each chapter should include the date and place, the question the scientist asked, a labeled drawing of the key experiment, the evidence in numbers, and the conclusion. Add your own data from the unit's home experiments: the bread test from Lesson 4.1, the broth jars from Lesson 4.2, the zone of inhibition dishes from Lesson 4.3 and the apple test from Lesson 4.4, each with a data table, a graph and a short conclusion. Finish with a one-page essay, "How We Learned to Fight Germs," explaining how each discovery built on the one before.

- **Accuracy**: Names, dates, places and experiments are correct, and the science of germs, antibiotics and immunity is explained correctly.
- **Data and calculations**: Every home experiment has a complete data table with units, a correctly made graph, and correct averages, percents or rates with work shown.
- **Scientific thinking**: Each experiment names the variable changed, the variables kept the same and the control; conclusions are supported by the data and weaknesses are honestly stated.
- **Care and presentation**: Drawings are neat, labeled and titled; the casebook is organized and easy to follow; the essay is clear and in complete sentences.

Answer key

1. (B).
2. Before: 220 ÷ 2,000 × 100 = 11%. After: 26 ÷ 2,000 × 100 = 1.3%. Percent decrease: (11 − 1.3) ÷ 11 × 100 = 9.7 ÷ 11 × 100 ≈ 88%.
3. Reasons (any two): it seemed to blame doctors for their patients' deaths; no one yet knew that living germs cause disease, so his "cadaverous particles" sounded vague; he was slow to publish his data; his angry letters turned people against him. Later, Pasteur showed that microbes come from other microbes and can cause disease, and Lister showed that antiseptics prevent infection, so germ theory explained why handwashing worked.
4. (B).
5. Flask 1: round flask with an S-shaped neck, broth in the bottom, dust dots in the low bend, an arrow showing air passing through the neck to the broth; the broth stays clear because microbes are trapped in the bend and never reach it. Flask 2: short, broken neck with dust falling straight in; the broth turns cloudy within days because microbes on the dust land in the broth and multiply. (1 point for each correctly labeled part in Flask 1, up to 4 points; 2 points for Flask 2 and its correct prediction.)
6. 3 hours = 180 minutes; 180 ÷ 20 = 9 divisions; 2^9 = 512 cells.
7. Fleming saw that on a culture dish of *Staphylococcus* spoiled by a *Penicillium* mold, the bacterial colonies around the mold had dissolved, leaving a clear zone. He named the substance penicillin. Florey, Chain and Heatley at Oxford learned to extract and purify it, proved it cured infected mice in 1940, and first tested it on human patients in 1941, turning it into a usable medicine.
8. (a) Strain A. (b) Strain B, since a 6 mm zone means no bacteria were killed beyond the disk. (c) (26 − 6) ÷ 2 = 10 mm.

9. (C) strep throat, because it is caused by bacteria (*Streptococcus*); the others are caused by viruses, which antibiotics cannot affect. Penicillin stops bacteria from building their cell walls, so they burst as they grow; human cells have no cell wall, so they are not harmed.
10. First line (barriers): skin, stomach acid, mucus. Second line (general responses): phagocytes, fever, inflammation. Third line (specific): antibodies, memory cells.
11. (a) 14 days after the first exposure; 7 days after the second. (b) 100 ÷ 20 = 5 times higher. (c) After the first exposure, memory cells remained that recognize the germ, so the second response is faster and stronger. Jenner gave James Phipps cowpox, a mild disease from cows; it trained the boy’s immune system so that when he was later exposed to the similar smallpox germ, his body responded quickly and he did not become ill.

UNIT 5

Photosynthesis

A great oak grows from one small acorn, and a willow from a cut stick. Where does all that wood come from? In this unit you will follow scientists who answered that question one careful step at a time. Jan Baptist van Helmont weighed a willow and its soil for five years and proved that the tree was not made of dirt. Jan Ingenhousz watched silver bubbles rise from leaves in sunlight and found that plants make good air only in light. Antoine Lavoisier put a guinea pig in a jacket of ice and discovered that living things burn their food slowly with oxygen. Theodor Engelmann used a prism and swarms of bacteria to show which colors of light a green plant uses.

Along the way you will learn the two great reactions of life. In **photosynthesis**, chloroplasts use light energy to turn carbon dioxide and water into glucose and oxygen. In **cellular respiration**, mitochondria use oxygen to release the energy stored in glucose, giving back carbon dioxide and water. You will float spinach disks under a lamp, measure heat from sprouting seeds, and separate the hidden colors in a green leaf, recording your data, graphing it and calculating rates like a real lab scientist.

“O LORD, how manifold are thy works! in wisdom hast thou made them all: the earth is full of thy riches” (Psalm 104:24). Every slice of bread and every breath of fresh air depends on green leaves catching sunlight. By the end of the unit you will be able to trace the food on your plate back to the light of the sun.

BEFORE YOU BEGIN

- Can name the main parts of plant and animal cells, including chloroplasts and mitochondria (Unit 2)
- Can use a kitchen scale and a ruler and record measurements in grams and centimeters
- Can divide and multiply decimals and find a percent
- Can read a simple data table and line graph

MASTERY CHECKLIST

- I can tell how van Helmont’s willow experiment showed that a plant does not build its body out of soil, and explain where most of a plant’s dry mass really comes from.
- I can write the word and chemical equations for photosynthesis and cellular respiration and show that their atoms balance.
- I can run the floating leaf disk experiment with a control, name its variables, graph my data and find a rate.
- I can explain the difference between breathing and cellular respiration, and explain that plants do both photosynthesis and respiration.
- I can explain why leaves look green and which colors of light drive photosynthesis best.

- I can use the ten percent rule to show how energy from the sun flows through a food chain.

OPTIONAL BOOKS AND RESOURCES

- ***Concepts of Biology*** **(OpenStax, free online textbook)** (https://openstax.org/details/books/concepts-biology): Free. The chapters on photosynthesis and on how cells obtain energy give clear diagrams and a step up toward high-school Biology. Best read with a parent, a section at a time, as a stretch for strong readers.
- **NASA Earth Observatory** (https://earthobservatory.nasa.gov/): Free. Search the site for “phytoplankton” or “chlorophyll” to see satellite pictures of green blooms of tiny algae in the ocean, the sea’s producers (Lesson 5.4).
- ***Biology 2e*** **(OpenStax, free online textbook)** (https://openstax.org/details/books/biology-2e): Free. A college-level text for parents who want background; its photosynthesis chapter explains the light reactions and the Calvin cycle in more depth. For the leaf disk lab (Lesson 5.2) you will also need a plastic medicine syringe with no needle, sold cheaply at pharmacies.

5.1 Van Helmont's Willow (published 1648) ★

OBJECTIVES

- Tell the story of Jan Baptist van Helmont's five-year willow experiment and explain what his before-and-after weighings showed.
- Calculate mass gained and lost from data, and use ratios to compare the willow's gain with the soil's loss.
- Explain that most of a plant's dry mass comes from carbon dioxide in the air, joined with water by photosynthesis, and not from the soil.

Warm-up. Hold an acorn, a bean or an apple seed in your hand. A seed like that can grow into a tree that weighs as much as a car. Write down your best guess: where did all that wood come from? Choose from soil, water, air, sunlight or something else, and write one sentence explaining your choice. Keep your guess; you will check it at the end of the lesson.

Video. Coming soon. Van Helmont's willow tree experiment and where a tree's mass comes from (carbon dioxide from the air); search Khan Academy, Crash Course Kids, Homeschool Pop (Alternate), Science Max, Moody Science classics, and science explainer channels that weigh plants or burn wood to show its carbon.

KEY TERMS

mass
The amount of matter in an object, measured in grams (g) or kilograms (kg). A balance or scale measures it.

dry mass
The mass of a plant or soil sample after all its water has been dried out. Scientists use dry mass to see how much real building material a plant has made.

photosynthesis
The process by which plants, algae and some bacteria use light energy to join carbon dioxide and water into sugar (glucose), giving off oxygen.

carbon dioxide
An invisible gas in the air made of one carbon atom and two oxygen atoms (CO_2). Animals breathe it out, and plants take it in through their leaves.

glucose
A simple sugar ($C_6H_{12}O_6$) made in photosynthesis. Plants use it for energy and build it into starch, cellulose and other materials.

chloroplast
The green organelle in plant cells where photosynthesis happens. Its green color comes from the pigment chlorophyll.

CORE IDEAS

The story. Jan Baptist van Helmont was born in Brussels around 1580, in what was then the Spanish Netherlands and is now Belgium. He trained as a physician but spent much of his life in his own laboratory at Vilvoorde, near Brussels, heating, weighing and testing. He wondered about an old question: where does a growing plant get its body? Most people assumed that plants eat the soil, the way a calf drinks milk. Van Helmont decided to find out with a balance. He dried 200 pounds of soil in a furnace (about 90.7 kg), put it into a large clay pot, moistened it with rainwater, and planted a willow stem that weighed 5 pounds (about 2.3 kg). He covered the pot with a metal plate punched full of small holes, so that rain and air could get in but dust could not, and for five years he added nothing but rainwater or distilled water. His account was published by his son in 1648, four years after his death, in a large book called *Ortus Medicinae* ("The Rise of Medicine"). We do not know the exact years of the experiment, only that it ended before he died in 1644.

How they figured it out. After five years van Helmont pulled up the willow and weighed it. It had grown to about 169 pounds and 3 ounces (about 76.7 kg), and that did not even count the leaves that had fallen each autumn. Then he dried the soil again and weighed it. It came to 200 pounds less only about 2 ounces, a loss of roughly 57 g. The tree had gained about 164 pounds (about 74.4 kg) while the soil had lost almost nothing. Van Helmont reasoned like a careful bookkeeper: the mass that went into the tree had to come from somewhere, and the only thing he had added was water. He concluded that the wood, bark and roots had come from water alone.

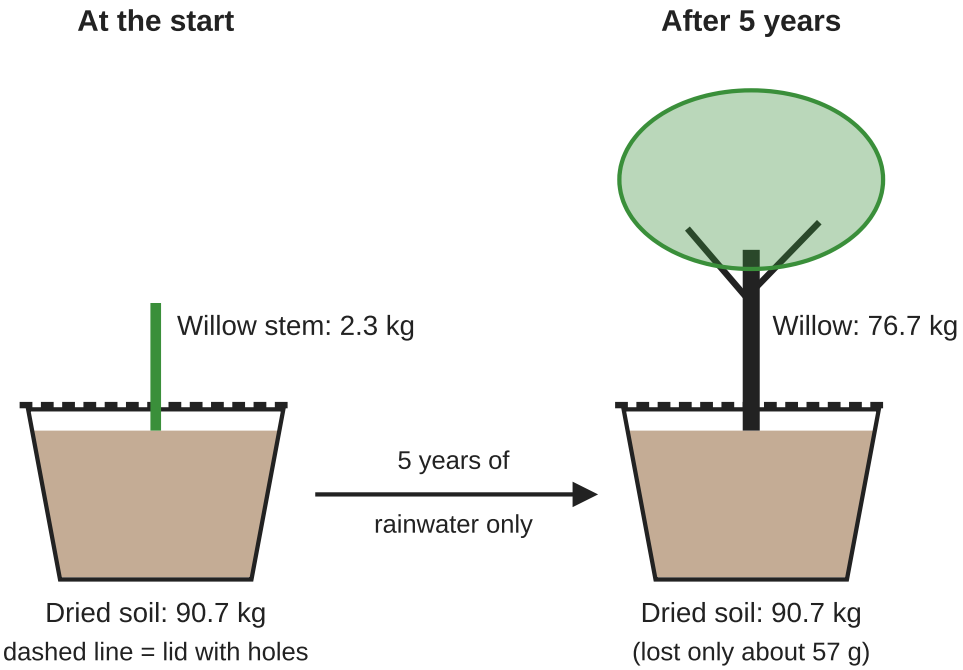
The big idea. Van Helmont was half right. He proved that a plant does *not* build itself out of soil. The 57 g the soil lost were mostly minerals, a few pinches of material a plant needs in small amounts. But water was not the whole answer. A living plant is full of water, yet if you dry the willow's wood, most of what is left came from a gas in the air: **carbon dioxide**. In green leaves, tiny organelles called **chloroplasts** use the energy of light to join carbon dioxide and water into the sugar **glucose**, and they give off oxygen. This is **photosynthesis**. Written as a chemical equation: $6\ CO_2 + 6\ H_2O + \text{light energy} \rightarrow C_6H_{12}O_6 + 6\ O_2$. The plant then links glucose into long chains to make starch for storage and cellulose for cell walls and wood. About half the dry mass of wood is carbon, and every bit of that carbon was pulled from the air.

A gas he could not weigh. Here is the surprise. Van Helmont himself invented the word *gas*. He burned 62 pounds of oak charcoal and found only about 1 pound of ash left; the rest, he wrote, had gone off as a "wild spirit," which he called *gas sylvestre*. That wild gas was carbon dioxide, the very stuff his willow had been drinking from the air through its leaves for five years. He had no way to trap and weigh a gas flowing in and out of a pot, so he never counted it. Good scientists can be careful and honest and still miss something they cannot yet measure. That is why science moves forward step by step, as later workers build on earlier ones.

Try it and see. You can repeat a small version of van Helmont's experiment with a bean plant, a pot of dried soil and a kitchen scale. Weigh the dry soil and the seed at the start,

water with measured amounts of water, and after four weeks weigh the plant and dry the soil again. Then dry the plant itself and weigh it, to see how much of its mass was only water. You will be doing what every good experimenter does: keeping a mass ledger with columns for what went in and what came out.

God's handiwork. On the third day of creation God said, "Let the earth bring forth grass, the herb yielding seed, and the fruit tree yielding fruit after his kind" (Genesis 1:11). A willow grows from a cut stick into a tree, and an oak from a single acorn, by catching sunlight and turning invisible air and plain water into wood, leaves and fruit. Every forest is built mostly out of the air around it. Van Helmont's patient weighing helps us see how wonderful that is.



Van Helmont's willow experiment, with his weights changed to kilograms. The tree gained about 74 kg while the soil lost only about 57 g.

PICTURE STUDY



The Large Piece of Turf, Albrecht Dürer, 1503 · Watercolor and gouache on paper · Albertina, Vienna

Albrecht Dürer of Nuremberg was one of the greatest artists of the German Renaissance, and he looked at nature as closely as any scientist. In 1503 he painted a simple clump of meadow plants, the kind you might step over without a glance, as if it were seen from the eye level of a beetle. Botanists can still name the plants: grasses, dandelions and broad-leaved plantain among them.

Dürer painted the roots and the dark soil at the bottom of the picture. He could not have known it, but the plants in his turf were doing exactly what van Helmont's willow did: building their leaves and stems out of air and water, using light.

Look closely

- Find the dandelions. How many flower heads can you count, and are they all open?
- Look at the bottom edge. What do you see of the roots and soil?
- Find the broadest leaves. Why might a broad, flat leaf be useful to a plant?
- Notice the light. Which parts of the turf are bright, and which are in shadow?

Talk about it

1. If you could weigh this clump of plants, where would most of its dry mass have come from?
2. Why do you think Dürer painted something so ordinary with so much care?
3. What can an artist's careful drawing teach a scientist?

> **Safety first.** If you dry soil in an oven, an adult must handle the oven and hot pans; use a low setting and oven mitts. Wash your hands after handling soil, and do not eat any seeds

> that have been soaked or planted.

EXPERIMENT: MY MINI WILLOW: A MASS LEDGER FOR A BEAN PLANT

Question: Does a growing plant take its mass out of the soil? You will repeat van Helmont's experiment on a small scale with a bean plant. Prediction: write whether you think the soil will lose about as much mass as the plant gains.

What we change: nothing on purpose; we let the plant grow. What we keep the same: the pot, the soil, the window, and the kind of water. What we measure: the dry mass of the soil before and after, the mass of the seeds, the plant's fresh mass and its dry mass, and the water we add.

You will need

- A kitchen scale that reads to 1 g (one that reads to 0.1 g is better)
- A plastic pot or yogurt cup with drainage holes and a saucer
- About 500 g of potting soil, dried (spread thin on a tray in the sun for several days, or in a low oven with an adult)
- 3 dry bean seeds (pinto, kidney or lima), soaked overnight
- A measuring cup marked in milliliters
- Rainwater or tap water, a sunny window, a ruler
- Lab notebook and pencil

Steps

1. Weigh the dry seeds before soaking and record their mass. Weigh the empty pot and saucer, then add the dried soil and weigh again. Subtract to find the dry soil mass.
2. Plant the soaked seeds about 2 cm deep. Set the pot in a sunny window.
3. Water with measured amounts of water, and record every milliliter you add. Water over the saucer so that no soil washes away.
4. Once a week, measure the height of the tallest plant and draw it in your notebook.
5. After four weeks, gently pull out the plants. Shake and rinse every bit of soil from the roots back into the pot.
6. Blot the plants dry with a paper towel and weigh them together. This is their fresh mass.
7. Spread the soil thin and let it dry completely (several sunny days), then weigh it again. Compare with the starting dry mass.
8. Lay the plants on a paper plate and let them dry for a week until they crumble. Weigh them again. This is their dry mass.
9. Fill in a mass ledger: seed mass in, water in, soil mass before and after, plant fresh mass, plant dry mass. Write a conclusion: where did the plant's dry mass come from?

Tips

- Small plants are light. If your scale reads only to 1 g, weigh all the plants together, and say in your conclusion that your scale may be off by 1 g.
- The fresh plant is mostly water. The dry mass is the real building material the plant made.
- Do not add fertilizer. Van Helmont added only water, and so should you.

Check your work

- I kept a complete mass ledger with units.
- I compared the soil's change in mass with the plant's gain in mass.
- I explained in my own words that most of the plant's dry mass came from carbon dioxide in the air.

NATURE NOTEBOOK

1. Draw van Helmont's pot at the start and after five years, side by side. Label each with its mass in kilograms.
2. Draw a leaf with three arrows going in (light, carbon dioxide, water) and two arrows coming out (glucose to the plant, oxygen to the air). Write the photosynthesis equation underneath.
3. Make a page titled "My Mass Ledger" with columns for Date, What went in, What came out, and Mass (g), and fill in the first row from your bean project.

> **From history.** More than a century after van Helmont, the English minister and chemist Joseph Priestley took the next step. On August 17, 1771, he put a living sprig of mint into a closed jar of air in which a candle had burned until it went out. After some days, a candle could burn in that air again. Priestley wrote that plants somehow restore air that burning and breathing have spoiled. Within a few years, other scientists showed that plants need light to do this and that they take carbon dioxide from the air, filling in the part of the story van Helmont had missed.

WORKED EXAMPLES

Example 1. Van Helmont's willow weighed about 2.3 kg when he planted it and about 76.7 kg after five years. How much mass did it gain in all, and how much on average each year?

(Use the map or drawing "Read the willow's mass at the start and after five years from the diagram." shown earlier in this lesson.)

1. Mass gained = final mass − starting mass = 76.7 kg − 2.3 kg = 74.4 kg.
2. Average gain per year = 74.4 kg ÷ 5 years = 14.88 kg per year.
3. Round sensibly: about 14.9 kg per year. (The real yearly gain was even larger, since the fallen leaves were never weighed.)

Answer: The willow gained about 74.4 kg, an average of about 14.9 kg per year.

Example 2. In the same five years the soil lost only about 57 g. How many times larger was the willow's gain than the soil's loss? What does this ratio tell you?

1. Put both masses in the same unit: 74.4 kg = 74.4 × 1,000 g = 74,400 g.
2. Ratio = 74,400 g ÷ 57 g ≈ 1,305.
3. So the willow gained about 1,300 times more mass than the soil lost. Even if every gram the soil lost went into the tree, the soil could account for less than 1/1,000 of the tree's gain.

Answer: About 1,300 times larger. The soil could not have been the main source of the tree's mass.

Example 3. A 100 g sample of completely dried willow wood is roughly 50 g carbon, 43 g oxygen, 6 g hydrogen and 1 g minerals. In photosynthesis the carbon and oxygen built into sugar come from carbon dioxide, the hydrogen comes from water, and minerals come from the soil. What percent of the dry wood came from the air, from water, and from the soil?

1. From the air (carbon dioxide): carbon + oxygen = 50 g + 43 g = 93 g. Percent = 93 ÷ 100 × 100% = 93%.
2. From water: hydrogen = 6 g, which is 6%.
3. From the soil: minerals = 1 g, which is 1%.
4. Check: 93% + 6% + 1% = 100%.

Answer: About 93% from the air, 6% from water and 1% from the soil. Van Helmont was right about the soil and the water mattered, but the air was the biggest source.

PRACTICE

1. EASY What kind of plant did van Helmont grow, what was the only thing he added to the pot, and how long did his experiment last?
2. EASY Why did van Helmont cover the pot with a metal plate full of small holes?
3. EASY Name the three things a plant needs for photosynthesis and the two things it makes.
4. MEDIUM Van Helmont's 200 pounds of soil is about 90.7 kg, which is 90,700 g. The soil lost about 57 g. What percent of the soil was lost? Round to two decimal places.
5. MEDIUM Van Helmont burned 62 pounds of charcoal and found about 1 pound of ash. What fraction of the charcoal's mass went off as gas? Where did that carbon originally come from?
6. MEDIUM Data reading. A student repeated van Helmont's experiment with radishes for 4 weeks.
   Dry soil at start: 500 g. Dry soil at end: 499 g.
   Seeds at start: 1 g. Radish plants at end, fresh: 38 g. Radish plants at end, dried: 3 g.
   Water added: 1,200 mL.
   Which statement is best supported by the data? (A) The plants took most of their mass from the soil. (B) The plants gained far more mass than the soil lost. (C) The plants did not grow. (D) The soil gained mass.
7. HARD Data reading. Using the radish data in problem 6: (a) How many grams of the fresh radish plants were water? (b) What percent of the fresh mass was water? (c) The 3 g of dry mass is mostly carbon, oxygen and hydrogen. Where did most of it come from?
8. HARD Experiment design. You want to test whether light is needed for a plant to gain dry mass. Describe your experiment: name the independent variable, the dependent variable, at least three things you keep the same, and how many plants you would use.
9. CHALLENGE Van Helmont concluded that the willow was made from water alone. In a short paragraph, explain what he got right, what he missed, and why he missed it.
10. CHALLENGE Van Helmont did not weigh the leaves that fell from the willow each autumn. Would including them make the tree's total gain larger or smaller? Does leaving them out weaken or strengthen his main conclusion that the mass did not come from the soil?

COMMON MISTAKES

- Thinking plants eat soil. Soil supplies water and small amounts of minerals, but most of a plant's dry mass comes from carbon dioxide in the air.
- Thinking a gas has no mass. Carbon dioxide is invisible, but it is matter and has mass; a tree is built mostly from it.
- Thinking a plant's fresh mass is all material it has built. Most of a fresh plant is water; dry mass shows how much sugar, starch and cellulose the plant actually made.

Make it a game · Where Did the Mass Come From?. A parent names something made by or from a plant: a log, a potato, a sheet of paper, a loaf of bread, a cotton shirt, a pumpkin. Answer within ten seconds with where most of its dry mass came from (the air, as carbon dioxide) and name one thing the plant also needed (light, water or minerals). Score one point for each correct answer and a bonus point if you can name the sugar chain it is made of (starch or cellulose). Play to ten points.

Scripture. “And God said, Let the earth bring forth grass, the herb yielding seed, and the fruit tree yielding fruit after his kind, whose seed is in itself, upon the earth: and it was so.” (Genesis 1:11, KJV)

God made plants able to grow and to reproduce after their kind. Van Helmont’s willow shows how they grow: by turning light, air and water into their own living bodies.

Practice answers

1. A willow; only rainwater (or distilled water); five years.
2. To keep dust and dirt from falling in and adding mass to the soil, while still letting rain and air reach the plant.
3. It needs carbon dioxide, water and light energy. It makes glucose (sugar) and oxygen.
4. 57 ÷ 90,700 × 100% ≈ 0.06%. The soil lost less than one tenth of one percent of its mass.
5. 61 out of 62 parts, or about 98%, went off as gas (carbon dioxide). The carbon in the charcoal originally came from carbon dioxide in the air, captured by the tree in photosynthesis.
6. (B). The plants went from 1 g to 38 g fresh, while the soil lost only about 1 g (which may be within the scale’s error).
7. (a) 38 g − 3 g = 35 g of water. (b) 35 ÷ 38 × 100% ≈ 92%. (c) Mostly from carbon dioxide in the air, joined with water in photosynthesis; only a tiny part came from soil minerals.
8. Independent variable: light (one group in a sunny window, one group in a dark closet). Dependent variable: dry mass of each plant after a set time, such as 3 weeks. Keep the same: kind of seed, seed mass, amount and kind of soil, amount of water, temperature, pot size and time. Use several plants (for example 5 or more) in each group and average the results, since one plant could be unusual. Expected result: plants in the dark lose dry mass (they use up the seed’s stored food) while those in light gain it.
9. He was right that the willow did not build itself out of soil, since the soil lost only about 57 g while the tree gained about 74 kg. Water did play a part: it supplies hydrogen and fills the living plant. But he missed carbon dioxide, a gas in the air that supplies most of a plant’s dry mass (its carbon and most of its oxygen). He missed it because a gas is invisible and flows in and out freely, and he had no way to trap and weigh it in his setup.
10. Including them would make the total gain larger. Leaving them out actually strengthens his conclusion: the tree made even more material than he measured, yet the soil still lost only about 57 g, so the soil was even less able to account for the growth.

5.2 Leaf Disk Lab ★

OBJECTIVES

- Tell how Jan Ingenhousz showed in 1779 that leaves give off oxygen only in light, and explain why leaf disks float as they photosynthesize.
- Carry out the floating leaf disk experiment with a control, identifying the independent, dependent and controlled variables.
- Record data in a table, draw a line graph, and use it to find the time for half the disks to float and the rate of floating in disks per minute.

Warm-up. Drop a fresh spinach or lettuce leaf into a glass of water. Does it float or sink? Push it to the bottom with a spoon and let go. Now look closely at the leaf's underside under the water with a magnifying glass, if you have one. Write down why you think the leaf floats, and what might be hidden inside it.

Video. Coming soon. Floating leaf disk photosynthesis lab with spinach, baking soda and a syringe, and bubbles of oxygen rising from Elodea pondweed in light; search Science Max, Homeschool Pop (Alternate), Khan Academy, Bozeman Science, and science-teacher lab demonstration channels with time-lapse footage.

KEY TERMS

stomata
Tiny pores, mostly on the underside of a leaf, that open and close to let carbon dioxide in and oxygen and water vapor out. One pore is a stoma.

mesophyll
The middle tissue of a leaf, packed with chloroplasts. Its lower, spongy part is full of air spaces, which is why leaves float.

independent variable
The one thing a scientist changes on purpose in an experiment, such as the distance of the lamp.

dependent variable
The thing a scientist measures to see the effect of the change, such as the number of disks floating.

control group
A setup that is the same as the test except for the factor being tested, used for comparison. Leaf disks in water with no baking soda are a control.

rate
How fast something happens, found by dividing a change by the time it took, such as 1 disk per minute.

CORE IDEAS

The story. In the summer of 1779 a Dutch-born physician named Jan Ingenhousz took a break from his work at the court of the Empress Maria Theresa in Vienna and spent several months in England doing experiments on plants. He had read that Joseph Priestley's sprig of mint could restore air that a candle had spoiled, and he wanted to know how. Ingenhousz dipped leaves under water in glass jars and set them in sunshine. Soon tiny silver bubbles formed on the leaves, most of all on their undersides, and slowly rose. When he moved the jars into shade, the bubbles slowed; at night they stopped. He collected the gas and plunged a glowing wick into it, and the wick burst into flame. The gas was the part of air we now call **oxygen**. Late that year he published his findings in a book called *Experiments upon Vegetables*.

How they figured it out. Ingenhousz ran hundreds of trials that summer, changing one thing at a time. He found three things. First, plants give off this good air only in light, and it is the light that matters, not the warmth of the sun. Second, only the green parts work: leaves and green stems made bubbles, but roots, flowers and fruits did not. Third, in the dark, plants do the opposite, like animals: they use up good air and spoil it. That last fact is respiration, which you will study in the next lesson. Ingenhousz had shown that light drives the work in green leaves.

The big idea. A leaf is built like a thin sandwich. Under the waxy top skin is a layer of tall cells crowded with chloroplasts. Below that is the **spongy mesophyll**, loosely packed cells with air spaces between them, opening to the outside through tiny pores called **stomata**. That trapped air is why a leaf floats. In the leaf disk lab you punch small disks from a spinach leaf and use a plastic syringe to pull the air out of their spaces, so that a solution soaks in and the disks sink. The solution contains a little baking soda (sodium bicarbonate), which supplies dissolved carbon dioxide, and a drop of dish soap, which helps the liquid soak into the leaf. Put the cup under a lamp, and the chloroplasts begin to photosynthesize. The oxygen they release collects in the air spaces, the disks become lighter than the water, and one by one they rise. The faster photosynthesis goes, the faster the disks float.

A fair test. This lab turns photosynthesis into something you can count. The thing you change on purpose, such as the distance from the lamp, is the **independent variable**. What you measure, the number of disks floating each minute, is the **dependent variable**. Everything else must stay the same: the kind of leaf, the size of the disks, the amount of solution, the water temperature and the number of disks. A **control group** of disks in water with soap but no baking soda tells you whether the carbon dioxide really matters. A handy result to compare is the time it takes for half the disks (5 out of 10) to float. A shorter time means a faster rate of photosynthesis.

Try it and see. When you run the lab, you will usually see that disks near a bright lamp float much sooner than disks far from it, and that the control disks with no baking soda mostly stay on the bottom. If you cover a cup of floating disks with foil, they slowly sink again, because in the dark they use up oxygen in respiration. You will also notice that more light does not speed things up forever: at some point the disks cannot go any faster, because they

run short of carbon dioxide or the temperature limits them. Scientists call that other factor the limiting factor.

God's handiwork. One square centimeter of a leaf's underside can hold tens of thousands of stomata, each opening and closing on its own to let air in and keep water from escaping too fast. "He causeth the grass to grow for the cattle, and herb for the service of man: that he may bring forth food out of the earth" (Psalm 104:14). Every green leaf in your yard is quietly making sugar and oxygen whenever the sun shines on it.

PICTURE STUDY



The Avenue at Middelharnis, Meindert Hobbema, 1689 · Oil on canvas · National Gallery, London

Meindert Hobbema was a Dutch painter who loved quiet country scenes of trees, mills and farm roads. This painting shows the road leading to the village of Middelharnis, on an island in South Holland, lined by rows of tall young trees that had been planted along it. The road seems to pull your eye straight to the church tower in the distance.

Look at all those trees and think of every leaf as a tiny food factory with its own pores, air spaces and chloroplasts, all working in the bright Dutch daylight under that great sky.

Look closely

- Follow the road with your eye. Where does it lead you?
- Look at the trees. Where are their leaves: all the way down the trunk, or bunched near the top? Why might that be?
- Find the man walking with his dog, and the people working in the field to the right.
- How much of the painting is sky? What does the sky tell you about the day?

Talk about it

1. On a bright day like this, what are the leaves of these trees giving off into the air?
2. Why do you think the painter made the trees so tall and thin, and the road so straight?
3. If you came back to this road at midnight, how would the trees' gas exchange be different?

> **Safety first.** Use a plastic syringe with no needle. Use an LED lamp if you can; an older bulb gets hot and can warm the water or burn your fingers. Keep the lamp and its cord away from the cups of water, and set the cups on a tray to catch spills. Baking soda and dish soap are safe, but do not drink the solution, and wash your hands afterward.

EXPERIMENT: THE FLOATING LEAF DISK EXPERIMENT

Question: Does the brightness of light change how fast leaves photosynthesize? Prediction: write which you think will float faster, disks close to the lamp or disks far from it, and why.

What we change: the distance from the lamp (10 cm and 40 cm). What we keep the same: the kind of leaf, disk size, number of disks, amount and recipe of solution, water temperature, and lamp. What we measure: the number of disks floating each minute. Control: disks in water and soap with no baking soda.

You will need

- Fresh spinach leaves (a few large ones)
- Baking soda and liquid dish soap
- A plastic syringe, 10 mL or larger, with no needle (from a pharmacy or the medicine aisle)
- A single-hole paper punch or a sturdy drinking straw to cut disks
- 3 clear cups or glasses, a measuring cup marked in mL, and a 1/8 teaspoon
- A desk lamp, an LED bulb if possible
- A ruler, a timer, a thermometer if you have one, aluminum foil
- Lab notebook, pencil and graph paper

Steps

1. Make the test solution: stir 1/8 teaspoon of baking soda (about 0.6 g) into 300 mL of room-temperature water, then add one small drop of dish soap and stir gently so it does not foam. Make the control solution the same way but with no baking soda.
2. Punch 30 disks from the spinach leaves, avoiding the big veins.
3. Pull the plunger out of the syringe, drop 10 disks into the barrel, and push the plunger back in until only a little air is left. Draw in about 5 mL of test solution and tap so the disks float in it.
4. Hold the syringe tip-up, push out the remaining air, then seal the tip with your finger. Pull the plunger back hard and hold for about 10 seconds to make a vacuum, then let it go. Repeat two or three times until most disks sink.

5. Pour the sunken disks and solution into a cup with about 3 cm of test solution. Do the same with 10 more disks in a cup of test solution and 10 disks in a cup of the control solution.
6. Set one test cup and the control cup 10 cm from the lamp, and the other test cup 40 cm from the lamp. Measure with a ruler. Check that the water temperatures are about the same.
7. Turn on the lamp and start the timer. Every minute for 20 minutes, count how many disks are floating in each cup and record it in a table.
8. Graph your results: time (minutes) on the horizontal axis and disks floating on the vertical axis, with one line for each cup. Find the time when 5 of 10 disks were floating in each cup.
9. Bonus: cover a cup of floating disks with foil and check it every 5 minutes. Record what happens.

Tips

- If the disks will not sink, give them a few more vacuum pulls and tap the syringe so they are not stuck to its sides.
- Gently swirl the cups if disks stick to the side or to each other.
- If the water near the lamp grows warm, move the lamp's light through a jar of plain water first, or use an LED bulb; otherwise you are testing heat as well as light.
- Run the whole experiment twice if you can. Repeated trials make results trustworthy.

Check your work

- I named my independent, dependent and controlled variables and my control group.
- I recorded data every minute and drew a line graph with labeled axes and units.
- I found the time for half the disks to float in each cup and explained what it shows about the rate of photosynthesis.

NATURE NOTEBOOK

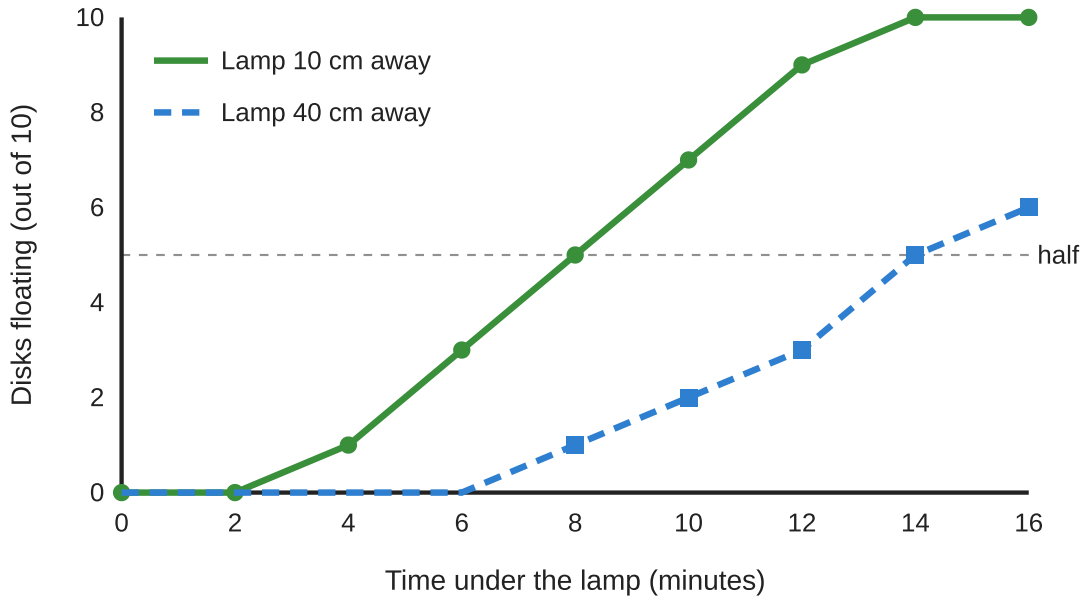
1. Draw a cross section of a leaf: top skin, the layer of tall green cells, the spongy layer with air spaces, a stoma on the bottom. Show carbon dioxide going in and oxygen coming out.
2. Draw three stages of a leaf disk: floating at the start, sunk after the syringe, rising under the lamp. Under each, write one sentence explaining what is in the air spaces.
3. Copy your data table into your lab notebook, then write a one-paragraph conclusion that begins, "My prediction was …"

> **From history.** Before he studied plants, Jan Ingenhousz was famous for fighting smallpox. He learned the method of inoculation in England, and in 1768 he traveled to Vienna, where he inoculated members of the family of the Empress Maria Theresa. The empress was so grateful that she made him her court physician and gave him a lifelong

income. That support gave him the freedom, years later, to spend a summer in England experimenting with leaves in jars of water.

WORKED EXAMPLES

Example 1. A student ran the leaf disk lab with one cup 10 cm from a lamp and another 40 cm away. Her data (disks floating out of 10):
Time (min): 0, 2, 4, 6, 8, 10, 12, 14, 16
10 cm: 0, 0, 1, 3, 5, 7, 9, 10, 10
40 cm: 0, 0, 0, 0, 1, 2, 3, 5, 6
Control (no baking soda): 0 at every time.
Using the graph, find the time for half the disks to float in each cup.



The dashed line marks 5 of 10 disks floating.

1. Half of 10 disks is 5 disks. Find 5 on the vertical axis (the dashed line on the graph).
2. Follow the 10 cm line until it reaches 5 disks: that happens at 8 minutes.
3. Follow the 40 cm line until it reaches 5 disks: that happens at 14 minutes.
4. The control cup never reached 5 disks; with no carbon dioxide supply, the disks could not photosynthesize enough to float.

Answer: 8 minutes at 10 cm and 14 minutes at 40 cm. The control never reached half.

Example 2. Using the same data, find the rate of floating, in disks per minute, for the 10 cm cup between 4 and 10 minutes, and for the 40 cm cup between 8 and 14 minutes. How many times faster was the near cup?

1. 10 cm cup: from 1 disk at 4 min to 7 disks at 10 min. Change = 7 − 1 = 6 disks in 10 − 4 = 6 minutes. Rate = 6 ÷ 6 = 1.0 disk per minute.
2. 40 cm cup: from 1 disk at 8 min to 5 disks at 14 min. Change = 5 − 1 = 4 disks in 14 − 8 = 6 minutes. Rate = 4 ÷ 6 ≈ 0.67 disk per minute.
3. Compare: 1.0 ÷ 0.67 ≈ 1.5.

Answer: About 1.0 disk per minute near the lamp and about 0.67 disk per minute far from it; the near cup floated about 1.5 times as fast.

Example 3. The recipe uses about 0.6 g of baking soda in 300 mL of water. A class of 4 teams needs 1,200 mL of solution. How much baking soda is needed? What is the concentration in grams per 100 mL?

1. Scale factor: 1,200 mL ÷ 300 mL = 4.
2. Baking soda needed: 0.6 g × 4 = 2.4 g (about 1/2 teaspoon).
3. Concentration: 0.6 g in 300 mL is 0.6 ÷ 3 = 0.2 g per 100 mL. The bigger batch is 2.4 ÷ 12 = 0.2 g per 100 mL, the same.

Answer: 2.4 g of baking soda; the concentration stays 0.2 g per 100 mL (a 0.2% solution).

PRACTICE

1. EASY What gas did Ingenhousz find bubbling from leaves in sunlight, and how did he test it?
2. EASY Why does a fresh leaf disk float at first, and why does it sink after you use the syringe?
3. EASY What is the job of the baking soda in the leaf disk lab? What is the job of the soap?
4. MEDIUM In a leaf disk lab testing lamp distance, name the independent variable, the dependent variable, and three controlled variables.
5. MEDIUM Data reading. A student counted disks floating (out of 10) after 10 minutes at different lamp distances:
   10 cm: 7. 20 cm: 5. 30 cm: 3. 40 cm: 2.
   (a) Describe the trend. (b) Predict the number floating at 50 cm. (c) Does the number drop by the same amount for every 10 cm?
6. MEDIUM Data reading. Two cups were tested under the same lamp.
   Cup A (water, soap, baking soda): 5 of 10 disks floating at 9 minutes.
   Cup B (water and soap only): 0 of 10 floating at 20 minutes.
   Which conclusion is best supported? (A) Light is not needed for photosynthesis. (B)

Soap makes disks sink. (C) The disks needed the carbon dioxide from the baking soda to photosynthesize and float. (D) Spinach cannot photosynthesize.

7. MEDIUM A cup of floating disks is covered with foil. After 30 minutes most disks have sunk. Explain why.
8. HARD Experiment design. Design a leaf disk experiment to test whether the color of light changes the rate of photosynthesis. Give your question, independent and dependent variables, what you keep the same, how you would make colored light, and what results would answer your question.
9. CHALLENGE A student puts one cup 5 cm from an old hot bulb and another 40 cm away. The near cup's water warms from 21 °C to 30 °C. Why is this a problem for the experiment, and how could the student fix it?
10. CHALLENGE Ingenhousz found that roots, flowers and fruits did not give off bubbles in the light, but leaves did. Using what you know about cells, explain why.

COMMON MISTAKES

- Thinking the bubbles from a leaf in light are carbon dioxide. The leaf takes carbon dioxide in; the bubbles are mostly oxygen.
- Thinking plants give off oxygen all day and all night. They make oxygen only in light; in the dark they use oxygen in respiration, just as animals do.
- Thinking more light always means faster photosynthesis without limit. When light is bright enough, something else, such as carbon dioxide or temperature, becomes the limiting factor.

Make it a game · Variable Sort. A parent writes 12 slips of paper for an experiment, such as "lamp distance," "disks floating," "kind of leaf," "water temperature," "cup with no baking soda," and "disk size." Draw a slip and place it under the right heading: Independent variable, Dependent variable, Controlled variable or Control group. Score a point for each correct sort and a bonus point if you can say why it belongs there. Then switch to a new experiment, such as light color, and play again.

Scripture. "He causeth the grass to grow for the cattle, and herb for the service of man: that he may bring forth food out of the earth;" (Psalm 104:14, KJV)

The psalmist praises God for making plants grow and feed both animals and people. The leaf disk lab lets us watch, minute by minute, the quiet work that makes that food.

Practice answers

1. Oxygen. He collected it and put a glowing wick into it; the wick burst into flame.
2. It floats because its spongy layer is full of air spaces. The syringe's vacuum pulls out that air and the solution soaks in, so the disk becomes heavier than the water and sinks.
3. The baking soda supplies dissolved carbon dioxide for photosynthesis. The soap helps the solution soak into the leaf's air spaces.

4. Independent: distance from the lamp. Dependent: number of disks floating over time (or time for half to float). Controlled: kind of leaf, disk size, number of disks, solution recipe and amount, water temperature, the same lamp.
5. (a) The farther the lamp, the fewer disks float, because dimmer light slows photosynthesis. (b) About 1 (any answer of 0 to 2 is reasonable). (c) No. It drops by 2, then 2, then only 1, so the change gets smaller as the light gets dimmer.
6. (C). The only difference between the cups was the baking soda, the source of carbon dioxide.
7. In the dark, photosynthesis stops, but the leaf cells keep doing respiration, which uses up oxygen. The oxygen in the air spaces is used up, so the disks become heavier than the water and sink.
8. Question: Does red, green or blue light make leaf disks float fastest? Independent: light color (cover cups or the lamp with red, green and blue cellophane, two layers each). Dependent: time for 5 of 10 disks to float. Keep the same: lamp, distance, disks, solution, temperature, number of disks, and include a white-light cup for comparison. Run at least two trials of each color. If disks under red and blue float sooner than those under green, then red and blue light drive photosynthesis better than green.
9. Temperature also changed, so the student cannot tell whether faster floating came from brighter light or warmer water; there are two variables at once. Fixes: use an LED bulb, put a jar of plain water between the lamp and the cup to absorb heat, or keep the cup in a pan of room-temperature water, and check temperatures with a thermometer.
10. Photosynthesis happens in chloroplasts, which contain the green pigment chlorophyll. Leaves and green stems are full of chloroplasts. Roots, most flowers and ripe fruits have few or none, so they cannot use light to make sugar and oxygen.

5.3 Cellular Respiration

OBJECTIVES

- Tell how Lavoisier and Laplace used an ice calorimeter and a guinea pig in 1782–1783 to show that respiration is like a slow, controlled burning.
- Write the word and chemical equations for cellular respiration, check that the atoms balance, and compare them with photosynthesis.
- Measure rates (breaths per minute, degrees per day) and explain how they show cellular respiration happening in animals and in sprouting seeds.

Warm-up. Sit still and count your breaths for 30 seconds while a parent times you. Then do 30 jumping jacks and count again right away. Put your hand on your chest or neck and feel your heartbeat too. Write both counts down, and write one sentence guessing why your body breathes faster when your muscles work harder.

Video. Coming soon. Cellular respiration in mitochondria for middle school, and how photosynthesis and respiration fit together as a cycle; search Amoeba Sisters, Crash Course Kids, Homeschool Pop (Alternate), Khan Academy, Bozeman Science, and history-of-science channels on Lavoisier and oxygen.

KEY TERMS

cellular respiration
The process in cells that uses oxygen to break down glucose, releasing energy the cell can use and giving off carbon dioxide and water.

mitochondrion
The organelle where most of cellular respiration happens; often called the powerhouse of the cell. The plural is mitochondria.

ATP
A small molecule (adenosine triphosphate) that carries energy in a cell, like a charged battery that can be spent wherever work is needed.

breathing
Moving air into and out of the lungs (or water over gills). It delivers oxygen to the body and removes carbon dioxide, but it is not the same as cellular respiration.

combustion
Burning: a fast reaction of a fuel with oxygen that gives off heat and light, along with carbon dioxide and water.

calorimeter
A device that measures how much heat something gives off. Lavoisier’s calorimeter measured heat by how much ice it melted.

CORE IDEAS

The story. In the cold winter of 1782–1783 in Paris, the chemist Antoine Lavoisier and the mathematician Pierre-Simon Laplace placed a live guinea pig inside a curious device. It was a metal chamber surrounded by a jacket packed with ice, and that jacket was surrounded by a second jacket of ice to keep out the warmth of the room. The only heat that could reach the inner ice came from the little animal's body. As the hours passed, the ice slowly melted and dripped out through a spout, and the two men caught the meltwater and weighed it. They had built an **ice calorimeter**, a heat meter, and they used it to ask a bold question: is the warmth of a living animal the same kind of thing as the heat of a fire?

How they figured it out. Lavoisier already knew that air contains a part that supports both burning and breathing, a gas he named *oxygène*. He knew that a candle and an animal shut in a jar both use it up and both give off "fixed air," which we call carbon dioxide. So the pair measured how much fixed air a guinea pig breathed out in a given time, and how much ice it melted. Then they burned charcoal in the calorimeter until it made about the same amount of fixed air, and measured the ice that melted. The two amounts of heat were close. Lavoisier concluded that respiration is a slow **combustion**: the animal burns its food with oxygen, very gently, inside its own body, and that is where its warmth comes from. A few years later, working with his assistant Armand Seguin, he measured that a man uses more oxygen after a meal and much more while doing work than when resting quietly.

The big idea. Today we call this process **cellular respiration**, and we know it happens inside cells, mostly in organelles called **mitochondria**. Glucose and oxygen go in; carbon dioxide, water and energy come out:

glucose + oxygen → carbon dioxide + water + energy

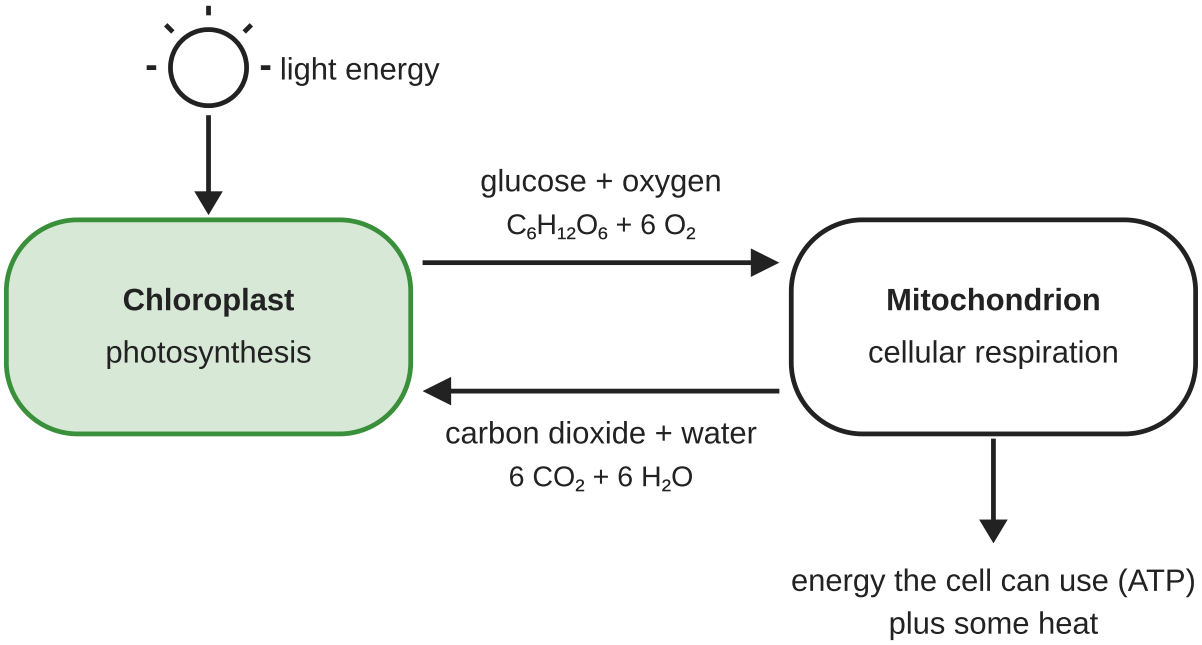
$C_6H_{12}O_6 + 6\ O_2 \rightarrow 6\ CO_2 + 6\ H_2O$ + energy

Unlike a fire, the cell does not let all the energy go at once. It breaks glucose down in many small steps, each guided by an enzyme, and stores much of the energy in small molecules called **ATP**, which the cell spends to move muscles, send nerve signals and build new parts. The rest escapes as heat, which is why your body stays near 37 °C. **Breathing** is the delivery service: lungs bring in oxygen and carry away carbon dioxide, and blood carries them to and from every cell. Cellular respiration is the chemistry that happens when the oxygen arrives.

Two halves of one circle. Look at the respiration equation beside the photosynthesis equation from Lesson 5.1, and you will see that one is the other run backward. Photosynthesis uses light energy to build glucose and oxygen from carbon dioxide and water; respiration releases that energy and returns carbon dioxide and water. Plants do *both*. Every living plant cell respires all day and all night, but plants photosynthesize only in light. On a sunny day a healthy plant makes far more oxygen than it uses, so it gives oxygen to the air. At night it uses oxygen, just as Ingenhousz noticed in 1779. Nearly every living thing respires: plants, animals, fungi and many microbes. Some, such as the yeast you met in Unit 3, can also get energy without oxygen by fermentation, but they get much less energy that way.

Try it and see. You cannot see a mitochondrion at home, but you can catch respiration in the act. Count your breaths before and after exercise: working muscles need more ATP, so they do more respiration and need more oxygen. Or soak dried peas until they sprout and seal them in a vacuum bottle with a thermometer. Sprouting seeds respire quickly to fuel their growth, and the heat they release warms the bottle a few degrees over a few days, while boiled (dead) seeds stay at room temperature. That is Lavoisier's calorimeter in a kitchen.

God's handiwork. "And the LORD God formed man of the dust of the ground, and breathed into his nostrils the breath of life; and man became a living soul" (Genesis 2:7). Every breath you take carries oxygen to trillions of cells, where it releases the sunlight energy that plants stored in food. The leaf and the lung, the chloroplast and the mitochondrion, fit together like two hands.



Photosynthesis and cellular respiration form a cycle: the products of one are the raw materials of the other.

PICTURE STUDY



An Experiment on a Bird in the Air Pump, Joseph Wright of Derby, 1768 · Oil on canvas · National Gallery, London

In the 1700s, traveling lecturers carried scientific instruments from town to town and showed experiments in people's homes. Joseph Wright of Derby, an English painter famous for scenes lit by a single candle, painted one of these evenings. The lecturer has placed a white cockatoo in a glass globe and is pumping out the air to show that a living creature needs air to live. Will he let air back in in time?

The painting was made a few years before Priestley and Lavoisier discovered which part of the air animals need. The people around the table see the effect with their own eyes, but no one in the room yet knows that it is oxygen the bird needs, or that its cells use oxygen to release energy from food.

Look closely

- Find the bird in the glass globe. What is happening to it?
- Look at the faces around the table. Find someone who is frightened, someone who is thinking hard, and someone who seems not to be paying attention.
- Find the man holding a watch. Why would a scientist time an experiment?
- Find the window and the moon. Where does the light in the room come from?

Talk about it

1. Using what you learned in this lesson, explain why the bird cannot live without air.
2. Was this a kind experiment? How should a scientist treat living animals used in experiments?
3. Why do you think Wright painted the lecturer looking straight out at us?

> **Safety first.** An adult should do the boiling of seeds for the control. Do not eat any of the soaked or sprouted seeds. If mold grows, keep the bottle closed and throw the seeds away in the trash, then wash the bottle with hot soapy water. If you exercise for the breathing count, stop if you feel dizzy or unwell.

EXPERIMENT: DO SPROUTING SEEDS GIVE OFF HEAT?

Question: Do living, sprouting seeds give off heat from cellular respiration? Prediction: write whether the sprouting seeds or the boiled seeds will be warmer after three days, and by about how much.

What we change: whether the seeds are alive (sprouting) or dead (boiled). What we keep the same: kind and amount of seeds, the containers, the thermometers, the room. What we measure: temperature in each container, once a day for three days.

You will need

- About 2 cups of dried whole peas, mung beans or lentils
- Two vacuum bottles (thermoses) of the same size, or two insulated cups with lids
- Two thermometers that read in °C (dial cooking thermometers work), or one used for both
- Cotton balls or a folded paper towel to plug the bottle necks loosely
- A pot and stove (adult only), a strainer, water
- Lab notebook and pencil

Steps

1. Soak all the seeds in water overnight. Next morning, drain them.
2. With an adult, boil half of the seeds for 10 minutes to kill them, then let them cool completely to room temperature.
3. Fill one bottle about three-quarters full with the living soaked seeds and the other with the same amount of boiled seeds.
4. Push a thermometer down into the middle of the seeds in each bottle, and plug the necks loosely with cotton so some air can get in.
5. Record the starting temperature of each bottle and of the room.
6. Read and record the temperatures at the same time each day for three days. Add a note on what the seeds look and smell like.
7. Make a double line graph of temperature (°C) against day for both bottles. Calculate how fast the living seeds warmed, in °C per day.
8. Write a conclusion explaining where the heat came from.

Tips

- Leave the necks loosely plugged, not sealed tight: the living seeds need oxygen for respiration.

- End the test after three days. If mold grows on the boiled seeds, the mold itself respires and can warm them, which would spoil the comparison.
- If you have only one thermometer, let it settle for a few minutes in each bottle before reading.

Check your work

- I recorded temperatures for both bottles and the room each day, with units.
- I graphed my data and calculated a rate in °C per day.
- I explained that cellular respiration in the living seeds releases energy, some of it as heat.

NATURE NOTEBOOK

1. Draw the photosynthesis and respiration cycle: a chloroplast and a mitochondrion with arrows for glucose, oxygen, carbon dioxide and water, and label where light energy goes in and ATP and heat come out.
2. Draw Lavoisier’s ice calorimeter as a cross section: the animal in the center, the inner ice jacket, the outer ice jacket, and the spout for meltwater. Label each part.
3. Make a two-column chart titled “Breathing versus Cellular Respiration.” In one column write where breathing happens and what it does; in the other, where cellular respiration happens and what it does.

> **From history.** Lavoisier’s wife, Marie-Anne Paulze Lavoisier, was his partner in the laboratory. She translated English scientific papers into French for him, kept records, and made careful drawings of the experiments, including sketches of Seguin breathing through a mask while Lavoisier measured the oxygen he used. Her engravings illustrated Lavoisier’s great chemistry textbook of 1789. Sadly, Lavoisier had also served as a tax collector for the king, and during the French Revolution he was arrested and executed in 1794. The mathematician Joseph-Louis Lagrange said that it had taken only a moment to cut off that head, but a hundred years might not produce another like it.

WORKED EXAMPLES

Example 1. Check that the cellular respiration equation is balanced: $C_6H_{12}O_6 + 6\ O_2 \rightarrow 6\ CO_2 + 6\ H_2O$. Count each kind of atom on both sides.

(Use the map or drawing "Respiration takes in glucose and oxygen and gives out carbon dioxide and water." shown earlier in this lesson.)

1. Carbon: left, 6 (in $C_6H_{12}O_6$). Right, $6 \times 1 = 6$ (in $6\ CO_2$). Balanced.
2. Hydrogen: left, 12. Right, $6 \times 2 = 12$ (in $6\ H_2O$). Balanced.
3. Oxygen: left, 6 (in glucose) + $6 \times 2 = 12$ (in $6\ O_2$), total 18. Right, $6 \times 2 = 12$ (in $6\ CO_2$) + $6 \times 1 = 6$ (in $6\ H_2O$), total 18. Balanced.
4. Atoms are not created or destroyed; they are only rearranged. The energy that was stored in glucose is released.

Answer: Balanced: 6 carbon, 12 hydrogen and 18 oxygen atoms on each side.

Example 2. At rest, a student counts 4 breaths in 15 seconds. Right after 2 minutes of jumping jacks, the student counts 9 breaths in 15 seconds. Find both breathing rates in breaths per minute and how many times faster the student breathes after exercise.

1. 15 seconds is 1/4 of a minute, so multiply each count by 4.
2. At rest: $4 \times 4 = 16$ breaths per minute.
3. After exercise: $9 \times 4 = 36$ breaths per minute.
4. Compare: $36 \div 16 = 2.25$.
5. Reason: working muscle cells do more cellular respiration to make ATP, so they use more oxygen and make more carbon dioxide; breathing speeds up to keep pace.

Answer: 16 breaths per minute at rest and 36 after exercise, about 2.25 times as fast.

Example 3. A student's sprouting-seed data (°C):
Day 0: living 21.0, boiled 21.0
Day 1: living 22.5, boiled 21.0
Day 2: living 24.0, boiled 21.5
Day 3: living 25.5, boiled 21.0
Find the rate at which the living seeds warmed and the difference between the bottles on Day 3.

1. Change for living seeds = 25.5 °C − 21.0 °C = 4.5 °C over 3 days.
2. Rate = 4.5 °C ÷ 3 days = 1.5 °C per day. (Each day the reading rose by exactly 1.5 °C, so the rate was steady.)
3. Difference on Day 3 = 25.5 °C − 21.0 °C = 4.5 °C.
4. The boiled seeds stayed within half a degree of room temperature, so the extra heat came from the living seeds' respiration.

Answer: The living seeds warmed at 1.5 °C per day and were 4.5 °C warmer than the boiled seeds on Day 3.

PRACTICE

1. EASY Write the word equation for cellular respiration.
2. EASY In which organelle does most cellular respiration take place? Do plant cells have this organelle?
3. EASY What is the difference between breathing and cellular respiration?
4. MEDIUM Why did Lavoisier compare a breathing guinea pig with burning charcoal? What did he conclude?
5. MEDIUM A resting student takes 15 breaths per minute. How many breaths is that in one hour? In one day?
6. MEDIUM Data reading. A scientist measured the oxygen a person used in different activities:
   Sitting: 0.25 L per minute. Walking: 1.0 L per minute. Jogging: 2.0 L per minute.
   (a) How many times more oxygen does jogging use than sitting? (b) How much oxygen does the person use in 30 minutes of walking? (c) What does the pattern tell you about cellular respiration in muscles?
7. HARD Data reading. A potted plant was sealed in a clear box with a carbon dioxide sensor.
   In bright light: CO_2 fell from 400 to 250 parts per million (ppm) in 2 hours.
   In darkness: CO_2 rose from 400 to 520 ppm in 2 hours.
   (a) Find the rate of change in each, in ppm per hour. (b) Explain both results. (c) Does the plant respire in the light?
8. HARD Experiment design. Design an experiment to find out whether temperature changes the rate of respiration in sprouting seeds. Name your independent variable, dependent variable, controlled variables, and what you would measure.

9. CHALLENGE Count the atoms in the photosynthesis equation $6\ CO_2 + 6\ H_2O \rightarrow C_6H_{12}O_6 + 6\ O_2$, and explain how it is related to the respiration equation.
10. CHALLENGE A friend says, “Plants do photosynthesis and animals do respiration.” Write a short paragraph correcting this statement, using evidence from this unit.

COMMON MISTAKES

- Thinking only animals respire. Plants, fungi and most microbes do cellular respiration too, all day and all night.
- Thinking respiration and breathing are the same thing. Breathing moves gases in and out of the lungs; cellular respiration is the chemical process inside cells.
- Thinking respiration makes energy. Energy is not created; respiration releases energy that was stored in glucose by photosynthesis and moves it into ATP and heat.

Make it a game · Photosynthesis or Respiration?. A parent calls out a clue, such as “happens in mitochondria,” “needs light,” “releases carbon dioxide,” “makes glucose,” “happens in a sprouting seed at night,” or “happens in a leaf at noon.” Answer “photosynthesis,” “respiration” or “both” and hold up one finger, two fingers or a fist. Score a point for each right answer. After ten clues, switch places and make up clues for the parent.

Scripture. “And the LORD God formed man of the dust of the ground, and breathed into his nostrils the breath of life; and man became a living soul.” (Genesis 2:7, KJV)

Breath and life go together in Scripture and in biology. Every cell in your body depends on the oxygen your breath brings, yet life itself is God’s gift, more than chemistry alone.

Practice answers

1. glucose + oxygen → carbon dioxide + water + energy
2. The mitochondrion. Yes, plant cells have mitochondria as well as chloroplasts.
3. Breathing moves air into and out of the lungs, bringing in oxygen and removing carbon dioxide. Cellular respiration is the chemical process inside cells that uses that oxygen to break down glucose and release energy.
4. Both use oxygen and give off carbon dioxide (fixed air) and heat. When they made the same amount of fixed air, they melted similar amounts of ice. He concluded that respiration is a slow combustion of food inside the body, and that it is the source of an animal’s body heat.
5. 15 × 60 = 900 breaths per hour; 900 × 24 = 21,600 breaths per day.
6. (a) 2.0 ÷ 0.25 = 8 times. (b) 1.0 × 30 = 30 L. (c) The harder the muscles work, the more cellular respiration they do and the more oxygen they need.
7. (a) Light: (250 − 400) ÷ 2 = −75 ppm per hour (falling 75 ppm per hour). Dark: (520 − 400) ÷ 2 = +60 ppm per hour. (b) In light, photosynthesis uses CO_2 faster than respiration makes it, so CO_2 falls. In darkness only respiration happens, so CO_2 rises. (c) Yes. Respiration goes on in light too, but photosynthesis is faster, so the net change is a fall.
8. Independent: temperature (for example, jars of sprouting seeds kept in a cool place at about 10 °C and a warm place at about 25 °C). Dependent: the rate of respiration, measured by the

carbon dioxide given off (with a CO_2 sensor or an indicator such as limewater turning cloudy) or by the loss of dry mass of the seeds over several days. Temperature of the jars cannot be the measurement here, because it is the thing being changed. Controlled: kind, number and mass of seeds, soaking time, jar size and test time. Use several jars at each temperature and include boiled seeds at each temperature as a control.

9. Left: C 6, H 12, O 12 + 6 = 18. Right: C 6, H 12, O 6 + 12 = 18. It is balanced. It is the respiration equation written backward: photosynthesis uses light energy to build glucose and oxygen from carbon dioxide and water, and respiration releases that energy while turning glucose and oxygen back into carbon dioxide and water.
10. Plants do both. Animals respire, but plant cells also have mitochondria and respire day and night. Ingenhousz found that in darkness plants spoil the air like animals do; leaf disks sink again in the dark as respiration uses up their oxygen; and a sealed plant in darkness makes the CO_2 in a box rise. Only in light do plants also photosynthesize, usually faster than they respire.

5.4 Food from Light

OBJECTIVES

- Describe Theodor Engelmann’s experiment with an alga, a prism and bacteria, and explain why leaves use red and blue light best and look green.
- Explain how plants turn the glucose made in photosynthesis into starch, cellulose and other materials, and why every food chain begins with producers using light.
- Separate leaf pigments by chromatography and calculate Rf values, and use the ten percent rule to calculate energy passed along a food chain.

Warm-up. Look at your last meal or snack and list every food in it. Next to each, trace it back step by step to a plant: bread to wheat, cheese to a cow to grass, an egg to a hen to grain. Can you find any food that does not lead back to a green plant (or an alga) in the end? Circle the plant at the start of each chain.

Video. Coming soon. Why leaves are green, chlorophyll absorbing red and blue light, Engelmann’s experiment, and leaf pigment paper chromatography; search Khan Academy, Crash Course Kids, Homeschool Pop (Alternate), Science Max, NASA Earth Observatory (ocean phytoplankton), and Moody Science classics.

KEY TERMS

chlorophyll
The green pigment in chloroplasts that absorbs light energy for photosynthesis. It absorbs red and blue light well and reflects green light.

pigment
A substance that absorbs some colors of light and reflects others, giving a thing its color. Leaves contain green chlorophylls and yellow-orange carotenoids.

producer
A living thing that makes its own food from light (or, rarely, from chemicals). Plants, algae and some bacteria are producers.

consumer
A living thing that gets its energy by eating other living things or their products. Animals and fungi are consumers.

starch
A long chain of glucose molecules that plants make to store food, found in seeds, potatoes and grains. It turns blue-black with iodine.

chromatography
A way of separating a mixture by letting a liquid carry it up paper or another material; different substances travel different distances.

CORE IDEAS

The story. In the early 1880s, in his laboratory at Utrecht in the Netherlands, the German scientist Theodor Wilhelm Engelmann set up one of the most beautiful experiments in the history of biology. On a microscope slide he laid a long, thin strand of the green alga *Cladophora*, a living chain of cells, in a drop of water. Into the water he put bacteria of a kind that swims toward oxygen. Then, using a small glass prism fitted to his microscope, he spread a tiny rainbow along the strand, so that one end lay in violet light, the middle in green and yellow, and the far end in red. After a while he looked again. The bacteria had crowded thickly around the parts of the alga lit by red light and by blue-violet light. Hardly any had gathered in the green.

How they figured it out. Engelmann had turned living bacteria into tiny oxygen meters. Where the alga made the most oxygen, the most bacteria gathered to breathe it, and the alga made the most oxygen where it was photosynthesizing fastest. So the crowds of bacteria showed which colors of light drive photosynthesis best: red and blue-violet. Green light did very little. That matched what chemists found when they shone light through leaf extracts: the green pigment **chlorophyll** absorbs red and blue light strongly but lets most green light pass through or bounce off. That is why leaves look green. The green you see is the light the leaf is *not* using.

The big idea. In photosynthesis, the light energy that chlorophyll catches is stored in the chemical bonds of glucose. Glucose is the plant's raw material for nearly everything. The plant burns some of it in respiration for energy. It links some into long chains of **starch** to store, packing it into seeds, roots and tubers: a wheat grain, a potato, a grain of rice. It links some into **cellulose** for cell walls and wood. And it combines some with nitrogen and other minerals from the soil to build proteins, oils and vitamins. Because plants, algae and some bacteria make their own food from light, they are called **producers**. Every other living thing is a **consumer** that depends on them. A slice of bread is stored sunlight from a wheat field. Even a steak is sunlight that passed through grass and then a cow.

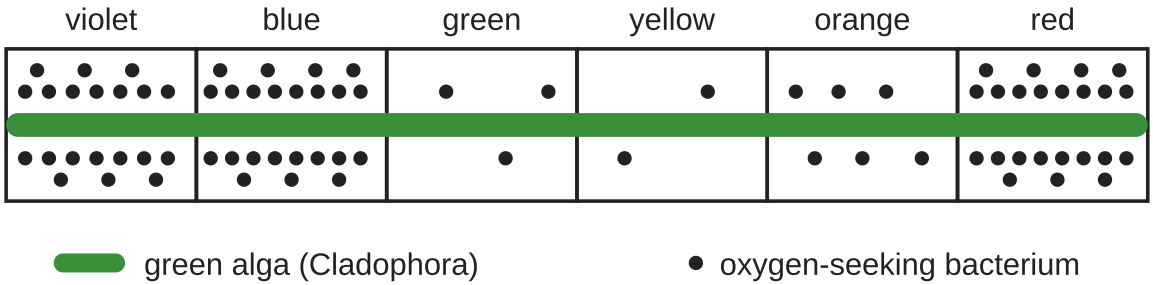
In the world today. Energy passes along a food chain, but not all of it. Each animal uses most of the energy in its food to live, move and stay warm, and that energy leaves as heat from respiration. As a rough rule, only about 10 percent of the energy at one level is stored in the bodies of the next. That is why a meadow can feed many grasshoppers, fewer shrews, and only a few owls. Matter cycles round and round (carbon dioxide to sugar and back again), but energy flows one way, from the sun, through living things, and out as heat. Producers are not only on land. Countless tiny algae drifting in the sea, called phytoplankton, carry out about half of all the photosynthesis on earth. And leaves hold more than chlorophyll: yellow and orange pigments called carotenoids help catch light too. In autumn, when a tree stops making chlorophyll, the green fades and those golden colors show through.

Try it and see. You can separate a leaf's pigments with a coffee filter and rubbing alcohol. Press a line of spinach juice near the bottom of a paper strip and stand it in a little alcohol. As the alcohol climbs, it carries the pigments with it, but not equally far, and the green line

spreads into bands of different colors. Scientists compare pigments by their **Rf value**: the distance a pigment traveled divided by the distance the liquid traveled.

God's handiwork. "Every good gift and every perfect gift is from above, and cometh down from the Father of lights" (James 1:17). Every meal you eat began as light from the sun God made, caught by a green leaf and packed into sugar. Each time you give thanks for your food, you can remember that it came to you through sunshine, a plant, and the care of the One who made them both.

Light from a prism falls along a strand of green alga



Bacteria crowd where the alga gives off the most oxygen:
in the red and blue-violet light.

Engelmann's experiment (simplified). Oxygen-seeking bacteria gathered where the alga made the most oxygen: in red and blue-violet light.

PICTURE STUDY



Wheat Field with Cypresses, Vincent van Gogh, 1889 · Oil on canvas · The Metropolitan Museum of Art, New York

Vincent van Gogh painted this picture in the summer of 1889 near the town of Saint-Rémy in the south of France. He was fascinated by the tall cypress trees of that region and wrote to his brother Theo that a cypress was as beautiful in line and shape as an Egyptian obelisk. He painted the wheat in thick strokes of yellow and gold, ripening under the hot southern sun.

Every grain of that wheat is a little storehouse of starch, made from carbon dioxide and water with the energy of sunlight. When we eat bread, we are eating sunshine that a field like this one caught and stored.

Look closely

- Find the cypress tree. What shape does it make against the sky, and what color is it?
- Look at the wheat. How did van Gogh use his brush strokes to show wind moving through it?
- Look at the sky and clouds. Are they still or moving?
- Find all the different greens in the painting. Which plants are still green, and which have turned gold?

Talk about it

1. The cypress is dark green and the ripe wheat is gold. Which plant is still busy photosynthesizing, and why might the ripe wheat have stopped?
2. Trace the energy in a loaf of bread back to this painting. What steps did it pass through?
3. Why do you think van Gogh chose bright yellows and swirling lines for a summer field?

> **Safety first.** Rubbing alcohol is flammable and poisonous to drink. Use it only with a parent, in a room with open windows or good air flow, far from any flame, stove or heater. Cover the glass while the strip develops, keep the alcohol away from your eyes, and pour leftover alcohol back into its bottle or let a parent dispose of it.

EXPERIMENT: HIDDEN COLORS IN A GREEN LEAF

Question: Is the green of a leaf made of just one pigment, or several? Prediction: write how many colored bands you expect and what colors they will be.

What we change: the kind of leaf (spinach first, then a second leaf of your choice, such as kale, parsley or a red-tinged leaf). What we keep the same: the paper, the size of the strip, the amount of alcohol, the starting line height and the time. What we measure: the distance each colored band travels and the distance the alcohol travels, to calculate Rf values.

You will need

- White coffee filters (or strips of white paper towel)
- Fresh spinach leaves and one other kind of leaf
- A coin
- Rubbing alcohol (70% or 91% isopropyl alcohol)
- 2 tall clear glasses, plastic wrap, tape, 2 pencils
- A ruler marked in centimeters, scissors
- Lab notebook and colored pencils

Steps

1. Cut strips of coffee filter about 2.5 cm wide and 12 cm long. With a pencil (not a pen), draw a light line across each strip 2 cm from the bottom.
2. Lay a spinach leaf across the pencil line. Roll the edge of a coin firmly over the leaf along the line, pressing green juice into the paper. Move to a fresh part of the leaf and repeat 8 to 10 times, until the line is dark green.
3. Pour rubbing alcohol into a glass to a depth of about 1 cm.
4. Tape the top of the strip to a pencil and lay the pencil across the rim of the glass so the bottom of the strip dips into the alcohol but the green line stays above it.
5. Cover the top of the glass loosely with plastic wrap and wait 20 to 40 minutes, watching every few minutes. Do the same with the second kind of leaf in the other glass.
6. When the alcohol is about 1 cm from the top, lift the strip out and immediately mark how high the alcohol reached (the solvent front) with a pencil.
7. Let the strip dry. Mark the middle of each colored band and record its color.
8. Measure from the starting line to the solvent front, and from the starting line to each band, in centimeters. Calculate Rf = distance of band ÷ distance of solvent for each band.
9. Tape your strips into your notebook and compare the two leaves.

Tips

- Use pencil for the lines; ink would dissolve and run up the strip with the pigments.
- A darker starting line gives clearer bands, so take your time pressing the juice in.
- The colors can fade in bright light, so sketch or photograph your strips soon after they dry.

Check your work

- I separated at least two colored bands from a green leaf.
- I measured distances in centimeters and calculated Rf values to two decimal places.
- I explained that a green leaf contains several pigments, with chlorophyll the main one.

NATURE NOTEBOOK

1. Draw a leaf with a sunbeam hitting it. Show red and blue light being absorbed and green light bouncing off to your eye. Label chlorophyll.
2. Draw a food chain from the sun to grass to grasshopper to shrew to owl. Write the energy at each level using the ten percent rule, starting with 10,000 kJ in the grass.
3. Draw a wheat plant and label where it stores starch (the grain), where it has cellulose (the stem), and where it photosynthesizes (the green leaves).

> **From history.** In the late 1940s and 1950s at the University of California, Berkeley, the chemist Melvin Calvin and his team worked out the steps by which a plant turns carbon dioxide into sugar. They gave a green alga called *Chlorella* carbon dioxide made with radioactive carbon-14, let it photosynthesize for just a few seconds, and then quickly killed it in hot alcohol. By finding which chemicals contained the radioactive carbon after 5 seconds, 10 seconds and longer, they traced the carbon’s path step by step. The cycle of reactions they discovered is called the Calvin cycle, and Calvin received the Nobel Prize in Chemistry in 1961.

WORKED EXAMPLES

Example 1. In a meadow, the grass stores 10,000 kJ of energy from sunlight. Using the ten percent rule, how much energy reaches the grasshoppers, the shrews that eat them, and the owls that eat the shrews? What percent of the grass's energy reaches the owls?

1. Grasshoppers: 10% of 10,000 kJ = 0.10 × 10,000 = 1,000 kJ.
2. Shrews: 10% of 1,000 kJ = 100 kJ.
3. Owls: 10% of 100 kJ = 10 kJ.
4. Percent reaching owls: 10 ÷ 10,000 × 100% = 0.1%.
5. The rest was used for living and given off as heat by respiration at each level.

Answer: 1,000 kJ to grasshoppers, 100 kJ to shrews and 10 kJ to owls, only 0.1% of the energy in the grass.

Example 2. In the simplified Engelmann figure, count the bacteria along the alga in each color band and find how many times more bacteria gathered in red light than in green light.

(Use the map or drawing "Count the dots in each band." shown earlier in this lesson.)

1. Count from the figure: violet 20, blue 23, green 3, yellow 2, orange 6, red 23.
2. Ratio of red to green: 23 ÷ 3 ≈ 7.7.
3. The colors with the most bacteria (blue and red) are where the alga made the most oxygen, so those colors drive photosynthesis best.

Answer: About 7.7 times as many bacteria in red light as in green light.

Example 3. On a student's chromatography strip, the alcohol traveled 8.0 cm from the starting line. A yellow-orange band traveled 7.2 cm, a blue-green band 4.0 cm, and a yellow-green band 2.8 cm. Calculate the Rf value of each band.

1. Rf = distance traveled by the pigment ÷ distance traveled by the solvent.
2. Yellow-orange: 7.2 ÷ 8.0 = 0.90.
3. Blue-green: 4.0 ÷ 8.0 = 0.50.
4. Yellow-green: 2.8 ÷ 8.0 = 0.35.
5. An Rf value is always between 0 and 1, because no pigment can travel farther than the liquid carrying it.

Answer: Rf values: yellow-orange 0.90, blue-green 0.50, yellow-green 0.35.

PRACTICE

1. EASY Why do most leaves look green?
2. EASY Name three things a plant makes from the glucose it produces in photosynthesis.
3. EASY What is the difference between a producer and a consumer? Give an example of each.
4. MEDIUM Why did Engelmann use bacteria that swim toward oxygen? What did they tell him?
5. MEDIUM A field of grass captures 50,000 kJ. Using the ten percent rule, how much energy reaches a cow that eats the grass, and how much would reach a lion that ate the cow? Why can the field support many cows but few lions?
6. MEDIUM Data reading. A leaf extract was tested to see how much of each color of light it absorbs:
   Violet: 80%. Blue: 90%. Green: 15%. Yellow: 25%. Orange: 40%. Red: 85%.
   (a) Which two colors are absorbed most? (b) Which color is absorbed least? (c) How does this table agree with Engelmann’s result?
7. HARD Data reading. Bean plants were grown for three weeks under different lamps of equal brightness.
   Red light: average dry mass gain 2.4 g.
   Blue light: 2.1 g.
   Green light: 0.6 g.
   White light: 2.8 g.
   (a) Which light gave the least growth, and why? (b) Why might white light give the most? (c) What is one controlled variable this experiment needs?
8. HARD Experiment design. A gardener thinks a houseplant will grow faster under a red lamp than under a green lamp. Design an experiment to test this idea. Include the question, independent and dependent variables, controls and how you would make the result trustworthy.
9. CHALLENGE A fertilizer bag says “plant food.” Using what you learned in this unit, explain why this name is misleading.
10. CHALLENGE Explain the sentence “Matter cycles, but energy flows.” Use carbon and sunlight as your examples.

COMMON MISTAKES

- Thinking plants get their food from the soil or from fertilizer. Plants make their own food, glucose, from carbon dioxide and water using light; soil supplies water and small amounts of minerals.
- Thinking green light is the most useful light for plants because plants are green. Green is the color chlorophyll uses least; red and blue light are used most.
- Thinking energy is recycled in a food chain the way matter is. Matter cycles, but energy flows one way and is lost as heat at each step, so it must be supplied again by the sun.

Make it a game · Trace It to the Sun. A parent names a food or product: popcorn, a hamburger, honey, maple syrup, a wool sweater, milk, a wooden chair. Trace it back step by step to sunlight in as few correct steps as you can (milk: cow, grass, sunlight). Score one point for each correct chain and a bonus point if you name the stored form of the sugar along the way (starch in corn, cellulose in wood, sucrose in maple sap). First to ten points wins.

Scripture. “Every good gift and every perfect gift is from above, and cometh down from the Father of lights, with whom is no variableness, neither shadow of turning.” (James 1:17, KJV)

James calls God the Father of lights, the Maker of the sun and stars. The energy in every meal began as light, and every good gift, our daily bread included, comes from Him.

Practice answers

1. Their main pigment, chlorophyll, absorbs red and blue light and reflects or lets through most green light, so green light reaches our eyes.
2. Any three: starch (stored food), cellulose (cell walls and wood), energy through respiration, and, with minerals from the soil, proteins, oils or vitamins.
3. A producer makes its own food from light (for example grass, wheat or an alga). A consumer gets energy by eating other living things (for example a grasshopper, a cow or a person).
4. They acted as living oxygen meters: they gathered wherever the alga gave off the most oxygen. Their crowds showed that red and blue-violet light drive photosynthesis best and green light the least.
5. Cow level: 5,000 kJ. Lion level: 500 kJ. Most energy is used and lost as heat at each step, so there is much less energy at higher levels to support animals.
6. (a) Blue (90%) and red (85%). (b) Green (15%). (c) Engelmann’s bacteria crowded in red and blue-violet light, the same colors the extract absorbs most, and avoided green, which is absorbed least. Light that is absorbed is the light used for photosynthesis.
7. (a) Green light, because chlorophyll absorbs little green light, so little energy is captured for photosynthesis. (b) White light contains red and blue together (plus other colors that carotenoids can use), so the plants can absorb more useful light. (c) Any one: same kind of bean, same soil, same water, same temperature, same brightness, same time, same distance from the lamp.
8. Question: Do plants gain more mass under red light than under green light? Independent: color of the lamp (red and green, same brightness and distance). Dependent: increase in mass (or number of new leaves, or height) after 3 to 4 weeks. Keep the same: plant kind and starting size, pot, soil, water, temperature, hours of light each day. Use at least 4 plants per color, average the results, and add a group under white light for comparison.
9. Fertilizer supplies minerals such as nitrogen, phosphorus and potassium, which plants need in small amounts to build proteins and other parts. It does not supply energy or most of the plant’s mass. A plant makes its own food, glucose, from carbon dioxide and water using light energy.
10. Carbon atoms go around and around: plants take carbon dioxide from the air and build it into sugar; animals eat the sugar and breathe the carbon back out as carbon dioxide, which plants use again. Energy does not go around: it comes in as sunlight, is stored in sugar, is passed

along the food chain, and at each step is used and lost as heat, which living things cannot use again. So the sun must keep shining for life to continue.

Unit 5 review

Answer each question. Show your work for calculations, include units, and use complete sentences for explanations. For the diagram item, draw neatly and label every part. The test is worth 60 points.

1. (4 pts) Multiple choice. Van Helmont’s willow gained about 74 kg in five years, while the dried soil lost only about 57 g. What did this show? (A) Plants build their bodies mostly from soil. (B) The soil was not the main source of the tree’s mass. (C) Willows do not need water. (D) The tree gained mass from the metal lid.
2. (6 pts) A student plants a 4 g bean seed in 600 g of dried soil. After 5 weeks the plant’s fresh mass is 52 g, its dry mass is 6 g, and the dried soil weighs 599 g. (a) How much fresh mass did the plant gain? (b) What percent of the fresh plant was water? (c) How much mass did the soil lose?
3. (5 pts) Van Helmont concluded that the willow was made from water alone. Explain what he missed, where most of a plant’s dry mass actually comes from, and why he could not detect it.
4. (5 pts) Multiple choice. In the leaf disk lab, why do the sunken disks rise when placed under a lamp? (A) The lamp heats the water and makes it heavier. (B) Photosynthesis makes oxygen that fills the air spaces in the leaf. (C) The baking soda makes the water lighter. (D) The disks absorb carbon dioxide bubbles from the air.
5. (6 pts) Data reading. Disks floating (out of 10):
   Time (min): 0, 3, 6, 9, 12, 15
   Bright lamp: 0, 1, 4, 7, 10, 10
   Dim lamp: 0, 0, 1, 3, 5, 7
   (a) At what time did half the disks float in the dim cup? (b) Find the rate in disks per minute for the bright cup between 3 and 9 minutes. (c) What do the data show about light and photosynthesis?
6. (5 pts) Experiment design. A student wants to test whether the amount of baking soda changes how fast leaf disks float. Name the independent variable, the dependent variable, three controlled variables, and the control group.
7. (4 pts) Multiple choice. Which living things carry out cellular respiration? (A) Animals only. (B) Plants only, at night. (C) Plants, animals, fungi and most microbes, all the time. (D) Only things that have lungs.
8. (6 pts) Write the chemical equation for cellular respiration. Count the carbon, hydrogen and oxygen atoms on each side, and explain how the equation is related to photosynthesis.
9. (6 pts) Diagram. Draw a chloroplast and a mitochondrion side by side. Add arrows and labels to show: where light energy enters, what passes from the chloroplast to the mitochondrion, what passes back, and what energy comes out of the mitochondrion. Label each organelle with the process that happens in it.
10. (5 pts) Explain what Engelmann’s bacteria showed, and use it to explain why leaves look green.

11. (8 pts) Grass in a field stores 20,000 kJ of energy from sunlight. (a) Using the ten percent rule, how much energy reaches the grasshoppers, the shrews that eat grasshoppers, and the owls that eat shrews? (b) Where does the rest of the energy go at each step? (c) Explain why a slice of bread can be called “stored sunlight.”

UNIT PROJECT: MY PHOTOSYNTHESIS LAB REPORT

Choose one question about photosynthesis that you can test with the floating leaf disk method, such as the effect of light color, lamp distance, baking soda amount or leaf type. Plan a fair test with a control and at least two trials, run it, and write a full lab report: question, prediction, variables, materials, procedure, data table, line graph with labeled axes, the time for half the disks to float in each setup, and a conclusion.

Add a final page titled “From Light to My Lunch” with a labeled diagram of the photosynthesis and respiration cycle and a food chain from the sun to your own meal, and include your leaf chromatography strip with its Rf values.

- **Fair test**: The question is testable; independent, dependent and controlled variables and a control group are named correctly; at least two trials are run.
- **Data and graph**: Data are recorded in a neat table with units; the line graph has a title, labeled axes and a key; times and rates are calculated correctly.
- **Science explanation**: The conclusion uses the data to answer the question and correctly explains photosynthesis, the role of light and carbon dioxide, and why the disks float.
- **Care and completeness**: The report includes the cycle diagram, the food chain and the chromatography strip; drawings are neat and labeled; spelling and units are correct.

Answer key

1. (B). The tree gained more than 1,000 times as much mass as the soil lost.
2. (a) 52 g − 4 g = 48 g. (b) Water = 52 − 6 = 46 g; 46 ÷ 52 × 100% ≈ 88%. (c) 600 g − 599 g = 1 g (which may be within the error of a 1 g scale).
3. He missed carbon dioxide in the air. In photosynthesis, a plant joins carbon dioxide with water to make glucose, which it builds into starch and cellulose, so most of the dry mass (the carbon and most of the oxygen) comes from the air. Carbon dioxide is an invisible gas that moved in and out of his pot freely, and he had no way to trap and weigh it.
4. (B).
5. (a) 12 minutes. (b) (7 − 1) ÷ (9 − 3) = 6 ÷ 6 = 1 disk per minute. (c) Brighter light makes photosynthesis go faster, so the disks float sooner.
6. Independent: amount of baking soda (for example 0, 1/16, 1/8 and 1/4 teaspoon in 300 mL). Dependent: time for 5 of 10 disks to float (or disks floating over time). Controlled: leaf type, disk size and number, lamp and distance, water volume and temperature, amount of soap. Control group: disks in water and soap with no baking soda.
7. (C).
8. $C_6H_{12}O_6 + 6\ O_2 \rightarrow 6\ CO_2 + 6\ H_2O$ + energy. Each side has 6 carbon, 12 hydrogen and 18 oxygen atoms. It is the photosynthesis equation run backward: photosynthesis stores light

energy in glucose, and respiration releases it, turning glucose and oxygen back into carbon dioxide and water.

9. The drawing should show: light energy entering the chloroplast (labeled photosynthesis); an arrow from chloroplast to mitochondrion labeled glucose + oxygen; an arrow from mitochondrion back to chloroplast labeled carbon dioxide + water; the mitochondrion labeled cellular respiration, with an arrow out labeled energy the cell can use (ATP) and heat. Award 1 point for each correct element.
10. The oxygen-seeking bacteria crowded along the alga where it was lit by red and blue-violet light, showing that the alga made the most oxygen, and so photosynthesized fastest, in those colors. Few gathered in green light. Chlorophyll absorbs red and blue light and reflects or passes most green light, so the green light reaches our eyes and leaves look green.
11. (a) Grasshoppers 2,000 kJ, shrews 200 kJ, owls 20 kJ. (b) Most is used for living, moving and keeping warm, and leaves as heat from respiration; some is in parts that are not eaten. (c) Wheat plants used light energy in photosynthesis to make glucose and stored it as starch in their grain. Flour is ground grain, so the energy in bread is light energy the wheat captured and stored.

UNIT 6

Fungi

Mushrooms on the lawn, fuzz on old bread, the blue veins in a cheese and the yeast in your bread dough all belong to one kingdom: the **fungi**. Fungi are not plants. They have no chlorophyll, their cell walls are made of chitin, and they feed by digesting food outside their bodies and absorbing it. Most of a fungus is hidden as a web of threads called a mycelium; the mushroom is only its fruiting body. Fungi are God's great recyclers, turning fallen leaves and logs back into soil, and many of them partner with tree roots to feed whole forests.

In this unit you will follow Elias Fries, "the Linnaeus of mycology," into the Swedish woods; look through Robert Hooke's microscope at a forest of blue mold; watch Pier Antonio Micheli grow molds from spores in 1729; and make spore prints like Beatrix Potter, who studied fungi before she wrote *Peter Rabbit*. You will measure mushroom growth, count mold on a centimeter grid, estimate millions of spores by sampling, and design fair experiments.

The last lesson is about safety. Some of the deadliest poisons in nature are made by plain-looking mushrooms, and no folk test can tell them apart from edible ones. The rule for this unit and for life is simple: **never eat or taste a wild mushroom.** We study fungi with our eyes, rulers, notebooks and microscopes, and we leave the wild ones on the ground.

BEFORE YOU BEGIN

- Can use a microscope and make a simple wet-mount slide (Unit 1)
- Knows the basic parts of a cell and the difference between plant and animal cells (Unit 2)
- Knows that yeast is a single-celled fungus and that microbes can be grown safely in sealed containers (Unit 3)
- Can convert between millimeters, centimeters and meters and calculate a percent

MASTERY CHECKLIST

- I can explain why fungi are a kingdom of their own and how they get their food.
- I can draw and label a mushroom and its mycelium, and the parts of black bread mold.
- I can design a fair mold experiment, measure growth on a grid and graph my data.
- I can make a spore print and use spore color in a dichotomous key.
- I can estimate a large number by sampling and scaling up.
- I can state the wild mushroom safety rule, describe a deadly Amanita and tell what to do if someone swallows a wild mushroom.

OPTIONAL BOOKS AND RESOURCES

- **North American Mycological Association** (https://namyco.org/): Free information on mushrooms and a directory of local mushroom clubs, many of which hold family-

friendly forays and shows where experts identify mushrooms. A good way to see wild fungi safely with experienced guides.

- **Mushroom Observer** (https://mushroomobserver.org/): Free. A large collection of photographed and identified mushroom observations. Browse it with a parent to compare your sketches and spore prints with real records. Never use any website or app to decide whether a mushroom is safe to eat.
- **Poison Control (National Capital Poison Center)** (https://www.poison.org/): Free help online and by phone at 1-800-222-1222 (US). Post the number by your phone during Lesson 6.4 and keep it there.

6.1 Mushrooms ★

OBJECTIVES

- Name the parts of a mushroom and explain that the mushroom is only the fruiting body of a much larger, hidden mycelium made of hyphae.
- Explain how fungi feed by digesting food outside their bodies and absorbing it, and give three reasons fungi are placed in their own kingdom rather than with plants.
- Measure fungal structures and calculate growth rates, percentages and scale using metric units.

Warm-up. Take one white button mushroom from the grocery store. Before you touch it with a knife, look for roots, leaves and anything green. Now gently pull the stalk lengthwise into strips. What do the strips look like? Peek under the cap with a magnifying glass. Write three observations and one question in your lab notebook.

Video. Coming soon. How mushrooms grow: mycelium, hyphae and fruiting bodies, time-lapse of mushrooms growing (search nature time-lapse footage, Smithsonian, SciShow Kids, Homeschool Pop (Alternate), Moody Science classics, US Forest Service).

KEY TERMS

fungus (plural fungi)
A living thing whose cells have walls made of chitin and that cannot make its own food; it digests food outside its body and absorbs it. Mushrooms, molds and yeasts are fungi.

hypha (plural hyphae)
One thin, branching thread of a fungus, usually only a few micrometers wide. Hyphae grow at their tips.

mycelium
The whole tangled network of hyphae, usually hidden inside soil, wood or food. It is the main body of the fungus.

fruiting body
The part of a fungus that grows up to make and release spores. A mushroom is a fruiting body.

decomposer
A living thing that breaks down dead plants and animals and returns their materials to the soil.

chitin
A tough material that makes up fungal cell walls. Insects and crabs use chitin in their outer skeletons too.

CORE IDEAS

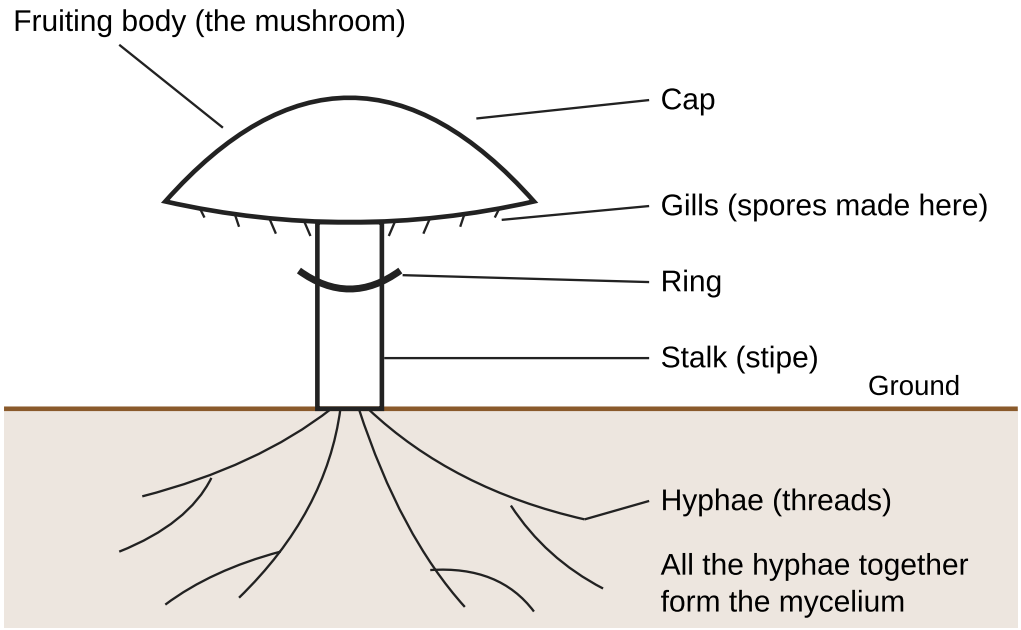
The story. In the woods of Småland, in southern Sweden, a boy named Elias Fries grew up in a pastor's house at the edge of the forest. When he was about twelve, he found a strange white fungus on a tree, branched like coral, and he never forgot it. Fries spent the rest of his life learning the names and shapes of fungi. He became a professor at Lund and later at Uppsala, the university where Linnaeus had taught. In 1821 he published the first volume of *Systema Mycologicum* (A System of Fungi), sorting thousands of mushrooms by their caps, gills and the color of their spores. Mushroom scientists still begin many names with his work, and he is often called "the Linnaeus of mycology." **Mycology** is the study of fungi, from the Greek word *mykes*, mushroom.

How they figured it out. For centuries people called mushrooms plants, because they stand still and grow out of the ground. But as microscopes improved, naturalists noticed big differences. Mushrooms have no green chlorophyll, so they cannot make food from light as you learned in Unit 5. Their cell walls are made of **chitin**, the same tough material in a beetle's shell, not the cellulose of plant walls. They store extra food as glycogen, as animals do, not as starch. And they do not swallow food or make it: they *absorb* it. In 1969 the American biologist Robert Whittaker proposed sorting living things into five kingdoms, and fungi were given a kingdom of their own. Biology textbooks still teach the fungi as a separate kingdom today.

The big idea. A mushroom is not the whole fungus. It is the **fruiting body**, the part that pushes up to scatter spores, the way an apple is the part of a tree that carries seeds. The real body of the fungus is the **mycelium**, a web of threads called **hyphae** spread through soil, a log or a pile of leaves. Each hypha is only a few micrometers wide, so thin that thousands lie hidden in a spoonful of forest soil. Hyphae grow at their tips and pour out *enzymes*, chemicals that break dead leaves and wood into small molecules. Then the hyphae soak those molecules in. A fungus digests its food on the outside and absorbs it, which makes most fungi **decomposers**. When conditions are right, usually after rain, the hyphae bundle together and swell with water to build a mushroom in just a few days. A typical gilled mushroom has a **cap**, thin **gills** underneath where spores are made, a **stalk** (stipe), and on some kinds a **ring** left from a skin that once covered the young gills. Not every fungus makes mushrooms: molds (Lesson 6.2) and the yeasts you met in Unit 3 are fungi too.

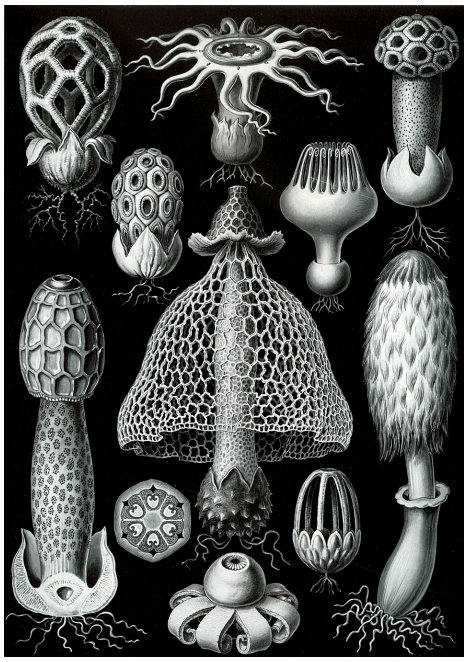
In the world today. In the Malheur National Forest of eastern Oregon, US Forest Service scientists in the late 1990s used genetic tests on samples of honey mushroom fungus (*Armillaria*) and found that one single mycelium had spread under about 965 hectares of forest, nearly 10 square kilometers. It is often called the "humongous fungus," one of the largest living things known, yet above ground you see only clusters of honey-colored mushrooms in the fall. Fungi also feed people. The white button, cremini and portobello mushrooms in a grocery store are all one species, *Agaricus bisporus*; a portobello is simply a brown one left to grow larger. Farmers grow them indoors on beds of compost, where the mycelium digests straw and manure.

God’s handiwork. Fungi also work quietly with trees. Many hyphae wrap around and enter tree roots in a partnership called **mycorrhiza** (“fungus root”): the fungus gathers water and minerals from far into the soil, and the tree pays it with sugar made by photosynthesis. Most land plants have fungal partners like this. Without decomposers, fallen leaves, dead trees and animal bodies would pile up and their minerals would stay locked away. The psalmist looked at the wide world and said, “O LORD, how manifold are thy works!” The mushroom on your lawn is a small sign of a great hidden network, made to clean the forest floor and feed the trees.



The mushroom above ground is the fruiting body. The main body of the fungus is the mycelium of hyphae hidden in the soil.

PICTURE STUDY



Basimycetes, Plate 63 from Kunstformen der Natur (Art Forms in Nature), Ernst Haeckel, 1899–1904 (published in parts) · Color lithograph (printed plate) from drawings · Published in Leipzig, Germany; copies in many natural history libraries

Ernst Haeckel was a German zoologist who drew living things from the sea and the land with great skill. Between 1899 and 1904 he published *Kunstformen der Natur*, a set of one hundred plates meant to show the beauty and order in nature. This plate gathers many kinds of mushroom-forming fungi into one balanced design, as if they were set out in a museum case.

Haeckel held views about the origin of life that Christians do not share, and some of his other drawings were criticized for exaggeration. This plate, though, is a careful picture of real fungi, and it shows how much variety is packed into the mushroom world.

Look closely

- Find a mushroom that has been cut in half. Can you see the cap, the stalk and the gills or pores underneath?
- Find at least two different kinds of spore-bearing surfaces: thin gills, tiny holes (pores) or something else.
- Look at how Haeckel arranged the page. Is it balanced on the left and right? Where is the largest fungus?
- Compare the shapes. Which fungi look like umbrellas, and which look like shelves, cups or clubs?

Talk about it

1. Why do you think Haeckel drew some mushrooms from underneath instead of only from the side?
2. Which part of the fungus is missing from every drawing on this plate? (Hint: where is the mycelium?)
3. Choose one fungus on the plate and describe it so clearly that a friend could find it without pointing.

> **Safety first.** Use only mushrooms bought at a grocery store. Never eat, taste or nibble a wild mushroom, even a small piece; some can kill (Lesson 6.4). Ask an adult to help with the knife and with the oven if you use one. Wash your hands after handling any mushroom.

EXPERIMENT: HOW MUCH OF A MUSHROOM IS WATER?

A mushroom pops up in a few days because its hyphae swell with water. In this experiment you will find out how much of a store mushroom's mass is water by weighing it fresh and again after it dries. You will also compare caps with stalks to see whether the two parts hold the same share of water.

Question: What percent of a mushroom's mass is water, and is it the same for caps and stalks? **Prediction:** Write your guess before you begin. **What we change:** the part of the mushroom (cap or stalk). **What we keep the same:** the kind of mushroom, the slice thickness, the drying place and the drying time. **What we measure:** mass in grams before and after drying.

You will need

- 6 to 8 white button or cremini mushrooms from the store
- Kitchen scale that reads to 1 gram (0.1 gram is even better)
- Knife and cutting board (with an adult)
- Two plates or a cooling rack, labeled "caps" and "stalks"
- Paper towels, a magnifying glass and your lab notebook
- Optional: an oven on its lowest setting, used only by an adult

Steps

1. Draw one whole mushroom from the side and from below. Label the cap, gills, stalk and ring (if your mushroom has one) using the diagram in this lesson.
2. Snap the stalks off all the mushrooms. Weigh all the caps together and write the mass. Then weigh all the stalks together. If your scale needs a dish, weigh the empty dish first and subtract.
3. Slice the caps and stalks about 3 millimeters thick. Keep the cap slices on one plate and the stalk slices on the other.
4. Let the slices dry in a warm, dry, airy place for 3 to 5 days, turning them once a day. (Or an adult may dry them in an oven at its lowest setting with the door slightly open for 2 to 3 hours.) They are dry when they snap instead of bend.

5. Weigh the dry caps and dry stalks. Record each mass.
6. Calculate: water lost = fresh mass − dry mass. Percent water = water lost ÷ fresh mass × 100.
7. Make a small bar graph with two bars: percent water in caps and percent water in stalks. Write two sentences comparing your result with your prediction.

Tips

- Weigh many pieces together. A single small slice may weigh less than one gram, which a kitchen scale cannot read well.
- Food scientists report that fresh white mushrooms are a little over 90 percent water. If your answer is far from that, check whether your slices were fully dry.
- Do not leave drying slices where pets can reach them.

Check your work

- I recorded fresh and dry masses for caps and stalks with units.
- I calculated percent water correctly for each part.
- I drew and labeled the parts of a mushroom.
- I can explain why so much water helps a mushroom grow up so quickly.

NATURE NOTEBOOK

1. Draw a gilled mushroom as if the ground were made of glass, showing the mushroom above and the mycelium spreading below. Label the fruiting body, hyphae and mycelium.
2. Make a three-column chart titled “Plant, Fungus or Animal?” and compare the three on food, cell walls and stored food.
3. Find a mushroom, shelf fungus or patch of fungus on a log outdoors (look, do not pick or taste). Sketch it, note the date, the place and what it was growing on, and measure its cap width with a ruler held beside it.

> **From history.** Before George Washington Carver became famous for his work with peanuts and sweet potatoes, he was a skilled collector of fungi. As a student at Iowa State and later as a teacher at Tuskegee Institute in Alabama, he gathered fungi that caused diseases in crops and sent specimens to plant scientists at the US Department of Agriculture. Experts thought so highly of his collecting that at least one fungus was named in his honor. Carver saw careful study of small things as a way to help poor farmers grow healthier crops.

WORKED EXAMPLES

Example 1. A student grows an oyster mushroom kit and measures the width of the largest cap each morning: Day 1, 12 mm; Day 2, 20 mm; Day 3, 31 mm; Day 4, 45 mm. What was the average growth rate from Day 1 to Day 4, and on which day did the cap grow fastest?

1. Total growth = 45 mm − 12 mm = 33 mm.
2. Time passed = Day 4 − Day 1 = 3 days.
3. Average rate = 33 mm ÷ 3 days = 11 mm per day.
4. Daily growth: Day 1 to 2 = 8 mm; Day 2 to 3 = 11 mm; Day 3 to 4 = 14 mm. The largest change is from Day 3 to Day 4.

Answer: An average of 11 mm per day; the cap grew fastest from Day 3 to Day 4 (14 mm), so the growth was speeding up.

Example 2. A hypha is about 5 micrometers (µm) wide. How many hyphae could lie side by side across 1 millimeter? Across a mushroom stalk 1 centimeter wide?

1. 1 mm = 1,000 µm.
2. Across 1 mm: 1,000 µm ÷ 5 µm = 200 hyphae.
3. 1 cm = 10 mm, so across 1 cm: 200 × 10 = 2,000 hyphae.

Answer: About 200 hyphae across 1 mm, and about 2,000 across a 1 cm stalk. A stalk is a bundle of a great many hyphae packed together.

Example 3. The humongous fungus in Oregon covers about 965 hectares. A soccer field is about 105 m long and 68 m wide. About how many soccer fields would fit over the fungus? (1 hectare = 10,000 m^2)

1. Area of the fungus: 965 × 10,000 m^2 = 9,650,000 m^2.
2. Area of one field: 105 m × 68 m = 7,140 m^2.
3. Number of fields: 9,650,000 ÷ 7,140 ≈ 1,352.
4. Round to a sensible estimate: about 1,350 fields.

Answer: About 1,350 soccer fields.

PRACTICE

1. EASY What part of a fungus is a mushroom, and what is its job?
2. EASY What material makes up fungal cell walls, and what animals use the same material?

3. EASY Give three reasons fungi are not classified as plants.
4. MEDIUM A hypha is 8 µm wide. How many would fit side by side across 2 mm?
5. MEDIUM Data reading. Two oyster mushroom kits were kept at different temperatures. Cap width (mm): Day 0: Kit A (20 °C) 0, Kit B (12 °C) 0. Day 2: A 8, B 4. Day 4: A 22, B 10. Day 6: A 40, B 18. (a) Which kit grew faster? (b) Find each kit's average growth rate from Day 2 to Day 6. (c) How many times wider was Kit A's cap than Kit B's on Day 6?
6. MEDIUM Data reading. Scientists put oak leaves in mesh bags on the forest floor. In some bags the fungi were left alone; in others a treatment slowed the fungi. Percent of leaf mass lost: Week 0: normal 0%, slowed 0%. Week 4: normal 15%, slowed 5%. Week 8: normal 32%, slowed 9%. Week 12: normal 50%, slowed 14%. (a) What is the independent variable? (b) How many more percentage points of mass did the normal bags lose by Week 12? (c) What conclusion does the data support?
7. MEDIUM Explain in two or three sentences how a fungus "eats" a fallen log, and how this is different from the way you eat.
8. HARD A button mushroom cap is 3 cm across and a portobello cap is 12 cm across. How many times wider is the portobello? About how many times more area does the underside of its cap have? (Treat both caps as circles.)
9. HARD Experiment design. A student asks, "Does temperature change how fast oyster mushrooms grow?" Describe a fair experiment. Name the independent variable, the dependent variable, at least three things to keep the same, and how to make the results more reliable.
10. CHALLENGE A tree seedling is planted in sterilized soil with no fungi, and an identical seedling is planted in forest soil full of mycorrhizal fungi. Predict which grows better and explain why both the fungus and the tree gain from their partnership.

COMMON MISTAKES

- Thinking the mushroom is the whole fungus. It is only the fruiting body; the main body is the mycelium hidden in soil or wood.
- Thinking fungi are plants. Fungi have no chlorophyll, have chitin cell walls and absorb food, so they are a kingdom of their own.
- Thinking all fungi are harmful. Most fungi are decomposers and tree partners that keep forests healthy, and some feed us.

Make it a game · Kingdom Sort. A parent reads clues one at a time: "has chitin walls," "makes food from light," "eats by swallowing," "absorbs food after digesting it outside," "stores starch," "stores glycogen." Stand by a sign marked Plant, Fungus or Animal (some clues fit two). Score a point for each correct sign and a bonus point if you can name an example living thing for the clue.

Scripture. "O LORD, how manifold are thy works! in wisdom hast thou made them all: the earth is full of thy riches." (Psalm 104:24, KJV)

“Manifold” means many and varied. The fungi are a whole kingdom of hidden workers that recycle the forest and feed the trees, part of the riches that fill the earth.

Practice answers

1. A mushroom is the fruiting body of the fungus. Its job is to make and release spores.
2. Chitin. Insects, crabs and other arthropods use chitin in their outer skeletons.
3. Any three: fungi have no chlorophyll and cannot make their own food by photosynthesis; their cell walls are chitin, not cellulose; they store food as glycogen, not starch; they digest food outside their bodies and absorb it.
4. 2 mm = 2,000 µm. 2,000 ÷ 8 = 250 hyphae.
5. (a) Kit A, the warmer one. (b) Kit A: (40 − 8) ÷ 4 days = 8 mm per day. Kit B: (18 − 4) ÷ 4 days = 3.5 mm per day. (c) 40 ÷ 18 ≈ 2.2 times wider.
6. (a) Whether the fungi were active or slowed. (b) 50% − 14% = 36 percentage points. (c) Fungi are responsible for much of the breakdown of fallen leaves; when fungi are slowed, leaves decompose far more slowly.
7. The hyphae grow into the wood and release enzymes that break the wood down into small molecules outside the fungus. Then the hyphae absorb those molecules through their walls. You take food inside your body first and digest it in your stomach and intestines; a fungus digests first, outside, and then absorbs.
8. 12 ÷ 3 = 4 times wider. Area grows with the square of the width, so 4 × 4 = 16 times more area for gills.
9. Independent variable: temperature (for example, three identical kits at 12 °C, 18 °C and 24 °C). Dependent variable: cap width in mm (or total mass harvested) measured every day at the same time. Keep the same: kind and size of kit, amount of water and misting schedule, light, air flow and the day the kits were opened. For reliability, use more than one kit at each temperature (at least two or three), average the results and graph cap width against days.
10. The seedling in forest soil should grow better. Its fungal partner’s hyphae reach far beyond the roots and bring in water and minerals such as phosphorus. The tree gives the fungus sugar made by photosynthesis, which the fungus cannot make. Each supplies what the other lacks, so both benefit.

6.2 Molds

OBJECTIVES

- Describe how a mold such as black bread mold is built (hyphae, stolons, rhizoids, sporangia and spores) and how its spores travel.
- Design and carry out a controlled bread-mold experiment, measure mold growth on a centimeter grid, and graph the results.
- Explain how molds help people (cheese, soy sauce, medicine, decay) and harm them (spoiled food, toxins, allergies), and state safe rules for moldy food.

Warm-up. Think of the last time you found something moldy in your kitchen: bread, fruit, cheese or leftovers. Without opening any moldy container now, list what food it was, what color the mold was, and where the food had been kept. Then answer: why does bakery bread often grow mold sooner than packaged sandwich bread?

Video. Coming soon. Bread mold time-lapse and how molds grow, Rhizopus and Penicillium under the microscope (search nature and science time-lapse footage, SciShow Kids, Homeschool Pop (Alternate), Science Max, Moody Science classics, USDA food safety).

KEY TERMS

mold
A fungus that grows as a fuzzy mat of hyphae on the surface of food, wood, leather or other damp material.

spore
A tiny reproductive cell that can grow into a new fungus. Spores are light enough to drift in the air.

sporangium (plural sporangia)
A round case at the tip of an upright hypha that fills with spores and breaks open to release them, as in black bread mold.

decomposition
The breaking down of dead material into simpler substances by decomposers such as fungi and bacteria.

preservative
A substance added to food to slow the growth of molds and bacteria, such as salt, sugar, vinegar or calcium propionate in bread.

mycotoxin
A poison made by some molds. For example, a mold called *Aspergillus flavus* can make aflatoxin on poorly stored grain and peanuts.

CORE IDEAS

The story. In 1665 Robert Hooke, curator of experiments for the Royal Society in London, published *Micrographia*, a book of drawings of things seen through his microscope. You met his cork cells in Unit 2. In his chapter on blue mold, he described small white spots of hairy mold he found on the red sheepskin cover of a small book, which looked like nothing more than a few small specks. Under the lens it turned into a tiny forest: clear stalks, each topped with a round knob, standing up from the leather like a field of little mushrooms. Hooke drew what he saw with great care. He did not know where the mold had come from, and he wondered whether it simply sprang up as the leather decayed.

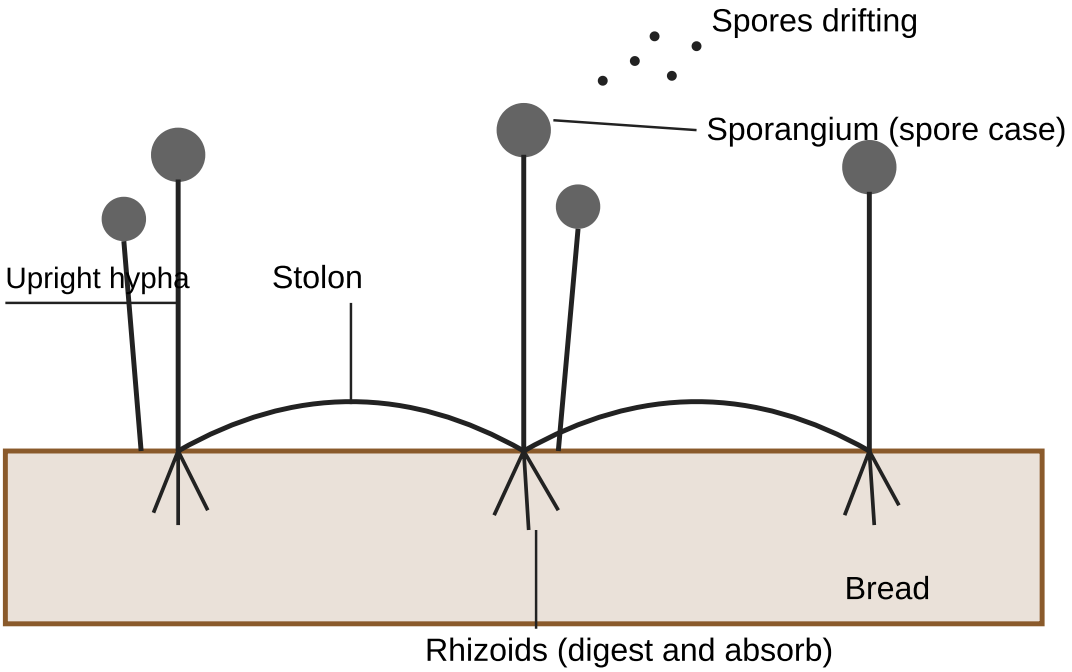
How they figured it out. The answer came from a botanist in Florence, Italy, named Pier Antonio Micheli. In 1729 he published *Nova Plantarum Genera* (New Kinds of Plants), with engraved plates of hundreds of living things, including molds. Micheli saw the tiny grains on the heads of the molds and tested them. He dusted these grains, which we call **spores**, onto fresh slices of fruit such as melon, and the same kind of mold grew from them. Molds come from spores, not from rotting food by itself. Micheli also named a mold *Aspergillus*, because its spore heads reminded him of the aspergillum, a brush used to sprinkle water in church. More than a century later, Pasteur's experiments (Unit 4) settled the same question for germs.

The big idea. A **mold** is a fungus that grows as a fuzzy surface mat of hyphae. Look at black bread mold (*Rhizopus*). Some hyphae, called **rhizoids**, grow down into the bread and pour out enzymes that digest it. Others, called **stolons**, arch across the surface to new spots. Upright hyphae rise into the air, and each carries a round **sporangium** that fills with black spores. When it breaks open, the spores drift away on the slightest breeze. Blue-green *Penicillium* grows its spores instead in little chains at the tips of brush-shaped branches; *penicillus* is Latin for a small paintbrush. Spores are everywhere in ordinary air, waiting. A mold grows when a spore lands where there is food, moisture and moderate warmth. Most food molds grow fastest at ordinary room temperatures; a refrigerator at about 4 °C slows them greatly but does not kill them, and dry food, salt, sugar, vinegar and added preservatives slow them as well.

In the world today. Molds are both helpers and spoilers. Cheesemakers use *Penicillium roqueforti* for the blue veins of Roquefort and *Penicillium camemberti* for the white rind of Camembert. In Japan and China, a mold called *Aspergillus oryzae* has long been used to brew soy sauce and miso. And you learned in Unit 4 how a *Penicillium* mold gave the world penicillin. But molds also spoil a great deal of food, cause sneezing and asthma in some people, and a few make poisons called **mycotoxins**. Food-safety experts at the US Department of Agriculture advise throwing away soft moldy foods such as bread, soft fruit and leftovers, because the hyphae reach far below the fuzz you can see. On hard cheese, cut away at least 2.5 centimeters around and below the moldy spot. Never sniff moldy food, because that pulls spores into your lungs.

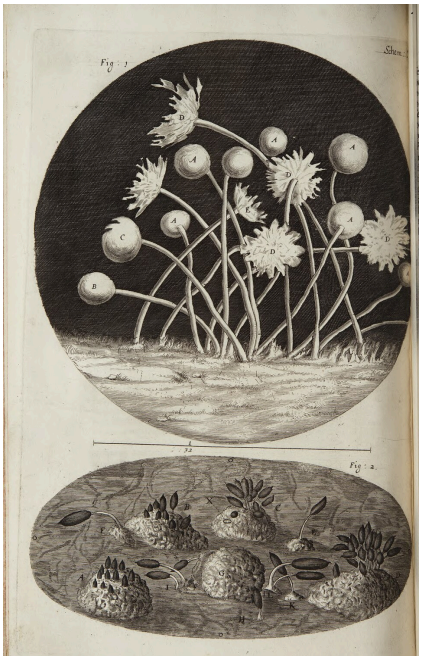
God's handiwork. Mold on your bread is annoying, but in the wider world it is part of a cleanup crew. Fallen fruit, dead leaves and old wood are broken down by molds and other

decomposers, and their carbon and minerals go back into the soil for new plants. The Lord Jesus pointed to this very fact of decay to teach about what lasts: earthly treasures are where “moth and rust doth corrupt.” A good steward stores food wisely, wastes little, and remembers that the things of this world do not last forever.



Black bread mold (Rhizopus). Rhizoids digest the bread, stolons spread across it, and sporangia release spores into the air.

PICTURE STUDY



Blue Mould, Schema XII from Micrographia, Robert Hooke, 1665 · Engraving in a printed book · Published in London; this scanned copy belongs to the National Library of Wales, Aberystwyth

Robert Hooke made the drawings in *Micrographia* from what he saw through his own microscope, and they were engraved on copper for printing. This plate goes with his chapter on blue mold. One figure shows the hairy mold he found on the red sheepskin cover of a small book, magnified until each tiny speck became a forest of stalks and knobs. Another shows small growths he found in the yellow spots on damask-rose leaves; we now know these are a rust, another kind of fungus.

Hooke did not know that molds grow from spores. More than sixty years later, Pier Antonio Micheli sowed mold spores on fresh fruit and showed where molds come from.

Look closely

- Find the mold drawing. How many stalks with round knobs can you count? What do you now know the knobs hold?
- Find the drawing of the growths from the rose leaf. How are they different from the mold?
- Look for letters or numbers beside the figures. Why would Hooke label his drawings this way?
- Look at the shading and fine lines. How did the engraver show which parts are round and which are thin threads?

Talk about it

1. Hooke wrote that the mold looked like a crowd of tiny mushrooms. Using what you learned in Lesson 6.1, was he right that molds and mushrooms are related?
2. How did careful drawings like this one help later scientists, such as Micheli, study where molds come from?

> **Safety first.** Seal every bread bag with tape before mold appears, and never open a moldy bag. Do not sniff moldy food. Anyone with asthma or mold allergies should observe through the bag only or skip the experiment. At the end, throw the sealed bags away in the outdoor trash. Wash your hands after handling the bags.

EXPERIMENT: THE BREAD MOLD RACE

You will test one factor that might change how fast mold grows on bread, and you will measure the mold every day on a 1-centimeter grid, just as a lab scientist would.

Question: Does moisture change how fast mold grows on bread? **Prediction:** Write yours, with a reason. **What we change:** whether the slice is dry or moistened. **What we keep the same:** the kind and age of bread, slice size, the bag, the place and its temperature, and how long the slice is exposed to the air. **What we measure:** the number of 1-cm squares with mold each day.

You will need

- 4 slices of the same bread (bakery bread with no preservatives grows mold sooner)

- 4 zip-top bags and clear tape
- A spray bottle or spoon, and water (about 5 mL per moist slice)
- Clear plastic sheet or tracing paper with a 1-cm grid drawn on it in permanent marker
- Marker, ruler, graph paper and lab notebook

Steps

1. Label the bags Dry A, Dry B, Moist A and Moist B. Two bags of each give you a repeat trial.
2. Leave all four slices open on a plate for 30 minutes so spores from the air can land on them equally.
3. Sprinkle about 5 mL of water evenly over each Moist slice. Leave the Dry slices alone.
4. Put each slice in its bag, squeeze out most of the air, seal it and tape the seal shut. Store all four bags side by side in the same dark cupboard at room temperature.
5. Each day at the same time, lay the grid over each bag and count the squares that have any mold in them. Record the counts in a table: Day, Dry A, Dry B, Moist A, Moist B.
6. Continue for 10 days or until one slice is mostly covered. Note mold colors (black, blue-green, white, orange).
7. Average the two Dry counts and the two Moist counts for each day. Draw a line graph with days on the x-axis and squares covered on the y-axis, one line for Dry and one for Moist.
8. Write a conclusion: Did moisture change the growth? By how much on the last day? Was your prediction supported?

Tips

- Count a square if any part of it has mold. Use the same rule every day.
- If no mold appears after a week, try again with bread that has no preservatives, or wipe a cotton swab across a dusty shelf and touch it to each slice before bagging (the same way on every slice).
- Mold you can see is only the part with spores. Hyphae have already spread through the bread before the fuzz appears.

Check your work

- I kept every bag sealed for the whole experiment.
- I changed only one variable and kept the others the same.
- My data table has a count for every bag on every day.
- My graph has a title, labeled axes with units and a key.
- My conclusion uses numbers from my data.

NATURE NOTEBOOK

1. Draw black bread mold as seen under a magnifier or microscope (or from the diagram), labeling rhizoids, stolons, sporangia and spores.

2. Make a “Mold Helpers and Mold Spoilers” T-chart with at least four examples on each side.
3. Keep a daily sketch log of one bread bag: draw the slice each day with the colored patches, dated, and write one sentence of what changed.

> **From history.** Roquefort blue cheese has been ripened for centuries in the natural limestone caves of Mount Combalou, near the village of Roquefort-sur-Soulzon in southern France, where cool, damp air helps the *Penicillium roqueforti* mold grow through the cheese. In 1411 King Charles VI of France granted the people of Roquefort a royal charter giving them the sole right to ripen cheese in those caves. Long before anyone knew what a spore was, cheesemakers had learned by careful observation how to put a mold to work.

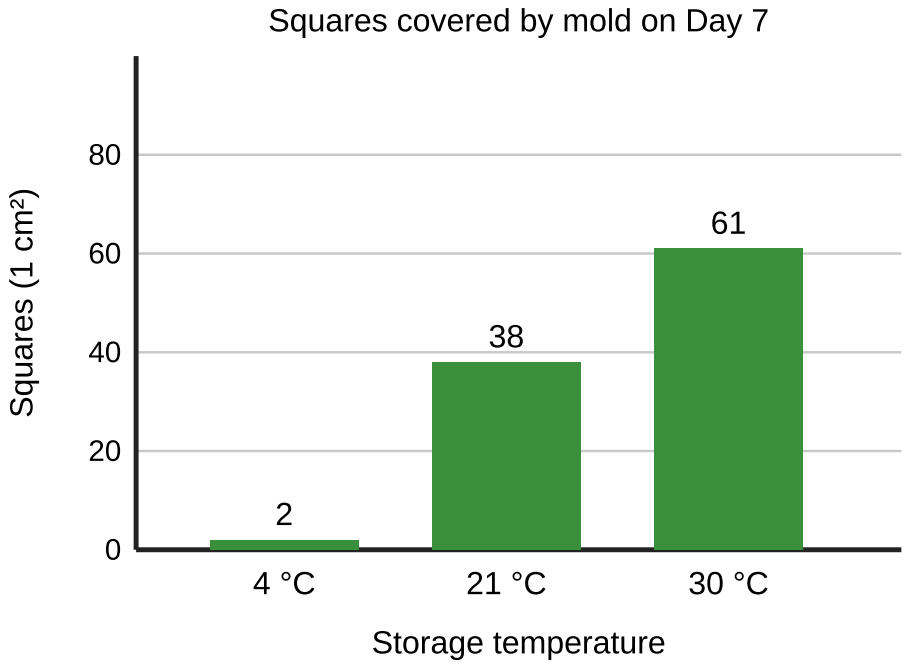
WORKED EXAMPLES

Example 1. A bread slice is traced on a 1-cm grid and fills 100 squares. On Day 5 mold covers 23 squares; on Day 7 it covers 47 squares. What percent of the slice is moldy on Day 7, and how fast did the mold spread between Day 5 and Day 7?

1. Percent covered on Day 7 = $47 \div 100 \times 100 = 47\%$.
2. Change in area = $47 - 23 = 24$ squares. Each square is 1 cm^2, so that is 24 cm^2.
3. Time = $7 - 5 = 2$ days.
4. Rate = 24 $cm^2 \div 2$ days = 12 cm^2 per day.

Answer: 47% of the slice; the mold spread about 12 cm^2 per day.

Example 2. A class kept identical bread slices at three temperatures for 7 days. The bar graph shows the squares covered (out of 100) on Day 7. Describe the pattern, find how many more squares were covered at 30 °C than at 21 °C, and state a conclusion.



Squares of bread covered by mold on Day 7 at three temperatures (class data).

1. Read the bars: 4 °C (refrigerator) = 2 squares; 21 °C (room) = 38 squares; 30 °C (warm) = 61 squares.
2. Pattern: the warmer the slice, the more mold grew, over this range of temperatures.
3. Difference: 61 − 38 = 23 more squares at 30 °C.
4. Compare cold and warm: 61 ÷ 2 ≈ 30, so the warm slice had about 30 times as much mold as the refrigerated one.
5. Notice that the refrigerated slice still grew a little mold, so cold slows mold but does not stop it.

Answer: Mold growth rose with temperature from 4 °C to 30 °C; the 30 °C slice had 23 more squares than the 21 °C slice. Conclusion: in this range, warmth speeds mold growth and refrigeration slows it greatly.

Example 3. A family compared bakery bread (no preservatives) with packaged bread (with calcium propionate). In three trials, the first visible mold appeared on these days: bakery 4, 5, 4; packaged 10, 12, 11. Find each average and compare them.

1. Bakery average = (4 + 5 + 4) ÷ 3 = 13 ÷ 3 ≈ 4.3 days.
2. Packaged average = (10 + 12 + 11) ÷ 3 = 33 ÷ 3 = 11 days.
3. Ratio = 11 ÷ 4.3 ≈ 2.5.
4. Using three trials and averaging makes one unusual slice less likely to mislead us.

Answer: Bakery bread averaged about 4.3 days and packaged bread 11 days; the bread with preservative lasted about 2.5 times as long before mold appeared.

PRACTICE

1. EASY What three things does a mold spore need to start growing?
2. EASY In black bread mold, what is the job of (a) the rhizoids and (b) the sporangia?
3. EASY What did Pier Antonio Micheli show in 1729 by placing mold spores on fresh slices of fruit?
4. MEDIUM A slice of bread covers 96 squares on a 1-cm grid. Mold covers 24 squares. What percent of the slice is moldy?
5. MEDIUM Data reading. Squares of mold on two slices: Day 3: Dry 0, Moist 4. Day 5: Dry 3, Moist 18. Day 7: Dry 9, Moist 41. Day 9: Dry 20, Moist 70. (a) On which day did mold first appear on each slice, as far as the data show? (b) Find the growth rate of the Moist slice from Day 5 to Day 9. (c) Which statement does the data best support? A. Moisture has no effect. B. Moist bread grows mold sooner and faster. C. Dry bread never grows mold.
6. MEDIUM Data reading. Days until mold appeared on strawberries stored at different temperatures: 2 °C: 9 days; 10 °C: 5 days; 20 °C: 2 days; 28 °C: 2 days. (a) Describe the trend from 2 °C to 20 °C. (b) What happens between 20 °C and 28 °C? (c) Where should strawberries be stored, and why?
7. MEDIUM Your brother finds a small green spot of mold on a loaf of bread and wants to cut that part off and eat the rest. What should you tell him, and why?
8. HARD Name two ways molds help people and two ways they harm people.
9. HARD Experiment design. A student wants to know whether vinegar slows mold on bread. Design the experiment: give the question, the independent and dependent variables, a control group, three things to keep the same and a safety rule.
10. CHALLENGE Hooke wondered whether the mold on his book cover came from the leather itself as it decayed. Explain what we now know about where it came from, and describe one observation that supports this.

COMMON MISTAKES

- Thinking mold is only the fuzz on top. The fuzz is mainly spores; the hyphae have already spread deep into soft food.
- Thinking a refrigerator kills mold. Cold only slows mold; it keeps growing slowly and grows fast again when warm.
- Thinking mold comes from the food itself. Mold grows from spores that are always floating in the air.

Make it a game · Spore Drift. One player is the sporangium and holds a handful of paper-punch dots (spores). Others hold paper plates labeled with foods: bread, cheese, a dry cracker, an apple in the fridge, a damp orange. The sporangium tosses the dots in front of a fan or by blowing. For each plate a dot lands on, the holder must say whether mold would grow fast, slowly or hardly at all, and why (food, moisture, temperature). A correct reason scores a point.

Scripture. “Lay not up for yourselves treasures upon earth, where moth and rust doth corrupt, and where thieves break through and steal:” (Matthew 6:19, KJV)

Everything on earth decays: bread molds, wood rots and iron rusts. Decomposers like molds are God’s way of recycling the world, and they also remind us to set our hearts on treasure that does not spoil.

Practice answers

1. Food (something to digest), moisture and a suitable, moderate temperature.
2. (a) Rhizoids grow down into the bread, release enzymes and absorb the digested food. (b) Sporangia make and hold spores and release them into the air.
3. That molds grow from spores: the same kind of mold grew where he sowed its spores. Mold does not simply arise from rotting food.
4. 24 ÷ 96 × 100 = 25%.
5. (a) Moist by Day 3; Dry between Day 3 and Day 5 (first counted on Day 5). (b) (70 − 18) ÷ 4 days = 13 squares per day. (c) B. Choice C is wrong because the dry slice did grow mold, only more slowly.
6. (a) The warmer the berries, the sooner mold appeared (9 days down to 2 days). (b) The time stays the same (2 days), so above about 20 °C extra warmth did not make mold appear any sooner in this test. (c) In the refrigerator, because cold slows mold growth and the berries last longest at 2 °C.
7. Throw the bread away. Bread is soft and porous, so the hyphae have already spread well beyond the visible spot, and some molds make toxins. Do not sniff it either. (Only hard cheese can be saved by cutting at least 2.5 cm around and below the mold.)
8. Helps (any two): flavoring cheeses such as Roquefort and Camembert; making soy sauce and miso; producing medicines such as penicillin; decomposing dead plants and returning nutrients to the soil. Harms (any two): spoiling food; causing allergies and asthma; making mycotoxins such as aflatoxin; damaging damp walls, books and wood.
9. Question: Does brushing bread with vinegar slow mold growth? Independent variable: vinegar or no vinegar (or different amounts). Dependent variable: squares covered by mold

each day (or days until mold first appears). Control: identical slices brushed with the same amount of plain water. Keep the same: kind and age of bread, liquid amount, air exposure time, bag type, storage place and temperature. Use at least two slices per group. Safety: seal and tape the bags and never open them once mold grows.

10. The mold grew from spores that drifted through the air and landed on the damp leather, which supplied food. Support: Micheli sowed mold spores on fresh fruit and the same kind of mold grew; also, bread sealed in a bag without exposure grows mold much more slowly, and sterilized food kept away from air (as in Pasteur’s experiments) does not spoil.

6.3 Spore Prints

OBJECTIVES

- Make a spore print from a store-bought mushroom and explain how basidia on the gills release spores that fall straight down.
- Use spore print color and other features with a dichotomous key to sort mushrooms into groups.
- Estimate very large numbers of spores by sampling a small area and scaling up, and convert between micrometers, millimeters and centimeters.

Warm-up. Hold a sheet of dark paper flat under the cap of a store mushroom whose gills are showing and tap the cap gently. Do you see anything fall? Now look at the gills with a magnifying glass and count how many gills are in a 1-centimeter stretch along the edge of the cap. Why might a mushroom have so many thin gills instead of a flat underside?

Video. Coming soon. How to make a mushroom spore print, and how mushrooms launch spores from the gills, slow-motion and microscope footage (search nature footage, Smithsonian, SciShow Kids, Homeschool Pop (Alternate), Science Max, mycological society and university extension channels).

KEY TERMS

spore print
The pattern of spores that falls from a mushroom cap onto paper placed under it. Its color is an important clue for identifying a mushroom.

gills
Thin, blade-like plates on the underside of many mushroom caps, lined with spore-making cells.

basidium (plural basidia)
A tiny club-shaped cell on the gills of most mushrooms that makes spores at its tip, usually four but in some kinds two.

pores
Tiny holes on the underside of some mushrooms, such as boletes, that open from tubes where spores are made instead of gills.

dichotomous key
An identification tool made of paired choices; at each step you pick the one that fits, until you reach a name.

micrometer (µm)
One thousandth of a millimeter. Mushroom spores are usually a few micrometers to about 20 micrometers long.

CORE IDEAS

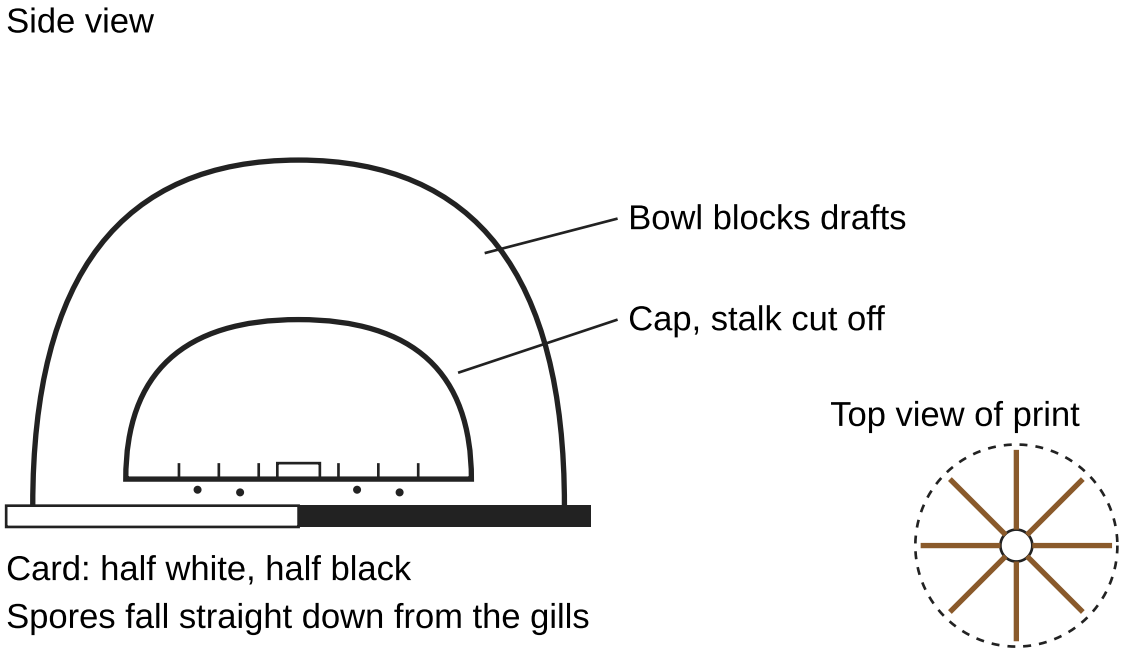
The story. Long before she wrote *The Tale of Peter Rabbit*, a young English woman named Beatrix Potter spent her summers in Scotland and the Lake District painting mushrooms. In the 1890s she made hundreds of exact watercolors of fungi, and she did not stop at drawing. With a microscope she watched spores swell and sprout threads, and she worked out how to grow them. A Scottish naturalist named Charles McIntosh, a country postman who knew the fungi of Perthshire, sent her specimens and encouraged her. Her uncle, the chemist Sir Henry Roscoe, helped her show her work to scientists at Kew Gardens in London. On April 1, 1897, her paper *On the Germination of the Spores of Agaricineae* was read at the Linnean Society of London. It was read by George Massee of Kew, because in those days women could not attend the society's meetings.

How they figured it out. Potter and other mushroom students of her day relied on a simple tool you can make at home: the **spore print**. Cut off the stalk, lay the cap gill side down on paper, cover it and wait. By the next day a pattern of fine dust lies under it, a perfect map of the gills. Under a microscope each gill is lined with millions of tiny club-shaped cells called **basidia**. Each basidium grows little prongs, and on each prong a spore forms; most mushrooms make four spores per basidium, but the grocery-store mushroom *Agaricus bisporus* makes only two, which is what *bisporus* means. When ripe, each spore is flicked off by a tiny drop of water, flies about a tenth of a millimeter into the gap between the gills, and then falls straight down out of the cap. That is why gills hang straight down and why a mushroom keeps its cap level.

The big idea. Spores are far too small to see one by one, usually just a few micrometers long, but piled by the millions they show their color. Spore print color is one of the first clues a mycologist checks. White prints come from many kinds, including shiitake and the deadly *Amanita* mushrooms you will meet in Lesson 6.4. Button, cremini and portobello mushrooms give dark chocolate-brown prints. Others print pink, rusty brown, purple-brown or black; inky caps print black. Oyster mushrooms usually print white to pale lilac. Not every mushroom has gills: boletes have **pores**, the openings of tubes, and their prints show a pattern of dots instead of lines. To sort mushrooms, scientists use a **dichotomous key**, a list of paired choices such as "print white or pale" versus "print dark," leading step by step to a name. Spore color, gill attachment, a ring, the base of the stalk, smell and where the mushroom grew all count. No single clue is ever enough to decide that a wild mushroom is safe to eat, and a key in a book is not a license to eat anything.

Try it and see. Buy a few mature mushrooms whose caps have opened so the gills show; portobellos work best. Cut off the stalk flush with the cap. Place the cap gills down on a card that is half white and half black, so both light and dark prints show up. Cover it with a bowl or cup to keep air currents from blowing the spores sideways, and leave it for 6 to 24 hours. Lift the bowl and the cap slowly and straight up. A light spray of hairspray from about 30 centimeters away will fix the print so it will not smudge. Under a microscope at 400 times, scrape a few spores onto a slide with a drop of water and look for their shape.

God’s handiwork. One large mushroom can drop billions of spores, and yet nearly all of them land where nothing grows. The Creator built fungi to sow generously so that a few reach the right damp log or patch of soil. Solomon gave the same advice for our own work: sow in the morning and in the evening, “for thou knowest not whether shall prosper, either this or that.” The quiet dust under a mushroom cap is a lesson in patience, abundance and order.



Making a spore print. Spores fall straight down from the gills and leave a radial pattern that matches the gills.

PICTURE STUDY



Hygrocybe coccinea (Scarlet Waxcap), Beatrix Potter, 1897 · Pencil and watercolor on paper · The Armitt Museum and Library, Ambleside, England

Beatrix Potter made this study of the scarlet waxcap in 1897, the same year her paper on the sprouting of spores was read at the Linnean Society. Like a scientist, she painted the mushrooms in a group as they grew, drew some cut lengthwise to show the gills and the flesh inside, and added spores drawn greatly magnified, as she saw them through her microscope.

Potter left many of her fungus paintings to the Armitt in Ambleside, near her Lake District farm, and mushroom experts still admire them for their accuracy. She was not trying to make the mushrooms cute, as she did with her rabbits and mice. She was trying to make them true.

Look closely

- Find the mushrooms drawn whole. What color are the caps, and how many can you count?
- Find a mushroom drawn cut in half. What can you see inside that you could not see from the outside?
- Look for the tiny drawings of spores. Why would Potter draw them so much larger than the mushrooms?
- Look at the ground around the base. What clues did Potter include about where the mushrooms grew?

Talk about it

1. Why would a scientist want a painting to show a mushroom whole, cut open and magnified?
2. How might Potter's careful mushroom paintings have helped her draw animals so well in her later books?
3. This waxcap is bright red. Using what you will learn in Lesson 6.4, explain why its color tells you nothing about whether it is safe.

> **Safety first.** Use store-bought mushrooms for spore prints. If an adult helps you make a print from a wild mushroom, use it only for study: wear gloves or wash your hands well afterward, keep it away from small children and pets, throw it in the trash when done, and never taste it. Spray hairspray or fixative only outdoors or in a well-ventilated room, away from your face.

EXPERIMENT: THE SPORE PRINT TEST

You will make several spore prints and test one factor that might change how good a print is.

Question: Does covering the cap with a bowl give a sharper, darker spore print? **Prediction:** Write yours with a reason. **What we change:** covered or uncovered. **What we keep the same:** kind and size of mushroom, how open the caps are, paper, room and time. **What we measure:** how sharp the gill lines are (rate 1 to 5) and how dark the print is (rate 1 to 5), plus the diameter of the print in millimeters.

You will need

- 4 portobello or open-capped cremini mushrooms of about the same size, plus 1 shiitake or oyster mushroom if you can find it
- Card stock or index cards, half colored black with marker (or half white and half black paper taped together)
- 2 bowls or cups large enough to cover a cap
- Knife (with an adult), ruler, magnifying glass, lab notebook
- Optional: hairspray, a microscope, slides and cover slips

Steps

1. Cut the stalks off flush with the caps. Lay each cap gill side down across the line between the white and black halves of a card.
2. Cover two caps with bowls. Leave two uncovered, side by side with the covered ones, in a still room away from fans and pets.
3. Put the shiitake or oyster cap on its own card, covered, so you can compare spore colors.
4. Wait 12 to 24 hours. Lift each cap slowly straight up.
5. Measure each print's diameter in millimeters. Rate sharpness and darkness from 1 (faint, blurry) to 5 (dark, crisp lines). Record in a table.

6. Write the spore color of each kind of mushroom on its card. Which half of the card shows it best?
7. If you have a microscope, touch a damp cover slip to the print, place it on a slide with a drop of water and draw five spores at 400 times. Describe their shape.
8. Fix your best print with a light spray of hairspray and glue it into your notebook.

Tips

- Older mushrooms with fully open caps drop far more spores than tight young buttons.
- If the cap dries out, add one drop of water on top before covering it.
- Rating scales are a real scientific tool when you cannot measure something with a ruler, but use the same rules for every print and, if you can, have a second person rate them without knowing which were covered.

Check your work

- I made at least four spore prints and recorded their sizes and ratings.
- I compared covered and uncovered prints fairly.
- I recorded the spore print color of each kind of mushroom.
- I can explain why the print matches the pattern of the gills.

NATURE NOTEBOOK

1. Glue in your best spore print and label the gill lines, the bare center where the stalk was, and the spore color.
2. Draw a single gill as if magnified 1,000 times, showing basidia with four little spores (and one with two, for *Agaricus bisporus*).
3. Copy the dichotomous key from this lesson and add one more mushroom from a field guide picture.

> **From history.** In 1909 Reginald Buller, a botanist at the University of Manitoba in Winnipeg, Canada, published the first volume of his *Researches on Fungi*. Buller turned a microscope on its side so he could watch spores leave the gills and fall, and he measured how mushrooms keep their gills pointing straight down. Buller also saw and described the tiny water drop that forms at the base of each spore just before it is launched; later scientists named it “Buller’s drop” in his honor.

WORKED EXAMPLES

Example 1. Under a microscope, a student counts 150 spores in one tiny square of a spore print that measures 0.1 mm by 0.1 mm. The whole print covers about 20 cm². Estimate how many spores are in the print.

1. Area of the small square: 0.1 mm × 0.1 mm = 0.01 mm².
2. Convert the print area to mm²: 1 cm² = 100 mm², so 20 cm² = 2,000 mm².
3. Number of small squares in the print: 2,000 mm² ÷ 0.01 mm² = 200,000 squares.
4. Total spores ≈ 150 × 200,000 = 30,000,000.
5. This counts only the spores that landed on the paper; many others drifted onto the bowl or stayed on the gills, so it is a low estimate.

Answer: About 30 million (3×10^7) spores on the paper.

Example 2. A portobello spore is about 6 µm long. (a) How many spores laid end to end would make a line 1 cm long? (b) If a drawing shows the spore magnified 1,000 times, how long is it in the drawing?

1. 1 cm = 10 mm = 10,000 µm.
2. (a) 10,000 µm ÷ 6 µm ≈ 1,667, so about 1,700 spores.
3. (b) 6 µm × 1,000 = 6,000 µm = 6 mm.

Answer: (a) About 1,700 spores. (b) 6 mm long in the drawing.

Example 3. Use this key to name a store mushroom with a white spore print, a brown cap on a centered stalk, and gills that stop where they meet the stalk. Key: 1a. Spore print dark chocolate brown → *Agaricus* (button, cremini, portobello). 1b. Spore print white or pale → go to 2. 2a. Gills run down onto the stalk; stalk short and off-center → oyster mushroom. 2b. Gills stop at the stalk; stalk centered → shiitake.

1. Step 1: The print is white, so choose 1b and go to 2.
2. Step 2: The gills stop at the stalk and the stalk is centered, so choose 2b.
3. The key ends at shiitake.

Answer: Shiitake. (This key works only for these three store mushrooms. It cannot be used to decide whether any wild mushroom is safe.)

PRACTICE

1. EASY Why do we put a mushroom cap on paper that is half white and half black when making a spore print?

2. EASY What are basidia, and how many spores does each usually make?
3. EASY What spore print color do you expect from a portobello? From a shiitake?
4. MEDIUM A spore is 8 µm long. How long would it look in a drawing magnified 500 times, in millimeters?
5. MEDIUM Data reading. A student made prints from four portobellos and left them for different times. Print darkness (1 to 5): 2 hours, 1; 4 hours, 2; 8 hours, 3; 12 hours, 4; 24 hours, 4. (a) What is the independent variable? (b) Describe the trend. (c) What is the shortest time in this test that gave the darkest print?
6. MEDIUM Data reading. Results for covered and uncovered prints: Covered: diameter 92 mm, sharpness 5, darkness 4. Uncovered: diameter 104 mm, sharpness 2, darkness 3. The caps were the same size. (a) Which print spread wider, and why might that be? (b) Which print would be more useful for identification? Explain.
7. MEDIUM Explain why a mushroom's gills must hang straight down for its spores to escape.
8. HARD A tiny square 0.1 mm by 0.1 mm of a spore print contains 80 spores. The print covers 15 cm². Estimate the number of spores on the paper.
9. HARD Experiment design. Does the age of a mushroom (tight closed cap versus fully open cap) change how many spores it drops? Describe a fair experiment, including how you will measure the result without counting millions of spores.
10. CHALLENGE A friend says, "My wild mushroom gave a brown spore print just like a store portobello, so it must be safe to eat." Explain why this reasoning is wrong.

COMMON MISTAKES

- Thinking spores are seeds. A seed holds a tiny plant with stored food inside a coat; a spore is a single cell with no embryo.
- Thinking the spore print shows the cap color. The print shows the spore color, which is often very different from the cap.
- Thinking a spore print or a key can prove a wild mushroom is safe to eat. Only many features together identify a mushroom, and no one should eat wild mushrooms without expert knowledge.

> **Make it a game · Key Detective.** A parent lays out pictures or real samples of store mushrooms (button, cremini, portobello, shiitake, oyster, enoki) along with their spore prints mixed up. Take turns matching each print to its mushroom and reading the key step by step aloud. Each correct match scores a point; reading the key without skipping a step earns a bonus.

> **Scripture.** "In the morning sow thy seed, and in the evening withhold not thine hand: for thou knowest not whether shall prosper, either this or that, or whether they both shall be alike good." (Ecclesiastes 11:6, KJV)

A mushroom scatters spores by the million because only a few will land in the right place. Solomon tells us to work and give generously in the same way, trusting God with which

efforts will prosper.

Practice answers

1. So that both light (white or cream) spores and dark spores show up clearly on one card.
2. Basidia are tiny club-shaped cells on the gills that make spores at their tips; most make four spores each (*Agaricus bisporus* makes two).
3. Portobello: dark chocolate brown. Shiitake: white.
4. 8 µm × 500 = 4,000 µm = 4 mm.
5. (a) The time the cap was left on the paper. (b) Darkness rose steadily from 2 to 12 hours, then leveled off; leaving it from 12 to 24 hours gave no darker print. (c) 12 hours.
6. (a) The uncovered print, because moving air blew some falling spores sideways past the edge of the cap. (b) The covered print, because its gill lines are sharp and its color is darker and easier to judge.
7. Each spore is shot only about a tenth of a millimeter sideways into the gap between gills and then falls by gravity. If the gills were tilted, the falling spores would hit the next gill and stick instead of dropping out below the cap.
8. Small square = 0.01 mm². Print = 15 × 100 = 1,500 mm². Squares = 1,500 ÷ 0.01 = 150,000. Spores ≈ 80 × 150,000 = 12,000,000 (12 million).
9. Use mushrooms of the same kind and similar cap size, some closed and some fully open (at least three of each). Independent variable: cap age (closed or open). Keep the same: paper, bowl covering, room, time (for example 12 hours). Dependent variable: spore amount, measured by counting spores in several 0.1 mm squares under a microscope and scaling up by print area, or by rating darkness on a 1 to 5 scale. Average each group and compare.
10. Spore print color is only one clue and many different mushrooms share the same color, including some that are poisonous. Identification needs many features checked together by an expert, and even then the course rule stands: never eat a wild mushroom. Matching one feature proves nothing about safety.

6.4 Which Are Poisonous (Never Eat Wild Ones)

OBJECTIVES

- State the safety rule for wild mushrooms and explain why no folk test, color rule or single feature can show that a wild mushroom is safe.
- Describe the features of deadly *Amanita* mushrooms and explain how amatoxins damage the liver after a delay of many hours.
- Use time, percent and body-mass calculations to explain why mushroom poisoning is so dangerous, especially for children.

Warm-up. Your parent writes five “rules” on cards: (1) Bright-colored mushrooms are poisonous and dull ones are safe. (2) If a squirrel eats it, it is safe. (3) Cooking makes any mushroom safe. (4) A mushroom that peels easily is safe. (5) Never eat a wild mushroom. Sort the cards into “true” and “false.” Keep your sorting and check it at the end of the lesson.

Video. Coming soon. Poisonous mushrooms and look-alikes: the death cap and destroying angel, why you should never eat wild mushrooms (search university extension services, poison control centers, US Forest Service, mycological society channels, Smithsonian, nature footage).

KEY TERMS

toxin
A poison made by a living thing.

amatoxin
A group of deadly toxins found in death caps, destroying angels and some other mushrooms. Amatoxins stop cells from making proteins and destroy the liver. Cooking, freezing and drying do not remove them.

Amanita
A group (genus) of mushrooms with white spore prints, often a ring on the stalk and a cup at the base. It includes the deadliest mushrooms in the world, such as the death cap.

volva
A cup or sac around the base of the stalk of an *Amanita*, left from the “egg” the young mushroom grew in. It is often buried in soil or leaves.

look-alike
A poisonous mushroom that closely resembles an edible one, such as the jack-o’-lantern mushroom and the chanterelle.

mycologist
A scientist who studies fungi.

CORE IDEAS

The story. In the year AD 54 the Roman emperor Claudius died in Rome after a dinner. The Roman historians Tacitus and Suetonius, writing some decades later, say he was poisoned with a dish of mushrooms, a food he loved. Whether poison was added to the mushrooms or the mushrooms themselves were deadly, no one today can be sure, and some modern writers have suggested a death cap. What is certain is that the ancient world already knew this hard truth: a mushroom that looks like dinner can kill. Nearly two thousand years later, mushroom poisonings still happen every year, and many of them begin with someone who was sure they knew what they had picked.

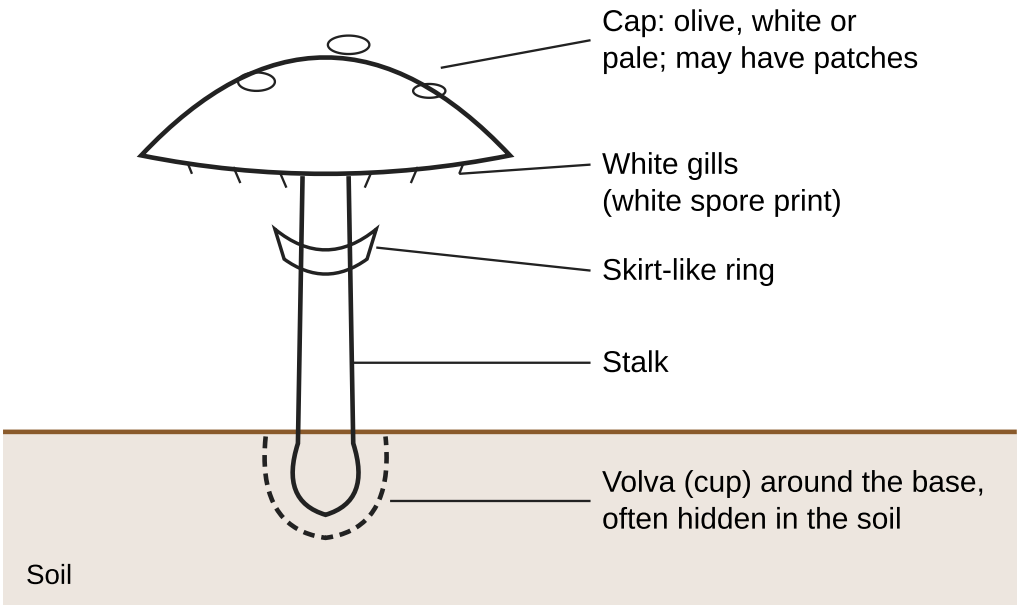
How they figured it out. In the 1900s chemists and doctors studied the death cap, *Amanita phalloides*, to learn why it is so deadly. The German chemist Heinrich Wieland and his students worked out a group of toxins in it, now called **amatoxins**. These poisons block an enzyme that every cell needs to read its DNA and build proteins. The cells that take in the most poison, especially liver cells, stop working and die. Doctors also noticed a cruel pattern. Symptoms usually begin about 6 to 24 hours after the meal, long after the mushrooms are digested: severe vomiting and diarrhea, then a day or so when the person may seem to get better, while the liver is being destroyed. Mushroom experts estimate that amatoxin mushrooms cause roughly nine out of ten deaths from mushroom poisoning worldwide.

The big idea. A deadly *Amanita* often looks clean and ordinary. The death cap has a smooth cap that is often greenish or yellowish olive but can be pale, white **gills**, a white spore print, a skirt-like **ring** on the stalk and a cup-shaped **volva** at the base, often hidden under soil or leaves. Destroying angels are pure white all over. Other mushrooms are dangerous too: a small brown mushroom on wood called *Galerina* carries the same amatoxins; the orange **jack-o'-lantern mushroom** is a **look-alike** of the edible chanterelle and causes severe vomiting; false morels can be deadly; and the red-and-white fly agaric is poisonous as well. None of the folk tests work. Color does not tell you: the deadliest kinds are plain. Animals do not tell you: slugs, insects and squirrels nibble mushrooms that would poison a person. Peeling, smell, taste, a silver spoon or cooking do not tell you or fix it. Even trained **mycologists** study for years and still check many features together.

In the world today. So the rule in this course, and in every safe family, is simple: **never eat or taste a wild mushroom.** Not a nibble, not a lick, not a "test bite." You may look, sketch, photograph, measure and make spore prints with an adult, then wash your hands. Keep little brothers and sisters and pets away from lawn mushrooms, and knock them over if small children play there. If anyone swallows part of a wild mushroom, call Poison Control right away at 1-800-222-1222 (in the United States), even if the person feels fine, and save the rest of the mushroom in a paper bag so an expert can identify it. Waiting for symptoms is the most dangerous choice of all, because with amatoxins the first signs come late.

God's handiwork. Poisonous mushrooms are not mistakes in the woods. The death cap is a partner of oak trees, trading minerals for sugar like other mycorrhizal fungi, and like all mushrooms it helps recycle the forest. God gave people dominion over the earth, and wise dominion includes knowing what not to touch. Proverbs says, "A prudent man foreseeth the

evil, and hideth himself; but the simple pass on, and are punished.” Long ago, a man in Elisha’s day gathered wild gourds “for they knew them not,” and the prophets cried out, “there is death in the pot.” A prudent naturalist admires the wild mushroom, records it carefully and leaves it on the ground.



Warning features of deadly Amanita mushrooms. The volva is often hidden in the soil, which is one reason a cut-off mushroom is so easy to misidentify.

PICTURE STUDY



Fly Agaric (Agaricus muscarius), from Coloured Figures of English Fungi or Mushrooms, James Sowerby, 1797–1803 (published in parts) · Hand-colored engraving · Published in London; copies in natural history libraries, with scanned copies online

James Sowerby was an English natural history artist who drew and engraved hundreds of plates of British plants, shells, minerals and fungi. His *Coloured Figures of English Fungi or Mushrooms* came out in parts over many years and showed each fungus life size, often from several angles and cut open. In his day the fly agaric was still placed in the large old genus *Agaricus*; mycologists now call it *Amanita muscaria*.

The fly agaric is the bright toadstool of fairy-tale pictures. It is poisonous, and it is a cousin of the plain-looking death cap, which is far deadlier.

Look closely

- Find the white warts on the red cap. These are bits of the veil that once wrapped the whole young mushroom.
- Look under the cap. What color are the gills?
- Find the ring on the stalk and the swollen base. Which features does this mushroom share with the death cap in this lesson’s diagram?
- Count the mushrooms Sowerby drew. Did he show them at different ages or from different sides? Why would that help a reader?

Talk about it

1. The fly agaric is bright and the death cap is dull. What does that tell you about the rule “bright mushrooms are poisonous, dull ones are safe”?
2. How could careful drawings like Sowerby’s help keep people safe?

> **Safety first.** Never eat, taste or lick any wild mushroom, and never let a younger child or pet do so. You may look at, photograph and sketch wild mushrooms; if you touch one, wash your hands with soap afterward. If anyone swallows part of a wild mushroom, call Poison Control at 1-800-222-1222 (US) right away, even with no symptoms, and keep the rest of the mushroom for identification. For this lesson's project, use only store-bought mushrooms and pictures.

EXPERIMENT: MYTH-BUSTER: DO FOLK TESTS WORK?

People have long believed in "tests" for poisonous mushrooms. You will test two of them safely, using only store-bought mushrooms that we know are edible, and see whether the tests could ever tell edible from poisonous.

Question: Do the "peel test" and the "silver spoon test" tell us anything about whether a mushroom is safe? **Prediction:** Write yours. **What we change:** the kind of store mushroom. **What we keep the same:** how we peel, how long the spoon stays in, the amount of water. **What we measure:** whether the cap peels, and whether the spoon darkens. Then we compare with what mycologists know about the death cap.

You will need

- Three or four kinds of store mushrooms (button, cremini, shiitake, oyster)
- A real silver or silver-plated spoon, or a stainless steel spoon if you have no silver
- A small pot, water and a stove (with an adult)
- Lab notebook, ruler, colored pencils
- Pictures of death caps and destroying angels from a field guide or library book

Steps

1. Make a data table with columns: Mushroom, Edible? (all yes for store mushrooms), Peels easily?, Spoon darkened?
2. Try to peel the skin from each cap, starting at the edge. Record yes or no.
3. With an adult, simmer a few slices of each mushroom in 250 mL of water with the spoon in it for 10 minutes. Let it cool and look at the spoon. Record any change.
4. Read in your field guide or this lesson whether death caps peel easily. (They do.) Add a row for the death cap from your reading: Edible? No. Peels easily? Yes.
5. Look at your table. Did either test separate the edible mushrooms from the deadly one?
6. Draw the death cap from a picture and label its cap, white gills, ring and volva.
7. Write a conclusion of at least three sentences: what your data show about folk tests, and the one rule that really keeps people safe.

Tips

- A test is useful only if it gives different results for the things you want to tell apart. If edible and deadly mushrooms both pass, the test is worthless.

- Silver darkens from sulfur compounds that appear in many foods, such as eggs, so a dark spoon tells you nothing about amatoxins.

Check your work

- I used only store-bought mushrooms and pictures.
- My data table is complete for each kind of mushroom.
- I explained why the peel test and the spoon test cannot tell edible from deadly mushrooms.
- I can state the safety rule and the Poison Control number.

NATURE NOTEBOOK

1. Draw a death cap and label the four warning features: pale or greenish cap, white gills, ring and volva. Write “NEVER EAT WILD MUSHROOMS” across the top.
2. Make a two-column chart of look-alikes: chanterelle and jack-o’-lantern, true morel and false morel. Note one difference for each pair from a field guide, and add “Only an expert can tell, and even then, do not eat.”
3. Write the Poison Control number and the three things to do if someone swallows a wild mushroom, and post the page by your family’s phone.

> **From history.** The name “fly agaric” comes from an old European custom: pieces of the red-and-white mushroom were crumbled into a saucer of milk to attract and stun flies. The German scholar Albertus Magnus mentioned this use in the 1200s, and the fly agaric’s scientific name, *Amanita muscaria*, still carries the Latin word for fly, *musca*.

WORKED EXAMPLES

Example 1. A family eats a wild mushroom dish at 6:30 pm. Amatoxin symptoms usually begin 6 to 24 hours after eating. Between what times would the first symptoms most likely start? What does this mean for the family?

1. Earliest: 6:30 pm + 6 hours = 12:30 am (just after midnight).
2. Latest: 6:30 pm + 24 hours = 6:30 pm the next evening.
3. So the family could feel perfectly well through the night and most of the next day.
4. Because the toxin is already working while they feel fine, they should call Poison Control right after eating an unknown wild mushroom, not wait for symptoms.

Answer: Between about 12:30 am and 6:30 pm the next day. Feeling fine for hours proves nothing; call Poison Control at once.

Example 2. A made-up practice table from a poison center lists 40 mushroom calls in one season. Symptoms began within 6 hours in 30 cases and after more than 6 hours in 10 cases. Doctors use the rule that late symptoms are a warning sign of the most dangerous mushrooms. What percent of cases were late-onset? What fraction?

1. Late-onset cases = 10 out of 40.
2. Fraction = 10/40 = 1/4.
3. Percent = 10 ÷ 40 × 100 = 25%.

Answer: 25%, or one case in four, had late symptoms, the pattern doctors take most seriously.

Example 3. Medical sources often give the deadly amount of amatoxin as about 0.1 mg for each kilogram of body mass. How much amatoxin could be deadly for a 40 kg child and for an 80 kg adult? Why are children at greater risk?

1. Child: 40 kg × 0.1 mg/kg = 4 mg.
2. Adult: 80 kg × 0.1 mg/kg = 8 mg.
3. Ratio: 8 mg ÷ 4 mg = 2, so the child is harmed by half the amount.
4. A single death cap can hold more than enough amatoxin for either one, and a child can be poisoned by a smaller piece.

Answer: About 4 mg for the child and 8 mg for the adult. Because the dangerous dose depends on body mass, a smaller body needs less poison to be harmed.

PRACTICE

1. EASY State the course safety rule for wild mushrooms in one sentence.
2. EASY Name four features of a death cap.
3. EASY Which organ do amatoxins damage most, and do cooking or freezing destroy them?
4. MEDIUM Someone eats a mushroom at noon and the first symptoms start at 10:00 the next morning. How many hours later is that, and does it fit the amatoxin pattern?
5. MEDIUM Data reading. A made-up practice table shows mushroom poisoning calls to a poison center by month: January 2, April 5, July 18, September 40, October 35, December 3. (a) Which month had the most calls? (b) How many times as many calls were there in September as in April? (c) Suggest a reason for the pattern.
6. MEDIUM Data reading. A field guide compares two mushrooms. Chanterelle: grows singly or scattered on the ground; ridges under the cap that are blunt and forked; flesh inside pale. Jack-o’-lantern: grows in tight clusters on wood or buried roots; true sharp-edged gills; flesh inside orange. (a) List two differences. (b) Why does this table still not make it safe for a beginner to eat a chanterelle found in the woods?

7. MEDIUM Explain why the rule “if animals eat it, it is safe for people” fails.
8. HARD A 30 kg child and a 75 kg adult each eat the same piece of a mushroom containing 5 mg of amatoxin. Using 0.1 mg per kg as the dangerous amount, who is at deadly risk?
9. HARD Experiment design. A neighbor says, “Poisonous mushrooms turn a silver coin black when cooked together.” Without anyone eating anything, design a fair test of this claim using mushrooms that experts have already identified.
10. CHALLENGE Your cousin feels sick 10 hours after eating wild mushrooms, then says the next day, “I’m better now, so I’m fine.” Explain using the science of amatoxins why this is dangerous thinking and what should have been done.

COMMON MISTAKES

- Thinking poisonous mushrooms are always brightly colored. The deadliest ones, the death cap and destroying angel, are plain green-olive or white.
- Thinking cooking, drying or freezing makes a poisonous mushroom safe. Amatoxins survive all of these.
- Thinking that if you feel fine a few hours after eating, the mushroom was safe. Amatoxin symptoms are delayed, and a false recovery can follow.

Make it a game · Safe or Sorry?. A parent reads situations aloud: “Your little brother puts a lawn mushroom in his mouth.” “You find a beautiful white mushroom in the park.” “A friend says a mushroom is safe because it smells nice.” “You want to make a spore print of a wild mushroom.” Give the safe action in ten seconds (for example: take it out, call Poison Control, keep the mushroom, wash hands, look and sketch only). Score a point for each correct action and a bonus for naming the science reason.

Scripture. “So they poured out for the men to eat. And it came to pass, as they were eating of the pottage, that they cried out, and said, O thou man of God, there is death in the pot. And they could not eat thereof.” (2 Kings 4:40, KJV)

During a famine, one of the sons of the prophets gathered wild gourds, “for they knew them not” (verse 39), and poisoned the stew. Wild foods we do not truly know can bring death to the table. Wisdom means leaving the wild mushroom where it grows.

Practice answers

1. Never eat or taste any wild mushroom.
2. A smooth cap that is often greenish or yellowish olive (but can be pale), white gills, a white spore print, a skirt-like ring on the stalk and a cup-shaped volva at the base (any four).
3. The liver. No; cooking, freezing and drying do not destroy amatoxins.
4. 22 hours. Yes; it falls inside the 6 to 24 hour delay typical of amatoxin poisoning, which is a dangerous sign.
5. (a) September. (b) 40 ÷ 5 = 8 times as many. (c) Most wild mushrooms fruit in late summer and fall after rains, so more people find and pick them then; few mushrooms appear in cold winter months.

6. (a) Any two: clusters on wood versus single on the ground; true gills versus blunt forked ridges; orange versus pale inner flesh. (b) Features vary with age, weather and damage, wood can be buried so the cluster looks like it grows from soil, and a beginner can misjudge; mistakes can mean serious poisoning. Only experts identify mushrooms for eating, and our rule is never eat wild mushrooms.
7. Different animals have different bodies; slugs, insects and squirrels can eat mushrooms that would poison a person. What an animal can digest safely tells you nothing about what a human can.
8. Child: 30 × 0.1 = 3 mg is dangerous, and 5 mg is more than that, so the child is at deadly risk. Adult: 75 × 0.1 = 7.5 mg; 5 mg is below that estimate, but it is still serious poisoning that needs emergency care. Both must call Poison Control at once.
9. Get mushrooms already identified by a mycologist: several known edible kinds and several known poisonous kinds (handled with gloves by an adult or expert). Independent variable: edible or poisonous. Cook each with an identical silver coin in the same amount of water for the same time. Dependent variable: whether the coin darkens (rate 0 to 3). Repeat with several samples of each kind. If both edible and poisonous mushrooms darken the coin (or neither does), the test cannot tell them apart and the claim is false. No one tastes any sample.
10. Amatoxin poisoning often follows a pattern: symptoms start after a long delay, then the person may seem to recover for a day or so while the toxin keeps destroying liver cells. Feeling better does not mean the poison is gone. Poison Control should have been called right after the meal (or at once when symptoms began), the leftover mushrooms saved for identification, and medical care sought immediately.

Unit 6 review

Answer each question. Show your work for calculations, include units, and use complete sentences for explanations. The test is worth 60 points.

1. (4 pts) Multiple choice. A mushroom is best described as: (A) the root of a fungus; (B) the fruiting body of a fungus that releases spores; (C) a small plant without leaves; (D) the whole fungus.
2. (5 pts) Give three reasons fungi are placed in their own kingdom instead of with plants.
3. (6 pts) Diagram. Draw a gilled mushroom standing on the ground with the soil below shown as if it were glass. Label six parts: cap, gills, ring, stalk, hyphae and mycelium. Draw an arrow showing where spores are made.
4. (5 pts) A mushroom cap measures 15 mm on Monday morning and 51 mm on Thursday morning. What was its average growth rate in mm per day?
5. (4 pts) Multiple choice. Which slice of bread will most likely grow mold fastest? (A) dry bread in a refrigerator; (B) moist bread in a refrigerator; (C) dry bread at room temperature; (D) moist bread at room temperature.
6. (6 pts) Data reading. Squares of mold (1 cm² each) on a slice: Day 2, 0; Day 4, 6; Day 6, 20; Day 8, 44. (a) Find the growth rate from Day 4 to Day 8. (b) The slice fills 110 squares. What percent was moldy on Day 8? (c) Was growth steady, speeding up or slowing down? Use numbers.
7. (6 pts) Design an experiment to test whether salt slows mold growth on cut potato slices. Name the independent variable, dependent variable, control group, two things to keep the same and one safety rule.
8. (4 pts) Short answer. Name one helpful mold and what people use it for, and explain why moldy bread should be thrown away rather than trimmed.
9. (5 pts) List the steps to make a spore print from a store mushroom, and explain why the cap is covered with a bowl.
10. (6 pts) A 0.1 mm × 0.1 mm square of a spore print holds 120 spores. The print covers 25 cm². Estimate the number of spores on the paper. Show each step.
11. (4 pts) Multiple choice. A mushroom gives a dark chocolate-brown spore print. Using the key from Lesson 6.3, it belongs to: (A) shiitake; (B) oyster mushroom; (C) *Agaricus*, such as a cremini or portobello; (D) *Amanita*.
12. (5 pts) A child eats part of a wild mushroom at 4:00 pm and feels fine at bedtime. (a) Using the 6 to 24 hour delay of amatoxins, between what times might symptoms begin? (b) What should the family do, and why should they not wait? (c) State the rule for wild mushrooms.

Unit project: my fungus field book

Make a small bound field book of the fungi you studied. Include your labeled mushroom and mycelium drawing, your bread-mold experiment (question, data table, line graph and conclusion), at least two fixed spore prints with their colors and sizes, a dichotomous key for the store mushrooms you tested, and at least three dated outdoor sketches of wild fungi with

location, what they grew on and measurements. End with a safety page: a labeled drawing of a deadly *Amanita*, the folk-test myths you busted, and the Poison Control number, with the rule written large: never eat a wild mushroom.

- **Accuracy**: Drawings are labeled correctly (cap, gills, ring, stalk, hyphae, mycelium, sporangia, volva), and science explanations are correct.
- **Data and math**: Mold data, spore estimates and measurements are complete, use metric units, are calculated correctly and are graphed with titles, labeled axes and keys.
- **Experiment design**: Each experiment states a question, prediction, independent and dependent variables and the things kept the same, and the conclusion uses the data.
- **Safety and care**: The safety page is clear and complete, observations of wild fungi were made without eating or tasting, and the field book is neat, dated and easy to read.

Answer key

1. (B) The fruiting body of a fungus that releases spores.
2. Any three: no chlorophyll, so they cannot make food by photosynthesis; cell walls of chitin rather than cellulose; food stored as glycogen rather than starch; they digest food outside their bodies and absorb it.
3. A drawing showing the cap on top, gills under the cap, a ring on the stalk, the stalk down to the soil line, and branching threads in the soil labeled hyphae, with the whole network labeled mycelium. The arrow points to the gills. One point per correctly placed label (6 points).
4. Growth = 51 − 15 = 36 mm over 3 days. Rate = 36 ÷ 3 = 12 mm per day.
5. (D) Moist bread at room temperature, because it has food, moisture and warmth.
6. (a) (44 − 6) ÷ 4 days = 9.5 cm^2 per day. (b) 44 ÷ 110 × 100 = 40%. (c) Speeding up: it grew 6, then 14, then 24 squares in each 2-day period.
7. Independent: salt or no salt (or amount of salt) sprinkled on the slices. Dependent: squares of mold (or days until mold appears). Control: identical slices with no salt. Keep the same (any two): potato kind and slice thickness, air exposure, bag type, temperature and storage place, number of slices. Safety: seal and tape the bags and never open them once mold grows. (Repeating with several slices per group earns full credit.)
8. Example: *Penicillium roqueforti* for blue cheese, *Penicillium camemberti* for Camembert, *Aspergillus oryzae* for soy sauce and miso, or the *Penicillium* that gave penicillin. Bread is soft, so the hyphae have spread far beyond the visible spot, and some molds make toxins.
9. Choose a mature mushroom with open gills; cut off the stalk; place the cap gill side down on paper that is half white and half black; cover with a bowl; wait 6 to 24 hours; lift the cap straight up. The bowl blocks air currents so the spores fall straight down and make a sharp print.
10. Small square = 0.01 mm^2. Print = 25 × 100 = 2,500 mm^2. Number of squares = 2,500 ÷ 0.01 = 250,000. Spores ≈ 120 × 250,000 = 30,000,000 (30 million).
11. (C) *Agaricus*. Shiitake, oyster and *Amanita* all give white or pale prints.
12. (a) Between 10:00 pm that night and 4:00 pm the next day. (b) Call Poison Control (1-800-222-1222 in the US) right away and save the rest of the mushroom for identification, because

amatoxins damage the liver before symptoms appear and feeling fine proves nothing. (c) Never eat or taste a wild mushroom.

UNIT 7

Animal Physiology

Every cell in an animal's body needs a steady supply of oxygen for cellular respiration, the process you studied in Unit 5. A single cell living in pond water can simply let oxygen diffuse in through its membrane. A trout, a goose, a beetle or a giraffe cannot; its cells are packed deep inside a large body. **Physiology** is the study of how the parts of a living body work, and in this unit you will see four very different solutions to the same problem: how to get oxygen in and move it to every cell.

You will follow Robert Boyle's air pump in 1670 as it draws hidden air out of water, and learn how a fish's gills pull that air back out with countercurrent flow. You will read John Hunter's 1774 report on the air sacs and hollow bones of birds, and trace a breath through a bird's one-way lungs to the bar-headed geese that cross the Himalayas. You will open a silkworm with Marcello Malpighi in 1669 to find the air tubes of insects, and you will measure blood pressure with the scientists who studied the giraffe's long neck and tight-skinned legs.

Along the way you will calculate ratios, percents, rates and pressures, read graphs of dissolved oxygen and air pressure, and run small experiments of your own with warm water, paper bones, mealworms and a leaking bottle. In each lesson ask the question a physiologist asks: **what problem does this body face, and how is it built to solve it?** Unit 8 carries these ideas into the dissection lab.

BEFORE YOU BEGIN

- Knows that cells use oxygen and give off carbon dioxide in cellular respiration (Unit 5)
- Can compute a percent, a ratio and a rate per minute
- Can read a bar graph and a line graph and name independent and dependent variables
- Can use a magnifying glass and record observations in a lab notebook

MASTERY CHECKLIST

- I can trace the path of water through a fish's gills and explain countercurrent exchange.
- I can trace one puff of air through a bird's air sacs and lungs in two breaths and explain why the airflow is one-way.
- I can describe the insect body plan and explain how spiracles, tracheae and molting work.
- I can calculate how gravity changes blood pressure with height and explain how a giraffe's body handles it.
- I can read graphs of dissolved oxygen and air pressure and design a fair experiment about an animal's body.

OPTIONAL BOOKS AND RESOURCES

- **All About Birds, from the Cornell Lab of Ornithology** (https://www.allaboutbirds.org/): Free. Photos, sounds and life histories of North American birds. Use it to identify the birds you sketch for Lesson 7.2 and to read about geese, flamingos and other birds in this unit.
- **Giraffe page, Smithsonian's National Zoo and Conservation Biology Institute** (https://nationalzoo.si.edu/animals/giraffe): Free. Facts, photos and care notes about giraffes from a zoo that keeps them. Good background for Lesson 7.4.
- **Jean-Henri Fabre's insect books (free at Project Gutenberg)** (https://www.gutenberg.org/ebooks/search/?query=Fabre): Free. English translations of Fabre's classic insect studies, such as *The Life of the Grasshopper* and *The Life of the Caterpillar*. Read a chapter aloud alongside Lesson 7.3.

7.1 How a Fish Breathes ★

OBJECTIVES

- Tell how Robert Boyle's air-pump experiments of 1670 helped show that water holds dissolved air, and explain why a fish must pass a great deal of water over its gills to get enough oxygen.
- Name and describe the parts of a fish's breathing system (mouth, gill arches, filaments, lamellae, operculum) and trace the path of water through them.
- Explain countercurrent exchange with numbers, and read a graph showing that cold water holds more dissolved oxygen than warm water.

Warm-up. Fill a clear glass with cold tap water and set it on the counter. Look at it closely now, then again at the end of the lesson. Tiny bubbles will gather on the inside of the glass as the water warms. Where did that air come from? Write a one-sentence guess. Then hold your breath for a few seconds and ask: if you had to get your air out of that glass of water instead of the room, how would you do it?

Video. Coming soon. How fish gills work and countercurrent exchange for middle school; search Smithsonian, NOAA Fisheries, aquarium channels (such as Monterey Bay Aquarium), Homeschool Pop (Alternate), Amoeba Sisters, and close-up nature footage of fish breathing and operculum movement.

KEY TERMS

gills
Feathery breathing organs that take oxygen out of water and give off carbon dioxide into it. A bony fish has four gill arches on each side of its head.

gill filament
One of the many thin, comb-like strands that hang from each gill arch. Each filament is covered with even thinner plates called lamellae.

lamellae
Tiny, thin plates on the gill filaments where oxygen passes from water into the blood. They give the gills a huge surface area. The singular is lamella.

operculum
The bony flap that covers and protects the gills of a bony fish. It opens and closes to pump water out over the gills.

countercurrent exchange
An arrangement in which two fluids flow past each other in opposite directions, so that one can pass oxygen (or heat) to the other along the whole length of their contact.

dissolved oxygen
Oxygen gas mixed into water as single molecules, too small to see. It is measured in milligrams per liter (mg/L). It is not the oxygen atom that is part of each water molecule.

CORE IDEAS

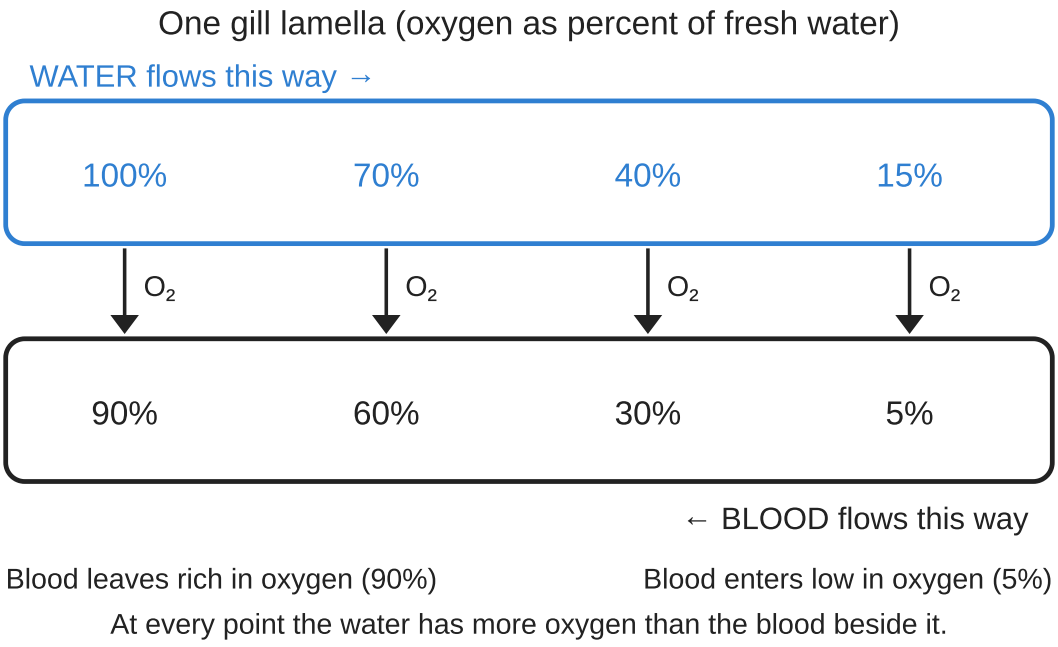
The story. In 1670 the Royal Society in London printed a report by Robert Boyle called *New Pneumatical Experiments about Respiration*. Boyle was a careful Christian experimenter who had built, with his assistant Robert Hooke, an air pump that could draw most of the air out of a glass jar. He used it to ask what living things need from the air. Among the creatures he tested were fish in water. As he pumped, something surprising happened: bubbles of air streamed out of the water itself, as if the water had been hiding air all along. The fish did poorly as the water lost its air. A fish swims in water, but Boyle's pump showed that what it truly lives on is the air dissolved in that water.

How they figured it out. Later chemists learned that the part of the air animals need is **oxygen**, and that water holds only a little of it. One liter of air at room temperature contains about 280 milligrams of oxygen. One liter of water at 20 °C holds only about 9 milligrams of **dissolved oxygen**, roughly 30 times less. Water is also about 800 times denser than air, so it takes real work to move it. A fish cannot breathe water in and out of a bag the way you breathe air into your lungs; that would be far too slow and heavy. Instead it pumps water in *one direction*. The fish opens its mouth and lowers the floor of its mouth to suck water in, closes its mouth and raises the floor to push water back over the **gills**, and then the **operculum**, the bony gill cover, swings open to let the water out. Fast swimmers such as tuna and many sharks simply swim with their mouths open and let the water rush through, which is called ram ventilation.

The big idea. Lift the operculum of a fish from the market and you will see four red, curved **gill arches**. Each arch carries two rows of thin **gill filaments**, and each filament is covered with hundreds of tiny plates called **lamellae**. Blood flows through the lamellae just one or two cell layers away from the water, so oxygen can diffuse in and carbon dioxide can diffuse out. Here is the clever part: the water flows across the lamellae in one direction and the blood flows through them in the *opposite* direction. This is called **countercurrent exchange**. Fresh water meets blood that is already well loaded with oxygen, and used water meets blood that is nearly empty, so all along the lamella the water still has more oxygen than the blood beside it, and oxygen keeps moving in. Blood can leave the gill carrying oxygen at around 80 to 90 percent of the level in the water. If the two flowed the same way, they would meet halfway and the blood could never get more than about 50 percent. A fish can take out as much as 80 percent of the oxygen in the water it pumps; you take only about a quarter of the oxygen from each breath of air.

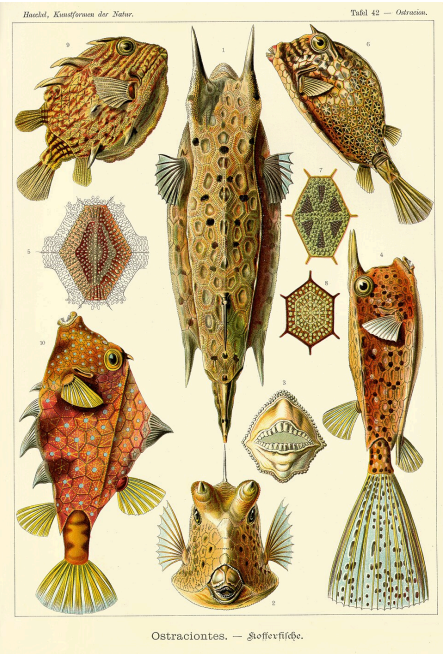
Try it and see. Water does not hold the same amount of oxygen at every temperature. Cold water holds more dissolved gas than warm water. At sea level, fresh water holds about 14.6 mg/L of oxygen at 0 °C but only about 7.6 mg/L at 30 °C. That is why the bubbles appear in your warm-up glass: as the cold water warms, it cannot hold as much air, and the extra comes out on the glass. It is also why trout need cool, fast-running streams, and why a goldfish in a warm, crowded bowl may gulp at the surface. If you have a pet fish, watch its operculum and count the beats in one minute. You are watching a living pump at work.

God's handiwork. "In whose hand is the soul of every living thing, and the breath of all mankind" (Job 12:10). The same Creator who gave you lungs gave the fish gills, and each is fitted to its home. The countercurrent design is so effective that engineers use the same idea in heat exchangers in power plants and buildings. When a trout holds still in a cold stream, its gills are quietly doing a job that took people thousands of years to understand.



Countercurrent exchange in a gill lamella. At every point the water has more oxygen than the blood beside it, so oxygen keeps moving into the blood. (Values are percent of the oxygen level in fresh water.)

PICTURE STUDY



Ostraciontes (Boxfishes), from Kunstformen der Natur (Art Forms in Nature), Ernst Haeckel, 1899–1904 · Color lithograph printed from Haeckel's drawings · Published in Leipzig and Vienna by the Bibliographisches Institut; copies are held in many libraries

Ernst Haeckel was a German zoologist who drew the shapes of living things with great care and published one hundred of his plates in a book called *Art Forms in Nature*. This plate shows boxfishes, also called trunkfishes. Their bodies are wrapped in a stiff armor of bony plates that fit together like tiles, so only their fins, eyes, mouths and tails can move.

A boxfish cannot flex its body to swim or to help pump water the way many fish do. It moves water over its gills by working its mouth and the small gill opening on each side, and it sculls slowly along with its fins. Look for those small openings as you study the plate.

Look closely

- Find the six-sided plates on the boxfish bodies. What other things in nature are built from six-sided shapes?
- Find a fish drawn head on. How is its body shaped in cross section?
- Look behind the eyes for the gill opening. Is it large or small compared with an ordinary fish's operculum?
- Find the mouth on several fish. Why might a small mouth matter for a fish that must pump water to breathe?

Talk about it

1. How does the boxfish's armor help it, and what might it cost the fish in breathing and swimming?
2. Why do you think Haeckel drew the same kind of fish from several angles?

3. If you drew a fish to show how it breathes, which parts would you label?

> **Safety first.** Use only warm tap water for the project, never boiling water. Do not take a pet fish out of its tank, tap on the glass or change the tank temperature to test it; just watch. Wash your hands after touching aquarium water or a market fish, and have an adult handle any knife if you look at the gills of a whole fish from the store.

EXPERIMENT: DOES WARM WATER HOLD LESS AIR?

Fish depend on the oxygen dissolved in their water. In this experiment you will find out whether temperature changes how much air water can hold, by watching air come out of water as it warms. Your question: *Does water that is warmed lose more of its dissolved air than water that stays cold?*

Before you start, write your prediction in your lab notebook as an if-then sentence.

You will need

- Three identical clear drinking glasses
- Cold tap water (let the tap run until the water is as cold as it gets)
- A kitchen thermometer
- A baking pan and warm tap water for a warm bath
- A bowl of ice water for a cold bath
- A magnifying glass, a ruler and a timer
- Lab notebook and pencil

Steps

1. Fill all three glasses to the same height with the same cold tap water, all at once. Measure and record the starting temperature.
2. Set glass A in a bowl of ice water. Set glass B on the counter at room temperature. Set glass C in a pan of warm tap water (about 40 °C).
3. Write down the variables. What you change: the temperature around the glass. What you keep the same: the kind of glass, the water source, the amount of water, the starting time. What you measure: the number of bubbles on the glass.
4. After 30 minutes, measure the water temperature in each glass.
5. Hold the ruler against the side of each glass and, with the magnifying glass, count the bubbles stuck inside the glass in a 2 cm by 2 cm square just below the waterline. Count two different squares and average them.
6. Record your results in a table with columns: Glass, Water temperature (°C), Bubbles in square 1, Bubbles in square 2, Average.
7. Make a bar graph of average bubbles for each glass. Write a conclusion that answers the question and connects it to fish.

Tips

- The bubbles are air that came out of solution as the water warmed. Water that stays cold should keep more of its air dissolved, so it should show fewer bubbles.
- Do not stir or bump the glasses; that knocks the bubbles loose.
- If you have a pet fish, add an observation: count how many times its operculum opens in 15 seconds, three times, and multiply the average by 4 to get beats per minute.

Check your work

- I wrote a prediction before I began.
- I named what I changed, what I kept the same and what I measured.
- My table and bar graph show the temperature and bubble count for each glass.
- My conclusion explains why a fish in warm water may have a harder time getting oxygen.

NATURE NOTEBOOK

1. Draw a fish from the side with its operculum lifted back. Label the mouth, operculum, gill arches and gill filaments, and draw blue arrows to show the one-way path of water.
2. Draw one gill filament close up with its lamellae. Draw a blue arrow for water flowing one way and a red arrow for blood flowing the other way, and write “countercurrent” beneath it.
3. Copy the dissolved-oxygen bar graph from this lesson and, beside each bar, draw a fish you might find in water of that temperature (trout in cold water, carp or goldfish in warm).

> **From history.** The Danish physiologist August Krogh, who won the Nobel Prize in 1920 for his work on how capillaries supply muscles with blood, spent much of his career comparing how different animals breathe, from insects to fish to people. He argued that for nearly every question in physiology there is some animal especially well suited to answer it. Gills, with their thin lamellae and one-way flow, became one of the best examples of how a body’s design matches its surroundings, and his 1941 book on breathing in many kinds of animals guided students for decades.

WORKED EXAMPLES

Example 1. One liter of air at room temperature contains about 280 mg of oxygen. One liter of fresh water at 20 °C holds about 9 mg of dissolved oxygen. How many times more oxygen is in the air? How many liters of water must a fish pass over its gills to meet the oxygen in just one liter of air?

1. Set up the ratio of air to water: 280 mg ÷ 9 mg.
2. 280 ÷ 9 = 31.1, so air holds about 31 times as much oxygen as the same volume of water.
3. To get 280 mg of oxygen from water at 9 mg per liter: 280 mg ÷ 9 mg/L = about 31 L of water.
4. That is why fish pump water through their gills in one direction all the time, instead of breathing in and out.

Answer: About 31 times more oxygen in air; about 31 L of water for the oxygen in 1 L of air.

Example 2. A student watches a goldfish and counts its operculum opening 23 times in 15 seconds. A second count gives 21 times in 15 seconds. What is the average breathing rate in beats per minute?

1. Average the two counts: (23 + 21) ÷ 2 = 22 beats in 15 seconds.
2. There are 60 ÷ 15 = 4 periods of 15 seconds in a minute.
3. 22 × 4 = 88 beats per minute.

Answer: About 88 beats per minute.

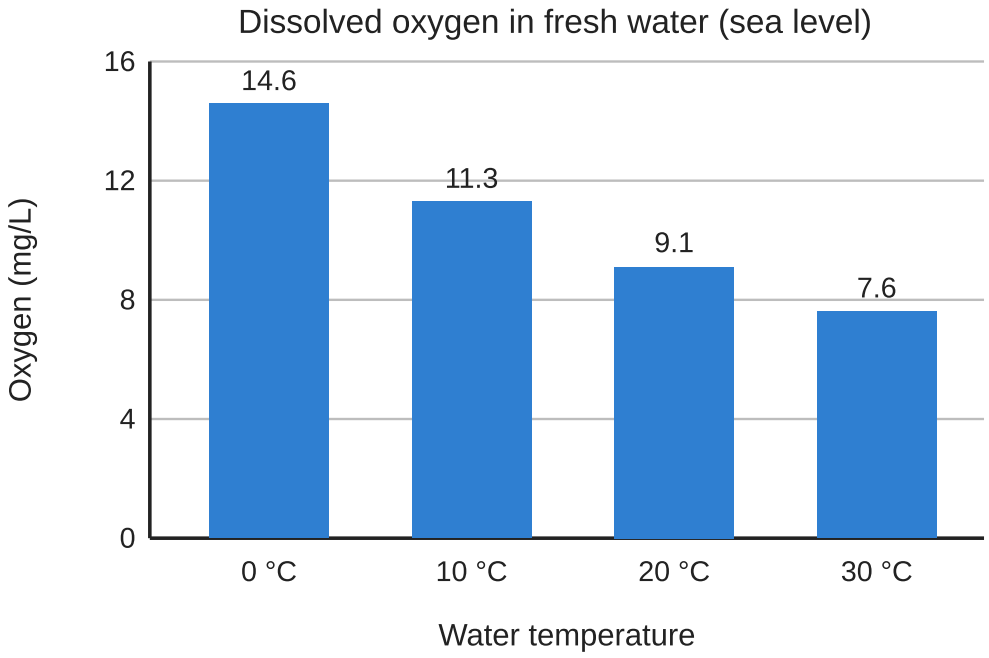
Example 3. Water enters a fish’s gills with 9.0 mg/L of dissolved oxygen and leaves with 1.8 mg/L. (a) What percent of the oxygen did the fish remove? (b) If the fish pumps 0.5 L of water over its gills each minute, how many milligrams of oxygen does it take in per minute?

1. Oxygen removed from each liter: 9.0 − 1.8 = 7.2 mg/L.
2. Percent removed: 7.2 ÷ 9.0 = 0.80, which is 80%.
3. Oxygen taken in per minute: 7.2 mg/L × 0.5 L/min = 3.6 mg per minute.

Answer: (a) 80% of the oxygen. (b) 3.6 mg of oxygen per minute.

PRACTICE

1. EASY Put these in the order water passes through them as a bony fish breathes: operculum opening, gill filaments and lamellae, mouth.
2. EASY What is the operculum, and what two jobs does it do?
3. EASY Why do the lamellae need to be so thin and so many?
4. MEDIUM A fish's operculum opens 18 times in 15 seconds. What is its rate in beats per minute?
5. MEDIUM Data reading. Use the bar graph of dissolved oxygen in fresh water at sea level (0 °C: 14.6 mg/L; 10 °C: 11.3 mg/L; 20 °C: 9.1 mg/L; 30 °C: 7.6 mg/L). (a) Describe the trend. (b) About how many milligrams less oxygen does a liter of water hold at 30 °C than at 0 °C? (c) Estimate the dissolved oxygen at 25 °C.



Dissolved oxygen in fresh water at sea level falls as the water warms.

6. MEDIUM Data reading. A student counted the operculum beats of the same goldfish on four mornings when the tank thermometer read different temperatures (the tank warmed and cooled with the room; the student did not heat it).
   18 °C: 52 beats/min
   20 °C: 60 beats/min
   22 °C: 69 beats/min
   24 °C: 78 beats/min
   (a) Which variable is independent and which is dependent? (b) Describe the pattern. (c) Using the graph from problem 5, give a reason for the pattern.
7. HARD Water enters the gills with 8.0 mg/L of oxygen and leaves with 2.4 mg/L. What percent of the oxygen did the fish remove?
8. HARD Explain in two or three sentences why countercurrent flow lets blood pick up more oxygen than flow in the same direction would.

9. CHALLENGE Experiment design. A student wants to know whether moving water (as from an aquarium bubbler) raises the dissolved oxygen in a jar of water. A pet store sells a simple dissolved-oxygen test kit. Design a fair experiment: give the question, the independent and dependent variables, at least three things to keep the same, and the control.
10. CHALLENGE Tuna swim with their mouths open almost all the time and cannot rest in one place for long. Use what you learned about ram ventilation to explain why.

COMMON MISTAKES

- Thinking fish breathe the oxygen atom inside the water molecule (H_2O). Fish use only the oxygen gas (O_2) dissolved in the water; they cannot split water apart.
- Thinking fish breathe water in and out like lungs. Water flows through a fish's gills in one direction: in at the mouth and out past the operculum.
- Thinking warm water holds more of everything. Warm water dissolves more sugar or salt, but it holds less dissolved gas, including oxygen.

Make it a game · Gill Relay. Set up a "lamella" with two lines of players or two rows of cups side by side. One row (the water) starts full of beans and is passed from left to right; the other (the blood) starts empty and is passed from right to left. At each station, if the water cup has more beans than the blood cup next to it, move one bean across. Then try it again with both rows moving the same direction. Count how many beans the blood row ends up with each time. Which way wins, and why?

Scripture. "In whose hand is the soul of every living thing, and the breath of all mankind." (Job 12:10, KJV)

Job speaks of "every living thing." The fish in the stream and the boy on the bank both live by God's hand, and both need oxygen, though He gave them very different ways to get it.

Practice answers

1. Mouth, then gill filaments and lamellae, then out through the operculum opening.
2. It is the bony flap that covers the gills of a bony fish. It protects the delicate gills and opens and closes to help pump water out over them.
3. Thin lamellae put the blood very close to the water so oxygen can diffuse in quickly, and having so many gives a huge surface area for oxygen to pass through.
4. $18 \times 4 = 72$ beats per minute.
5. (a) As the temperature rises, the water holds less dissolved oxygen. (b) $14.6 - 7.6 = 7.0$ mg/L less, about half as much. (c) About halfway between 9.1 and 7.6, so roughly 8.3 to 8.4 mg/L.
6. (a) Independent: water temperature. Dependent: operculum beats per minute. (b) As the water gets warmer, the goldfish breathes faster, about 4 to 5 more beats per minute for each degree. (c) Warmer water holds less dissolved oxygen, so the fish must pump more water each minute to get enough. (A goldfish's body chemistry also runs faster in warm water, so it needs more oxygen.)
7. $8.0 - 2.4 = 5.6$ mg/L removed; $5.6 \div 8.0 = 0.70$, so 70%.

8. In countercurrent flow the water and blood move in opposite directions, so all along the lamella the water beside the blood still has more oxygen than the blood, and oxygen keeps diffusing in until the blood leaves nearly as rich as fresh water. If both flowed the same way, the water would lose oxygen and the blood would gain it until they were equal, about halfway, and then no more oxygen would move.
9. Sample design. Question: Does bubbling air through water raise its dissolved oxygen? Independent variable: bubbler on or off. Dependent variable: dissolved oxygen in mg/L measured with the test kit. Keep the same: the same water source, the same jar size and amount of water, the same temperature and room, the same time (for example 1 hour), the same test kit and method. Control: an identical jar with no bubbler. Use at least three jars of each kind and average the results.
10. Tuna use ram ventilation: their swimming forces water in through the open mouth and over the gills. They depend on that flow and pump water poorly on their own, so if they stopped swimming, too little water would pass over the gills and they could not get enough oxygen.

7.2 How a Bird Breathes

OBJECTIVES

- Tell how the surgeon John Hunter described the air sacs and air-filled bones of birds in 1774, and name the main parts of a bird’s breathing system.
- Trace one breath of air through a bird’s two-cycle, one-way breathing system and explain why fresh air passes through the lungs during both inhaling and exhaling.
- Use percents and a graph of air pressure to calculate how much oxygen is available at high altitude, and explain how birds such as the bar-headed goose fly where people struggle to breathe.

Warm-up. Take a deep breath and blow it all out. Now think: when you breathe out, the air leaves the same way it came in, so some old air always stays in your lungs. Blow up a balloon, then let it go flat. Is any air left inside? Then press on a squeeze bottle with a straw in it and watch which way the air goes. Write one sentence guessing how an animal might design a lung where air flows only one way.

Video. Coming soon. How birds breathe with air sacs and one-way airflow, and bar-headed geese flying over the Himalayas; search Smithsonian, Cornell Lab of Ornithology, Homeschool Pop (Alternate), Amoeba Sisters, university biology channels, and nature footage of geese and flamingos.

KEY TERMS

air sacs
Thin-walled, balloon-like bags connected to a bird’s lungs. They work like bellows, storing and pushing air, but oxygen does not pass into the blood there. Most birds have nine.

parabronchi
The many tiny tubes that run through a bird’s lung. Air flows through them in one direction, and oxygen passes into the blood from them.

pneumatic bones
Hollow bones filled with air and connected to the air sacs, such as the upper wing bone (humerus) in many birds.

unidirectional airflow
Air moving in only one direction through the lung, instead of in and out the same way.

trachea
The windpipe, a tube stiffened by rings that carries air from the throat toward the lungs.

altitude
Height above sea level. As altitude increases, air pressure drops, so each breath holds less oxygen.

CORE IDEAS

The story. In 1774 the Royal Society in London published a paper by John Hunter, a Scottish surgeon who loved to take animals apart to see how they worked. Its long title began *An Account of Certain Receptacles of Air, in Birds, Which Communicate with the Lungs*. Hunter had found that a bird's lungs open into a set of thin, clear bags spread through its body, and that some of these bags reach right into the hollow bones of the wings. To prove the bones were part of the breathing system, he blocked a fowl's windpipe and opened its wing bone, and the bird drew air through the bone. It was a hard experiment to read about, but it settled the question: in a bird, air goes places it never goes in a mammal.

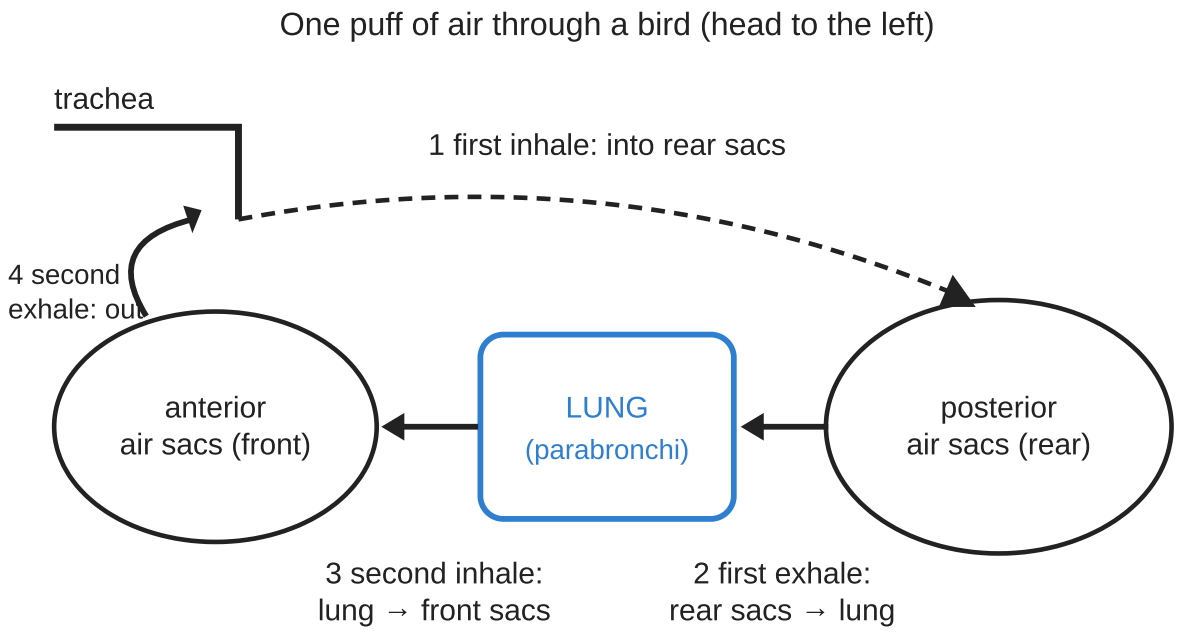
How they figured it out. For nearly two hundred years after Hunter, scientists argued about how the air moved through those bags. In the 1960s and 1970s, researchers such as Knut Schmidt-Nielsen at Duke University put small gas sensors into the air sacs of ostriches, ducks and other birds and had them breathe a puff of air marked with a harmless tracer gas. By timing when the tracer showed up in each sac, they worked out the path. A single breath of air takes *two full breaths* to pass through a bird. On the first inhale, it goes mostly into the rear (posterior) **air sacs**. On the first exhale, it is pushed from those sacs forward through the lungs. On the second inhale, it moves from the lungs into the front (anterior) air sacs, and on the second exhale it leaves through the **trachea**. Of course the bird is breathing all the time, so all four steps are happening at once to different puffs of air.

The big idea. The result is **unidirectional airflow**: air moves through the lung in one direction, during the inhale *and* the exhale. Your lungs are dead-end bags. Air goes in and comes back out the same way, so stale air always mixes with fresh. A bird's lungs are small and stiff and hardly change size. The air sacs do the pumping like bellows, while the lungs are threaded with thousands of tiny tubes called **parabronchi**, where oxygen passes into the blood through air capillaries. Blood crosses these tubes at an angle (a cross-current arrangement), so it can pick up a great deal of oxygen. Birds have no diaphragm; they breathe by moving the breastbone and ribs, which squeezes and expands the air sacs. Many also have **pneumatic bones**, hollow and connected to the air sacs, which keep the skeleton light without making it weak.

In the world today. At sea level, air pressure is about 101 kilopascals (kPa) and oxygen is about 21 percent of the air. At 6,000 meters the air is just as much oxygen by percent, but the pressure is only about 47 kPa, so each breath carries less than half as much oxygen. Most people feel ill above about 3,000 meters until their bodies adjust. Yet bar-headed geese migrate between Central Asia and India each year, flapping across the Himalayas. Researchers who fitted geese with small satellite tags and reported their results in 2011 found that the birds cross the mountains in a single day's flight, usually at night and early morning when the air is cool and dense, and some climbed above 6,000 meters. Their one-way lungs, large hearts and blood that grips oxygen tightly make it possible.

God's handiwork. Flapping flight is some of the hardest work any backboned animal does, and the bird's breathing system is built for it: light, constant, and always flushing the lung with fresh air. "Doth the hawk fly by thy wisdom, and stretch her wings toward the south?"

(Job 39:26). God asked Job that question to show him how much in creation is beyond human design. When you watch a flock of geese pass overhead in a V, remember that every bird in it is breathing in a way no human engineer thought of first.



One puff of air takes two breaths to pass through a bird. Air flows through the lung in the same direction on both the inhale and the exhale.

PICTURE STUDY



American Flamingo, Plate 431 from The Birds of America, John James Audubon (painting); engraved by Robert Havell Jr., 1838 · Hand-colored engraving and aquatint · Printed in London for *The Birds of America*; Audubon's original watercolors are kept by the New-York Historical Society

John James Audubon set out to paint every bird in North America life-size. His prints were made on huge sheets called double elephant folio, nearly a meter tall, and even so, the flamingo was too tall to fit standing straight. Audubon bent its neck down toward the ground so the whole bird would fit on the page.

That long neck holds a long trachea. A longer windpipe holds more stale air that must be moved with every breath, and birds have wider tracheas than mammals of the same size, which helps. The flamingo's one-way lungs and air sacs move air through that long tube day and night.

Look closely

- Trace the line of the flamingo's neck with your finger. How long would its trachea be compared with its body?
- Find the bill. How is its shape different from a goose's or a hawk's?
- Look at the small flamingos in the background. How did Audubon show distance?
- Look at the legs and feet. Where does this bird live and feed?

Talk about it

1. Why did Audubon bend the flamingo's neck? What does that tell you about his goal of painting birds life-size?
2. How would a long trachea make breathing harder, and how does a bird's design help with that?
3. Audubon shot birds and wired them into lifelike poses to paint them. How do bird artists and scientists study birds today?

EXPERIMENT: ARE HOLLOW BONES WEAKER?

Many birds have pneumatic bones: hollow tubes full of air from the air sacs. You might think a hollow bone would be weaker than a solid one. In this experiment you will test paper "bones" to find out. Your question: *Does a hollow paper tube hold more weight than a solid paper rod made from the same amount of paper?*

Write your prediction before you begin.

You will need

- Six sheets of the same printer paper
- Tape and a ruler
- A thick marker or a broom handle about 2 to 3 cm across, to roll paper around
- A light, stiff board or a hardcover book to set on top of the columns
- A stack of paperback books or bags of dried beans of known mass
- A kitchen scale, lab notebook and pencil

Steps

1. Make three hollow “bones”: roll one sheet of paper around the marker the long way, tape the edge, and slide the marker out.
2. Make three solid “bones”: roll one sheet as tightly as you can into a thin rod and tape it.
3. Trim all six to the same height, for example 20 cm. Weigh one of each to check that they have the same mass.
4. Write the variables. What you change: hollow or solid. What you keep the same: the paper, the amount of paper, the height, the tape, the way you load it. What you measure: the mass in kilograms that the column holds before it buckles.
5. Stand one column upright, set the board on top, and add books or bean bags one at a time, gently, until the column bends or collapses. Weigh the load it held just before it failed.
6. Test all six columns. Record the results in a table and find the average for hollow and for solid.
7. Make a bar graph of the two averages and write a conclusion that connects your result to a bird’s wing bones.

Tips

- Steady the column with your fingertips only until the board is balanced, then let go.
- Engineers use hollow tubes for bicycle frames and lampposts for the same reason God gave birds hollow bones: for the same mass, a wide tube resists bending and buckling much better than a thin solid rod.
- Real pneumatic bones are not empty pipes. Many have thin bony struts crossing the inside, like the braces in a bridge.

Check your work

- I wrote a prediction and named my variables.
- I tested at least three of each kind and averaged them.
- My bar graph and conclusion compare hollow and solid columns of the same mass.
- I can explain how hollow, air-filled bones help a flying bird.

NATURE NOTEBOOK

1. Draw the four-step path of one breath of air through a bird as a comic strip with four boxes: first inhale, first exhale, second inhale, second exhale. Color the air puff in each box.
2. Draw a person’s lungs and a bird’s lungs and air sacs side by side. Under each, write one sentence explaining which way air flows.
3. Watch birds outside for ten minutes. Sketch one bird in flight and one at rest. Write the time, place and weather, and note whether you could see its breast moving as it breathed.

From history. Knut Schmidt-Nielsen, a Norwegian-born physiologist who spent most of his career at Duke University, studied how animals survive hard conditions: camels and kangaroo rats in the desert, and ostriches in the heat. He found that a hot ostrich pants many times faster than its slow resting rate, yet does not wash too much carbon dioxide out of its blood, because the quick, shallow breaths mostly move air in and out of the trachea and air sacs rather than through the lungs. He also wrote a well-known 1971 article for *Scientific American* titled “How Birds Breathe,” which helped bring the one-way airflow of bird lungs to a wide audience.

WORKED EXAMPLES

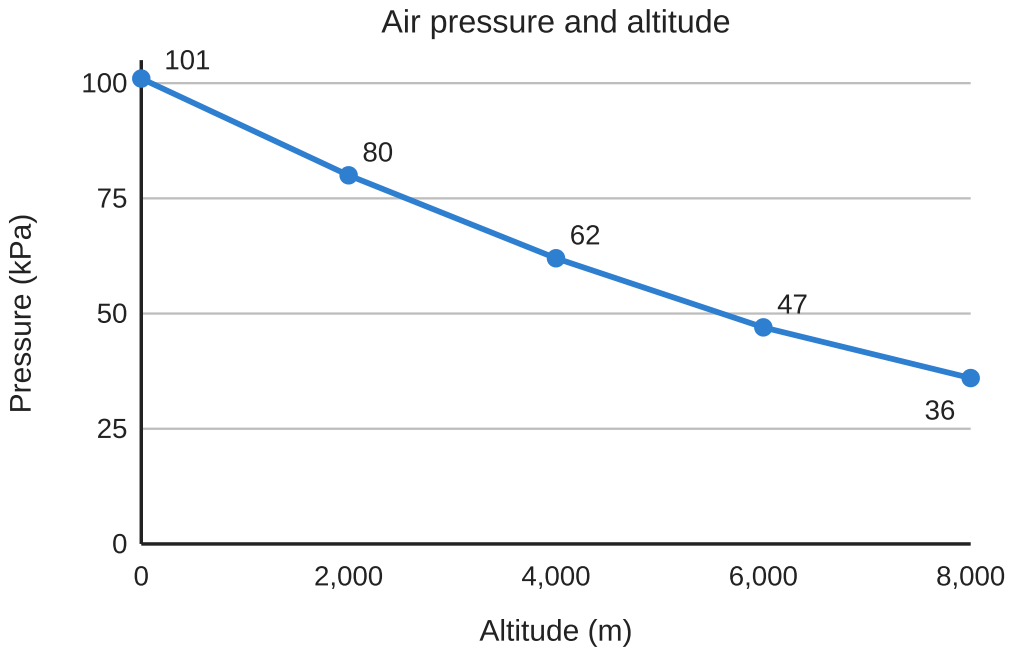
Example 1. A pigeon at rest takes 30 breaths per minute. One puff of air needs two complete breaths (two inhales and two exhales) to pass all the way through its breathing system. How many seconds does one breath take, and how long does the puff of air spend inside the bird?

(Use the map or drawing “Steps 1 through 4 take two full breaths.” shown earlier in this lesson.)

1. Time for one breath: 60 seconds ÷ 30 breaths = 2 seconds per breath.
2. The puff needs 2 complete breaths: 2 × 2 seconds = 4 seconds.
3. During those 4 seconds it passes through the posterior air sacs, the lung, and the anterior air sacs, in that order.

Answer: 2 seconds per breath; the puff spends about 4 seconds inside the bird.

Example 2. Oxygen makes up about 21% of the air at every altitude. Air pressure at sea level is about 101 kPa; at 6,000 m it is about 47 kPa. Find the pressure of the oxygen in each place, and what fraction of the sea-level oxygen is left at 6,000 m.



Air pressure drops as altitude increases.

1. Oxygen pressure at sea level: 0.21 × 101 kPa = 21.2 kPa.
2. Oxygen pressure at 6,000 m: 0.21 × 47 kPa = 9.9 kPa.
3. Fraction left: 9.9 ÷ 21.2 = 0.47, a little less than one half.
4. Notice that you could also divide the total pressures, 47 ÷ 101 = 0.47, because the 21% is the same in both places.

Answer: About 21.2 kPa at sea level and 9.9 kPa at 6,000 m, so only about 47% (a little under half) as much oxygen per breath.

Example 3. On a cool morning a zookeeper counts an ostrich’s breaths: 3 breaths in 30 seconds. On a hot afternoon, while the ostrich pants to cool off, the keeper counts 24 breaths in 30 seconds. Find both rates in breaths per minute and how many times faster the ostrich breathes when panting.

1. Cool morning: 3 × 2 = 6 breaths per minute.
2. Hot afternoon: 24 × 2 = 48 breaths per minute.
3. Compare: 48 ÷ 6 = 8.

Answer: 6 and 48 breaths per minute; the ostrich breathes 8 times as fast when panting.

PRACTICE

1. EASY What are air sacs, and does oxygen pass into the blood there?
2. EASY Put these in order for one puff of air: anterior air sacs, trachea (going in), lung, posterior air sacs, trachea (going out).
3. EASY What did John Hunter discover about bird bones in 1774?
4. MEDIUM How is airflow in a bird’s lung different from airflow in your lung? Why does that help the bird?
5. MEDIUM A small songbird takes 90 breaths per minute. How many seconds does one puff of air spend passing through it (two breaths)?
6. MEDIUM Data reading. Use the altitude graph: sea level 101 kPa; 2,000 m about 80 kPa; 4,000 m about 62 kPa; 6,000 m about 47 kPa; 8,000 m about 36 kPa. (a) By how much does pressure drop in the first 2,000 m, and in the last 2,000 m? (b) Is the graph a straight line? (c) At about what altitude is the pressure half of sea level?
   (Use the map or drawing “Air pressure at different altitudes (standard values, rounded).” shown earlier in this lesson.)
7. MEDIUM Data reading. A student timed a pet parakeet’s breathing (by watching its tail bob) before and after it flew around a room for one minute.
   At rest: 20 breaths in 15 s
   Just after flying: 38 breaths in 15 s
   2 minutes later: 30 breaths in 15 s
   4 minutes later: 22 breaths in 15 s
   (a) Convert each count to breaths per minute. (b) Describe what happened. (c) Why did the rate rise?
8. HARD At the top of Mount Everest (about 8,850 m), air pressure is about 33 kPa. What is the oxygen pressure there (21% of the air), and what fraction is that of sea level (21.2 kPa)?
9. CHALLENGE Experiment design. A student wonders whether a bird’s breathing rate depends on the air temperature. They can watch the backyard chickens through the fence each day without disturbing them. Design an observational study: the question, the independent and dependent variables, how to measure each, what to keep the same, and how many observations to make.
10. CHALLENGE Write a short paragraph explaining how three features let bar-headed geese fly over the Himalayas, where the air has less than half the oxygen of sea level.

COMMON MISTAKES

- Thinking a bird’s air sacs are where oxygen enters the blood. The air sacs only store and move air; gas exchange happens in the lungs.
- Thinking a bird’s lungs swell and shrink like ours. A bird’s lungs are small and stiff; the air sacs do the pumping.
- Thinking hollow bones are weak. For the same mass, a wide hollow tube resists bending better than a thin solid rod, and many bird bones are braced inside with tiny struts.

Make it a game · Two-Breath Relay. Mark four spots on the floor: Trachea, Posterior Sacs, Lung, Anterior Sacs. Each player is a puff of air. A caller shouts “Inhale!” or “Exhale!” in turn, and on every call each puff must move one step along the correct one-way path (trachea, posterior sacs, lung, anterior sacs, out the trachea). New puffs enter on every inhale. Anyone who goes backward or moves on the wrong call sits out. Play until everyone can do it without a mistake, then speed up the calls the way a bird breathes faster in flight.

Scripture. “Doth the hawk fly by thy wisdom, and stretch her wings toward the south?” (Job 39:26, KJV)

God reminded Job that the hawk’s migration was not Job’s invention. The more we learn about the hawk’s hollow bones and one-way lungs, the more clearly we see whose wisdom it flies by.

Practice answers

1. Air sacs are thin, balloon-like bags connected to a bird’s lungs that work like bellows to store and move air. Oxygen does not pass into the blood in the air sacs; that happens in the lungs (in the parabronchi).
2. Trachea (going in), posterior air sacs, lung, anterior air sacs, trachea (going out).
3. That some bird bones are hollow and filled with air from the air sacs, so they are part of the breathing system (pneumatic bones); a bird could even draw air through an opened wing bone.
4. In your lung air goes in and out the same way, so stale air mixes with fresh air in dead-end sacs. In a bird’s lung air flows one way through tubes, during both the inhale and the exhale, so the lung always has fresh air passing through and the bird gets more oxygen.
5. 60 ÷ 90 = 0.67 seconds per breath; 2 × 0.67 = about 1.3 seconds.
6. (a) First 2,000 m: 101 − 80 = 21 kPa. Last 2,000 m: 47 − 36 = 11 kPa. (b) No. It curves; the pressure drops quickly near the ground and more slowly higher up. (c) Half of 101 is about 50 kPa, which is between 4,000 and 6,000 m, at roughly 5,500 m.
7. (a) 80, 152, 120 and 88 breaths per minute. (b) The rate nearly doubled right after flying, then fell back toward the resting rate within about 4 minutes. (c) Flying muscles worked hard and needed more oxygen for cellular respiration and gave off more carbon dioxide, so the bird breathed faster.
8. 0.21 × 33 = 6.9 kPa; 6.9 ÷ 21.2 = 0.33, about one third of the sea-level value.
9. Sample design. Question: Do chickens breathe faster on hotter days? Independent variable: air temperature, read from an outdoor thermometer in the shade. Dependent variable: breaths per minute, counted by watching a resting hen’s body rise and fall for 30 seconds and doubling. Keep the same: the same hens, resting (not just after running), the same time of day if possible, the same counting method, observer stays quiet outside the pen. Make counts on at least 10 different days covering cool and hot weather, count each hen twice and average, then graph temperature against breathing rate.
10. Sample: Bar-headed geese have one-way airflow through their lungs, pushed by air sacs, so fresh air passes over the gas-exchange surfaces on both the inhale and the exhale. Their blood holds on to oxygen tightly, so it can load up even when there is little oxygen in the air,

and their large hearts pump that blood quickly to the flying muscles. They also tend to fly in the cool night and early morning, when the air is denser.

7.3 Insect Bodies

OBJECTIVES

- Tell how Marcello Malpighi's 1669 study of the silkworm revealed the tracheal tubes and spiracles insects use to breathe.
- Describe the insect body plan (head, thorax, abdomen, six legs, exoskeleton) and explain how insects breathe, circulate fluid and grow by molting.
- Use surface-area-to-volume ratios, growth ratios and scale to explain why insects stay small and how they grow in steps.

Warm-up. Find an empty drinking straw and a small sealed plastic bag. Blow gently through the straw into a glass of water and watch the bubbles. Now imagine your whole body had no lungs and no blood vessels for carrying oxygen, only thousands of tiny straws running from holes in your skin straight to every muscle. Write two sentences: What would be good about that design? What would limit how big you could grow?

Video. Coming soon. Insect anatomy for middle school: exoskeleton, spiracles and tracheae, open circulatory system and molting; search Smithsonian National Museum of Natural History, Amoeba Sisters, Homeschool Pop (Alternate), Science Max, university entomology extension channels, and close-up nature footage of a cicada or mealworm molting.

KEY TERMS

exoskeleton
A hard outer covering that supports and protects an animal's body from the outside. Muscles attach to its inner surface.

chitin
A tough, flexible material, built from chains of sugar-like units, that forms much of the exoskeleton of insects and other arthropods (and the cell walls of fungi).

molting
Shedding the old exoskeleton so the animal can grow. The new, soft exoskeleton stretches before it hardens. Scientists also call it ecdysis.

spiracles
Small openings along the sides of an insect's thorax and abdomen that let air into the tracheal tubes. Many can open and close like valves.

tracheae
A branching system of air-filled tubes that carry oxygen from the spiracles directly to the insect's cells. The finest branches are called tracheoles.

open circulatory system
A system in which the blood-like fluid (hemolymph) is pumped by a tube-shaped heart along the back and then flows loosely around the organs instead of staying inside vessels.

CORE IDEAS

The story. In the 1660s in Bologna, Italy, the physician Marcello Malpighi took up a question the Royal Society in London had asked him: what is inside a silkworm? Silk was a precious trade in Italy, yet no one had looked carefully at the caterpillar that made it. Malpighi worked with tiny scissors, needles and a microscope, and in 1669 the Royal Society published his long letter on the silkworm, the first full study of an insect's inside parts. Four years earlier, in 1665, Robert Hooke had amazed English readers with a huge engraving of a flea in his book *Micrographia*, showing its armored plates and jointed legs. Hooke drew the outside of an insect. Malpighi opened one up.

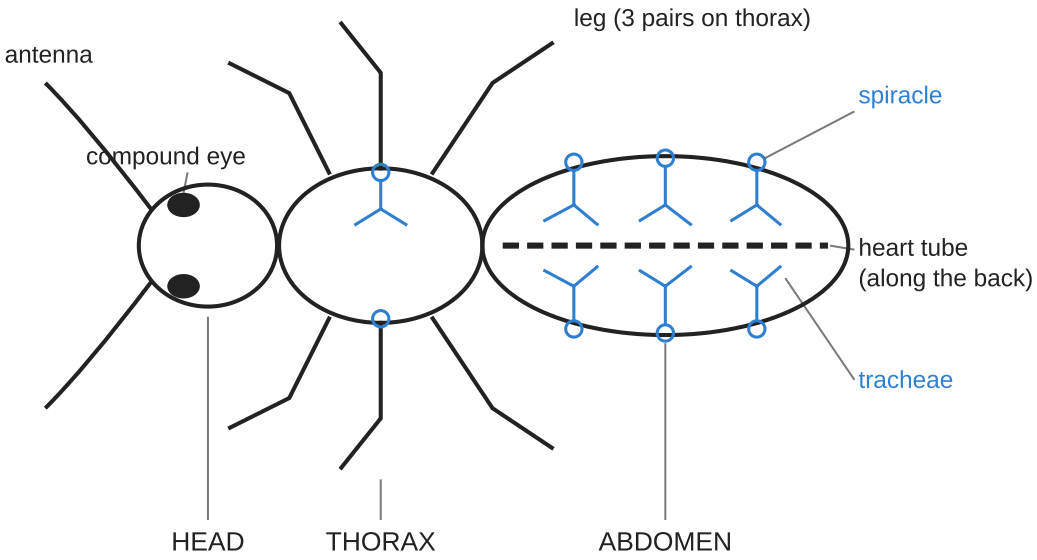
How they figured it out. Inside the silkworm, Malpighi found a network of shining, silvery threads running everywhere: along the gut, into the muscles, around the silk glands. They were tubes filled with air, and they branched again and again like the twigs of a tree. He traced them back to a row of small holes along each side of the body, the **spiracles**. To test whether air really entered there, he smeared oil over the spiracles, and the caterpillar soon died, as if it had been smothered. Insects, he concluded, breathe not through their mouths but through their sides, by a system of air tubes we now call **tracheae**. Malpighi also found the thin tubes that clean wastes from an insect's body fluid; to this day they are called Malpighian tubules.

The big idea. Every adult insect has three body parts: a **head** with antennae, eyes and mouthparts; a **thorax** that carries six jointed legs (three pairs) and, in most adults, two pairs of wings; and an **abdomen** that holds most of the digestive and reproductive organs. The whole body is wrapped in an **exoskeleton** made largely of **chitin**, light but strong, with muscles fastened to its inside. Air enters the spiracles and travels through the tracheae right to the cells, so oxygen does not have to ride in the blood. Insect blood, called **hemolymph**, is usually clear, green or yellow rather than red, and it carries food and wastes rather than much oxygen. A long tube-shaped heart along the back pushes it forward, and it then washes loosely back through the body: an **open circulatory system**.

Try it and see. An exoskeleton cannot stretch much, so an insect grows in steps. It forms a new soft covering underneath, splits the old one down the back, climbs out, swallows air or water to swell up, and then lets the new exoskeleton harden. This is **molting**. Between molts the insect may eat and fatten, but its hard parts, such as the head capsule, stay the same size. A caterpillar may molt four or five times before it becomes a pupa. If you keep mealworms (the young of a darkling beetle) in a container of oats, you will find their pale, empty skins and can measure how much bigger each new stage is. Look along the side of a mealworm or caterpillar with a magnifying glass and you may see the dots of its spiracles.

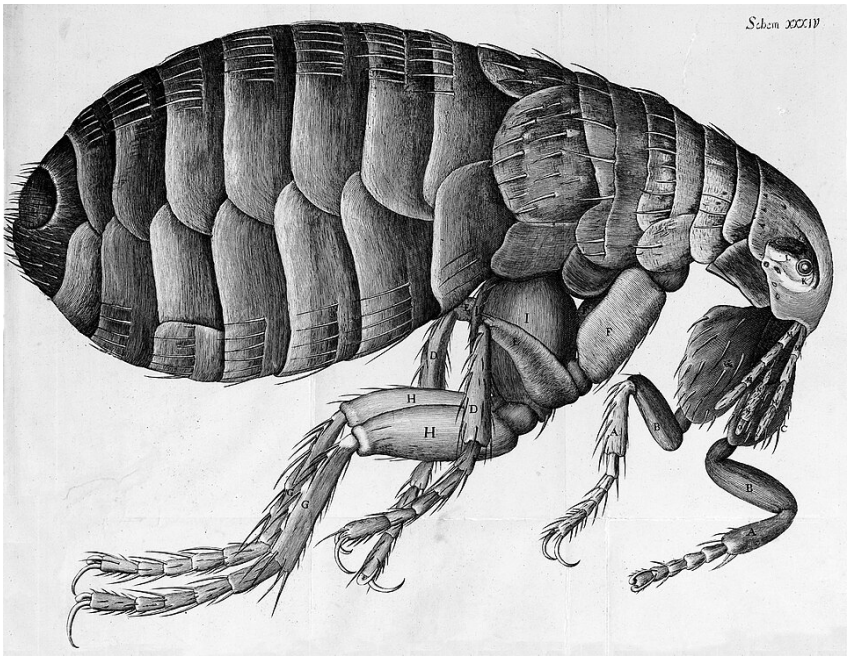
Why insects stay small. Air moves through the finest tracheal tubes mostly by diffusion, which works well over a few millimeters but slowly over long distances. Large, active insects help it along by pumping their abdomens, but the design still works best in small bodies. Small bodies also have a large surface area compared with their volume, which makes it easy to exchange gases but also easy to dry out; the waxy outer layer of the exoskeleton helps keep water in. "Go to the ant, thou sluggard; consider her ways, and be wise"

(Proverbs 6:6). A carpenter ant's body is a tiny marvel of engineering: armor, air pipes and a pump, all fitted together in a creature only about a centimeter long.



The insect body plan. Air enters at the spiracles and travels through branching tracheae straight to the cells.

PICTURE STUDY



A Flea, from Micrographia, Robert Hooke, 1665 · Engraving on a large fold-out plate · Printed in London for the Royal Society; copies are kept in many libraries, and the whole book is free to read online

Photo: Wellcome Collection · CC BY 4.0 (https://creativecommons.org/licenses/by/4.0) · via Wikimedia Commons

Robert Hooke was the Royal Society's Curator of Experiments, and in 1665 he published *Micrographia*, a book of the things he had seen with his microscope. His flea was drawn so large that the page had to fold out, and readers who had only ever slapped at fleas suddenly saw them as finely built creatures. Hooke praised the flea's strength and the beauty of its polished armor.

A flea is an insect, so it has every part you learned about in this lesson: head, thorax and abdomen, six jointed legs and an exoskeleton. Hooke could not see its tracheae from outside, but with a good lens you can sometimes spot spiracles along the abdomen.

Look closely

- Find the three body parts. Where does the thorax end and the abdomen begin?
- Count the legs. Which pair is built for jumping, and how can you tell?
- Look at the overlapping plates of the exoskeleton. Why would a hard covering be built in sections instead of one piece?
- Find the stiff hairs and claws. How might they help a flea hold on to an animal's fur?

Talk about it

1. Hooke worked hard to draw an animal most people thought was only a pest. What does that tell you about how a scientist looks at the world?
2. If the flea's exoskeleton cannot stretch, how did this flea grow to its full size?
3. How would you draw an insect to show what is inside it, as Malpighi did?

> **Safety first.** Handle live insects gently and only harmless ones such as mealworms from a pet or bait store. Never use the freezer; a refrigerator for no more than 10 minutes is enough to cool them, and they recover at room temperature. Wash your hands with soap after touching insects or their bedding. Anyone with an allergy to insects or dust should wear gloves or let someone else handle them. Do not collect stinging insects.

EXPERIMENT: DOES TEMPERATURE CHANGE HOW FAST A MEALWORM MOVES?

Insects do not make much of their own body heat, so their bodies are about as warm as the air around them. Their muscles, like all muscles, depend on cellular respiration, and those chemical reactions run more slowly when cool. Your question: *Do mealworms crawl more slowly when they are cool than when they are at room temperature?*

Write your prediction as an if-then sentence before you begin.

You will need

- 6 to 10 mealworms from a pet or bait store, with a container of oats or bran
- A sheet of white paper with a 1-cm grid drawn on it, or a ruler and pencil
- A small plastic cup with a lid with air holes
- A refrigerator, a kitchen thermometer and a timer
- A magnifying glass, lab notebook and pencil

Steps

1. Look at one mealworm with a magnifying glass. Count its legs and look for spiracles along its sides. Sketch it in your notebook.
2. Write the variables. What you change: the mealworm’s temperature (room temperature or cooled). What you keep the same: the same mealworms, the same paper, the same light, the same time allowed. What you measure: the distance in centimeters a mealworm crawls in 30 seconds.
3. At room temperature, place one mealworm in the center of the grid and start the timer. Follow its path lightly with a pencil for 30 seconds, then measure the length of the path. Test at least five mealworms and record each distance and the room temperature.
4. Put the same mealworms in the lidded cup in the refrigerator for 10 minutes. Record the refrigerator temperature.
5. Take them out and test each one the same way right away.
6. Find the average distance at each temperature and change each to a speed in centimeters per minute (multiply the 30-second distance by 2).
7. Make a bar graph of the two average speeds. Return the mealworms to their oats and write a conclusion.

Tips

- Mealworms are larvae. They have six true legs near the head end, and you may find their shed skins in the oats, proof that they molt.
- If a mealworm curls up and does not move, gently nudge it once with a soft brush and start timing again.
- For an extra trial, let the cooled mealworms warm up for 20 minutes and test them again. Do they speed back up?

Check your work

- I named my independent variable, my dependent variable and what I kept the same.
- I tested at least five mealworms at each temperature and averaged the results.
- My graph and conclusion compare the speeds and explain the result using what I know about insect bodies.
- I handled the mealworms gently and returned them safely.

NATURE NOTEBOOK

1. Draw an insect from above, as large as your page. Label the head, thorax, abdomen, antennae, compound eyes, six legs and wings (if it has them). Add spiracles along the sides and draw the tracheae branching inside in blue pencil.
2. Find a real insect outdoors (an ant, beetle, grasshopper or butterfly). Watch it without touching it for five minutes. Sketch it, write the date, place and temperature, and record one thing it did.
3. Draw a three-panel strip of a molting insect: old exoskeleton splitting, insect climbing out, new exoskeleton hardening. Write one sentence under each panel.

From history. Jean-Henri Fabre, a French schoolteacher, spent most of his long life watching insects in the fields of southern France and, later, in the walled garden of his home at Sérignan, which he called his *harmas*. He published ten volumes of *Souvenirs entomologiques* (Entomological Memories) between 1879 and 1907, describing dung beetles, wasps, crickets and spiders with patience and delight. Fabre preferred watching living insects in their own homes to studying dead ones in drawers, and his books, translated into many languages, have taught generations of young naturalists to sit still, watch closely and record what they see.

WORKED EXAMPLES

Example 1. A caterpillar's head capsule is 0.50 mm wide after it hatches. Entomologists have noticed that in many insects the head capsule grows by about the same ratio at each molt. If this caterpillar's head grows by a ratio of 1.4 at each molt, how wide will it be after each of the next three molts?

1. After molt 1: 0.50 mm × 1.4 = 0.70 mm.
2. After molt 2: 0.70 mm × 1.4 = 0.98 mm.
3. After molt 3: 0.98 mm × 1.4 = 1.372 mm, which rounds to 1.37 mm.
4. Notice that the head does not grow between molts. It jumps in size each time the old exoskeleton is shed.

Answer: 0.70 mm, 0.98 mm and about 1.37 mm.

Example 2. Model three "animals" as cubes with sides of 1 cm, 2 cm and 4 cm. Find the surface area, the volume and the ratio of surface area to volume for each. What happens to the ratio as the cube gets bigger?

1. A cube has 6 faces. Surface area = 6 × side × side. Volume = side × side × side.
2. 1 cm cube: area 6 × 1 = 6 cm^2; volume 1 cm^3; ratio 6 ÷ 1 = 6 to 1.
3. 2 cm cube: area 6 × 4 = 24 cm^2; volume 8 cm^3; ratio 24 ÷ 8 = 3 to 1.
4. 4 cm cube: area 6 × 16 = 96 cm^2; volume 64 cm^3; ratio 96 ÷ 64 = 1.5 to 1.
5. Each time the side doubles, the ratio is cut in half: big bodies have much less surface for each cubic centimeter of living tissue to exchange gases through.

Answer: Ratios of 6:1, 3:1 and 1.5:1. The ratio falls as size increases, which is one reason a body that breathes by diffusion through tubes from its surface works best when small.

Example 3. A flea 2 mm long jumps 20 cm. How many body lengths is that? If a student 1.5 m tall could jump the same number of body lengths, how far would the student jump?

1. Use the same units: 20 cm = 200 mm.
2. Body lengths: 200 mm ÷ 2 mm = 100 body lengths.
3. Student: 100 × 1.5 m = 150 m.

Answer: 100 body lengths; the student would jump 150 m, longer than a soccer field.

PRACTICE

1. EASY Name the three body parts of an adult insect and one thing attached to each.
2. EASY A spider has eight legs and two body parts. Is it an insect? Explain.
3. EASY Through what openings does air enter an insect, and through what tubes does it reach the cells?
4. MEDIUM Why must an insect molt in order to grow? What is the insect's greatest danger right after molting?
5. MEDIUM A grasshopper nymph's head is 1.0 mm wide. If it grows by a ratio of 1.3 at each molt, how wide is it after two molts?
6. MEDIUM Data reading. A student timed mealworms crawling on a grid at different temperatures (average of 5 mealworms each).
   8 °C: 1.2 cm/min
   14 °C: 3.5 cm/min
   20 °C: 6.1 cm/min
   26 °C: 8.4 cm/min
   (a) Name the independent and dependent variables. (b) Describe the trend. (c) Predict the speed at 17 °C.
7. MEDIUM Data reading. A student measured the head-capsule widths of mealworms and sorted them into groups by size:
   Group A: 0.40 mm
   Group B: 0.52 mm
   Group C: 0.68 mm
   Group D: 0.88 mm
   (a) Find the ratio of each group to the one before it (B÷A, C÷B, D÷C). (b) What do these ratios suggest about the groups? (c) Predict the width of the next group.
8. HARD A cube-shaped model insect has sides of 3 cm. Find its surface area, its volume and its surface-area-to-volume ratio. Compare it with a 1 cm cube (ratio 6:1).
9. CHALLENGE Experiment design. Malpighi blocked a silkworm's spiracles with oil. Design a kinder experiment, using mealworms and doing them no harm, to show that insects take in air for respiration and that they use more when warm. (Hint: animals that use oxygen give off carbon dioxide.) Give the question, the variables, a control and what you would measure.

10. CHALLENGE Explain in a short paragraph why a beetle the size of a dog could not breathe well with an insect's tracheal system.

COMMON MISTAKES

- Thinking insects breathe through their mouths. Air enters through spiracles on the sides of the thorax and abdomen.
- Thinking insects grow a little every day like a puppy. Their hard parts grow in jumps, only when they molt.
- Calling spiders, centipedes and pill bugs insects. Only arthropods with three body parts and six legs are insects.

Make it a game · Insect or Not?. One player names or shows a picture of a small creature: ant, spider, beetle, centipede, butterfly, tick, pill bug, grasshopper, scorpion, bee, earthworm, dragonfly. The others must shout "Insect!" or "Not!" and give the reason (count the legs and body parts). Earn one point for the right call and one more for the right reason. Bonus point: name the place where that creature's air goes in.

Scripture. "Go to the ant, thou sluggard; consider her ways, and be wise:" (Proverbs 6:6, KJV)

Solomon told the lazy man to study an insect. Scientists like Malpighi and Fabre took that advice literally, and found in the ant and the silkworm both lessons for living and wonders of design.

Practice answers

1. Head: antennae, eyes or mouthparts. Thorax: six legs (and wings in most adults). Abdomen: spiracles (it also holds most of the digestive and reproductive organs).
2. No. Insects have six legs and three body parts. A spider is an arachnid, another kind of arthropod.
3. Air enters through the spiracles along the sides of the thorax and abdomen and reaches the cells through the branching tracheae (tracheal tubes).
4. Its exoskeleton is hard and cannot stretch much, so it must shed the old one and grow a larger one. Right after molting the new exoskeleton is soft, so the insect is weak, slow and easily hurt or eaten until it hardens.
5. 1.0 × 1.3 = 1.3 mm; 1.3 × 1.3 = 1.69 mm, about 1.7 mm.
6. (a) Independent: temperature. Dependent: average crawling speed (cm/min). (b) The warmer the temperature, the faster the mealworms crawled; speed rose by roughly 2 to 2.6 cm/min for every 6 degrees. (c) About halfway between 3.5 and 6.1, so about 4.8 cm/min.
7. (a) 0.52 ÷ 0.40 = 1.30; 0.68 ÷ 0.52 = 1.31; 0.88 ÷ 0.68 = 1.29. (b) The ratio is nearly the same each time, about 1.3, which suggests each group is one molt (one growth stage) larger than the one before. (c) 0.88 × 1.3 = about 1.14 mm.
8. Area = 6 × 9 = 54 cm^2; volume = 27 cm^3; ratio = 54 ÷ 27 = 2:1. That is one third of the 1 cm cube's ratio, so the larger body has much less surface for its volume.
9. Sample design. Question: Do mealworms use more oxygen (and give off more carbon dioxide) when warm than when cool? Independent variable: temperature (for example 15 °C and 25

°C). Dependent variable: carbon dioxide given off, measured by the color change of a few drops of bromothymol blue indicator in a small dish sealed in a jar with 10 mealworms for 20 minutes (or with an inexpensive CO_2 sensor). Control: an identical jar with oats but no mealworms. Keep the same: the number and size of mealworms, the jar size, the time, the amount of indicator. The mealworms are removed and returned unharmed. A faster color change at higher temperature shows the insects' tracheae are taking in air for respiration.

10. Insects get oxygen to their cells by air moving through tracheae, mostly by diffusion in the smallest tubes, which is quick only over short distances of a few millimeters. In a dog-sized beetle the tubes would have to reach much farther into a much larger volume of tissue, and its surface area would be small compared with its volume, so oxygen could not reach the deep cells fast enough. Large animals need lungs and a closed system of blood vessels with oxygen-carrying blood to deliver oxygen over long distances.

7.4 The Giraffe's Neck

OBJECTIVES

- Tell how Robert Goetz in the 1950s and Alan Hargens in the 1980s measured blood pressure and leg pressure in living giraffes, and what they found.
- Explain how gravity changes blood pressure with height, and describe the giraffe's strong heart, thick-walled arteries, vein valves and tight leg skin.
- Calculate pressure differences from height using about 77 mmHg per meter of blood, and use them to compare a giraffe with a person.

Warm-up. Hang your arm straight down by your side for one minute and look at the veins on the back of your hand. Then raise your hand high above your head for one minute and look again. What changed? Now imagine a neck two meters long. Write one sentence about what the heart at the bottom of that neck would have to do.

Video. Coming soon. How a giraffe's heart and blood vessels handle its long neck and legs, and giraffe anatomy (seven neck bones, drinking posture); search Smithsonian's National Zoo, San Diego Zoo Wildlife Alliance, NASA (giraffe research for astronaut health), Homeschool Pop (Alternate), and African wildlife nature footage.

KEY TERMS

blood pressure
The push of blood against the walls of the blood vessels, made by the heart's pumping. It is highest in the arteries and changes with height because of gravity.

millimeters of mercury (mmHg)
The unit doctors and biologists use for blood pressure: how high (in millimeters) the pressure could push a column of mercury. A column of blood 1 meter tall presses with about 77 mmHg.

cervical vertebrae
The bones of the neck. Giraffes, people and nearly all mammals have seven.

left ventricle
The strongest chamber of the heart, which pumps blood out to the whole body. In a giraffe its wall is especially thick.

valve
A flap in the heart or in a vein that lets blood flow only one way. Valves in a giraffe's jugular veins help stop blood from rushing back to the head when it lowers its neck.

edema
Swelling caused when fluid is pushed out of the blood vessels into the surrounding tissues, often in the lower legs.

CORE IDEAS

The story. In South Africa in the 1950s, a heart surgeon named Robert Goetz wanted to know how a giraffe gets blood to its brain. A full-grown male giraffe can stand about 5.5 meters tall, and its brain may sit some two meters above its heart. Goetz and his colleagues worked with giraffes in the field, placed thin tubes in their arteries and recorded the pressure. The readings near the heart were roughly twice as high as a healthy person's, among the highest blood pressures known in any animal. Thirty years later, in the 1980s, a team led by Alan Hargens, a scientist who studied how gravity affects the body and whose work interested NASA, measured the pressure in giraffes' legs and asked a new question: why don't a giraffe's feet swell up?

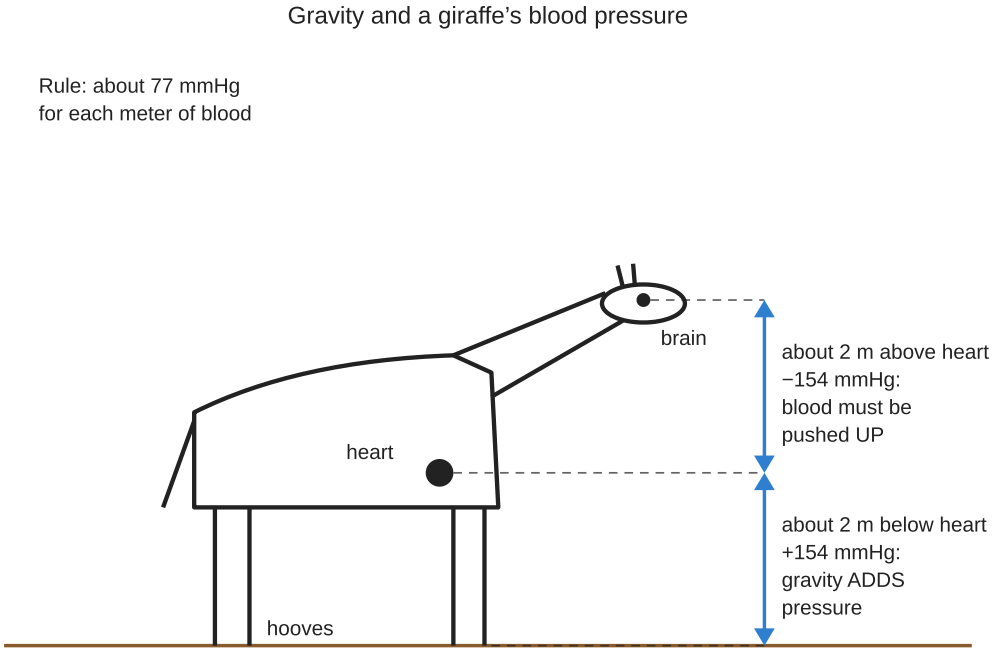
How they figured it out. Blood is a liquid, and every liquid presses harder the deeper you go, the way your ears feel the pressure at the bottom of a pool. A column of blood one meter tall presses on the bottom with about 77 millimeters of mercury (mmHg). So to push blood 2 meters *up* to the brain, a giraffe's heart needs about 2 × 77 = 154 mmHg just to lift it, before it has anything left over to drive the blood through the brain. Going *down*, gravity adds the same amount: the arteries in the lower legs, about 2 meters below the heart, carry pressures far higher than any in your body. Hargens found that the giraffe's legs are wrapped in thick, tight skin and tough tissue under it, which press inward like a pilot's pressure suit or a nurse's compression stocking. That squeeze keeps fluid from leaking out of the vessels and causing **edema**.

The big idea. A giraffe's whole body is built for life at two heights at once. Its heart can weigh around 11 kilograms, and the wall of its **left ventricle** is unusually thick, so it can pump at high pressure. Its leg arteries have thick, muscular walls that stand up to that pressure. When it lowers its head to drink, its head drops below its heart, and a sudden rush of blood could harm the brain; valves in the large jugular veins of the neck, together with other adjustments in how blood flows to the head, help protect it. Astonishingly, its long neck has only **seven cervical vertebrae**, the same number as yours. Each one is simply much longer, some more than 25 centimeters.

In the world today. Giraffes use their height to browse leaves from the tops of acacia trees, where few other animals can reach, and males swing their heavy necks at one another in contests called necking. Giraffes vary in height, spot pattern and neck length, and they belong to the same family as the forest-dwelling okapi. NASA scientists have studied giraffe legs because astronauts face the opposite problem: in orbit, without gravity pulling fluid down, it shifts toward their heads. Doctors, too, use what giraffes teach about compression stockings to help patients whose legs swell after long hours of standing or sitting.

Try it and see. You can feel the effect of height on pressure in your own body. When your hand hangs down, the veins on its back fill and bulge; raised overhead, they flatten. Fill a plastic bottle with water, poke holes at different heights, and the water squirts farther from the lower holes because the pressure is greater there. That is exactly what happens in a giraffe's legs.

God’s handiwork. A long neck by itself would be a problem; a long neck with a strong heart, pressure-proof arteries, one-way valves and a built-in compression suit is a solution. “Hearken unto this, O Job: stand still, and consider the wondrous works of God” (Job 37:14). When you see a giraffe bend gracefully to drink and lift its head again without fainting, you are seeing a design that took scientists decades of careful measurement to understand.



Gravity changes a giraffe’s blood pressure by about 77 mmHg for every meter above or below the heart.

PICTURE STUDY



The Nubian Giraffe, Jacques-Laurent Agasse, 1827 · Oil on canvas · Royal Collection, United Kingdom

In 1827 the ruler of Egypt, Muhammad Ali, sent a young giraffe as a gift to King George IV of England. It was the first living giraffe most English people had ever heard of, and the king kept it at Windsor. The Swiss-born painter Jacques-Laurent Agasse, who was famous for painting animals accurately, was asked to paint it soon after it arrived.

Agasse showed the giraffe with the two keepers who had traveled with it from Africa, the English animal dealer Edward Cross, and the cows whose milk was used to feed it. The giraffe was still young and not yet full height, but the long neck and legs that puzzled scientists for so long are already plain to see.

Look closely

- Compare the giraffe's height with the men beside it. About how many times taller is its head than a man's?
- Look at the slope of the back from shoulders to tail. Why do the front legs look longer than the back legs?
- Find the cows in the background. Why might they be in a painting of a giraffe?
- Look at the giraffe's head and neck. Where would its heart be, and how far would its blood have to climb?

Talk about it

1. Agasse painted the giraffe carefully, like a naturalist, rather than making it look fierce or strange. Why would accuracy matter to people who had never seen a giraffe?
2. Using what you learned, what might happen to this giraffe's blood when it lowers its head to drink?
3. How do zoos today help people learn about animals from far away?

> **Safety first.** An adult should poke the holes in the bottle with a pushpin or the tip of a nail. Do the bottle experiment outdoors or in a sink, and wipe up spills so no one slips. If you do the blood-flow warm-up, stop if you feel dizzy, and do not press on your neck.

EXPERIMENT: DOES WATER PRESSURE GROW WITH DEPTH?

A giraffe's feet feel far more blood pressure than its head because of the weight of the blood above them. You can measure the same effect with water. Your question: *Does water squirt farther from a hole lower in a bottle, where there is more water above it?*

Write your prediction first. Then connect your results to a giraffe standing on the savanna.

You will need

- A tall, empty 2-liter plastic bottle with the label removed
- A pushpin or small nail (adult use)
- Waterproof tape (such as duct tape) and a ruler
- A permanent marker
- A sink, tub or outdoor table, and a long tray or cookie sheet to catch water

- Water and a pitcher; lab notebook and pencil

Steps

1. With a ruler and marker, mark four spots in a straight line up the side of the bottle, 5 cm, 10 cm, 15 cm and 20 cm above the bottom. Have an adult poke a hole of the same size at each mark.
2. Cover all four holes with one strip of tape. Fill the bottle to a line 25 cm above the bottom, and set it at the edge of the tray.
3. Write the variables. What you change: the depth of the hole below the water surface. What you keep the same: the hole size, the starting water level, the bottle and the table height. What you measure: the distance each stream lands from the bottle, in centimeters.
4. Hold the water level at the 25 cm line by pouring in more water as needed. Pull off the tape and, right away, mark or measure where each stream lands on the tray.
5. Repeat three times and average the distances for each hole.
6. Record your data in a table: Hole height (cm), Depth below surface (cm), Trial 1, Trial 2, Trial 3, Average distance (cm). Then make a bar graph.
7. Write a conclusion that explains the pattern and compares the bottom hole to a giraffe’s feet and the top hole to its head.

Tips

- Depth below the surface = 25 cm − the hole height. The hole at 5 cm is 20 cm deep.
- If you cannot keep the level steady, measure the streams in the first two seconds after removing the tape, while the level has barely changed.
- The streams are a sign of pressure, not an exact measure of it, but a stronger push sends water farther.

Check your work

- I wrote a prediction and named my variables.
- I did three trials and averaged them.
- My table and graph show that deeper holes squirt farther.
- I can explain why a giraffe’s legs have the highest blood pressure in its body and how its tight skin helps.

NATURE NOTEBOOK

1. Draw a giraffe from the side and mark its brain, heart and hooves. Draw arrows showing which way gravity works against the blood and which way it adds pressure.
2. Draw a person’s neck skeleton and a giraffe’s neck skeleton side by side, each with seven vertebrae. Label the cervical vertebrae and write the count beside each.
3. Draw a giraffe bending to drink, with its front legs spread. Label the jugular vein and draw tiny valves in it, and write one sentence about why they matter.

From history. In 1809 the French naturalist Jean-Baptiste Lamarck published *Zoological Philosophy*, in which he used the giraffe as an example of his idea that animals change their bodies by use and pass those changes to their offspring. Lamarck was a careful classifier who did important work on invertebrates (he helped give the word “biology” its modern meaning), but his giraffe idea did not survive testing. When Gregor Mendel’s work on heredity was rediscovered in 1900, scientists learned that traits are passed on through inherited factors, now called genes, not through what parents do with their bodies.

WORKED EXAMPLES

Example 1. A column of blood 1 m tall presses with about 77 mmHg. A giraffe’s brain is about 2.0 m above its heart. A person’s brain is about 0.3 m above the heart. How much pressure does each heart need just to lift blood up to the brain?

(Use the map or drawing “Lifting blood 2 m takes about 154 mmHg.” shown earlier in this lesson.)

1. Giraffe: 2.0 m × 77 mmHg per m = 154 mmHg.
2. Person: 0.3 m × 77 mmHg per m = 23.1 mmHg, about 23 mmHg.
3. Compare: 154 ÷ 23 = about 6.7. The giraffe’s heart needs almost 7 times as much pressure just for the climb.

Answer: Giraffe about 154 mmHg; person about 23 mmHg.

Example 2. Suppose the average pressure in a giraffe’s arteries at heart level is 200 mmHg, and its hooves are 2.0 m below its heart. Estimate the average artery pressure at its hooves. Then compare it with a person whose average artery pressure at the heart is 95 mmHg and whose feet are 1.2 m below the heart.

1. Giraffe: going down adds pressure. 2.0 m × 77 = 154 mmHg. 200 + 154 = 354 mmHg at the hooves.
2. Person: 1.2 m × 77 = 92.4 mmHg. 95 + 92.4 = 187.4, about 187 mmHg at the feet.
3. Ratio: 354 ÷ 187 = about 1.9. The giraffe’s feet feel nearly twice the pressure of yours, which is why its tight leg skin and thick-walled arteries matter.

Answer: About 354 mmHg at the giraffe’s hooves, compared with about 187 mmHg in a person’s feet.

Example 3. A giraffe’s neck has 7 cervical vertebrae averaging about 25 cm long. A person’s neck also has 7, averaging about 2 cm long. Find the length of each neck’s column of vertebrae and how many times longer the giraffe’s is.

1. Giraffe: 7 × 25 cm = 175 cm, or 1.75 m.
2. Person: 7 × 2 cm = 14 cm.
3. Ratio: 175 ÷ 14 = 12.5.

Answer: About 175 cm for the giraffe and 14 cm for the person; the giraffe’s is about 12.5 times longer, with the same number of bones.

PRACTICE

1. EASY How many neck vertebrae does a giraffe have? How many do you have?
2. EASY Why do a giraffe’s legs not swell with fluid even though the pressure in them is very high?
3. EASY What job do the valves in a giraffe’s jugular veins do?
4. MEDIUM How much pressure does a column of blood 1.5 m tall add? (Use 77 mmHg per meter.)
5. MEDIUM Data reading. A student made this table using 77 mmHg per meter of blood:
   0.5 m: 39 mmHg
   1.0 m: 77 mmHg
   1.5 m: 116 mmHg
   2.0 m: 154 mmHg
   2.5 m: ?
   (a) What kind of graph would these points make? (b) Fill in the missing value. (c) A calf’s brain is 1.2 m above its heart. How much pressure does its heart need just to lift blood that high?
6. MEDIUM Data reading. In the bottle project, a student got these average distances (water level held at 25 cm):
   Hole at 20 cm (5 cm deep): 6 cm
   Hole at 15 cm (10 cm deep): 11 cm
   Hole at 10 cm (15 cm deep): 15 cm
   Hole at 5 cm (20 cm deep): 18 cm
   (a) Name the independent and dependent variables. (b) Describe the trend. (c) Which hole is most like a giraffe’s hooves, and why?
7. HARD When a giraffe lowers its head to drink, its head can drop about 1 m below its heart. If its brain was 2 m above its heart before, by how much does the pressure from gravity at its brain change?
8. HARD In 1809 the French naturalist Lamarck suggested that giraffes got long necks because each generation stretched to reach leaves and passed the longer necks on to their young. Explain why this idea fails, using an example from people.

9. CHALLENGE Experiment design. Some travelers wear compression socks on long car or plane trips. Design a fair test of whether compression socks reduce ankle swelling during 2 hours of sitting. Give the question, the variables, what to keep the same, how to measure, and the control.
10. CHALLENGE In a short paragraph, explain the two opposite pressure problems a giraffe faces at its head and at its feet, and name one feature of its body that solves each.

COMMON MISTAKES

- Thinking a giraffe has more neck bones than a person. Both have seven; the giraffe's are much longer.
- Thinking blood pressure is the same all over the body. Gravity makes it lower above the heart and higher below it, by about 77 mmHg per meter of height.
- Thinking a giraffe got its neck by stretching, and passed the stretch to its calves. Traits gained by use are not inherited.

> **Make it a game · Up or Down?.** A caller names a spot on a giraffe: brain, heart, knee, hoof, jaw, shoulder, ankle, tip of the tail. Players stand tall for spots above the heart, crouch for spots below it, and stand still with hands on chest for the heart itself. Then the caller gives a height, such as "1 meter below the heart," and the first player to call out the pressure change (plus or minus 77 mmHg per meter) wins a point.

> **Scripture.** "Hearken unto this, O Job: stand still, and consider the wondrous works of God." (Job 37:14, KJV)

Elihu told Job to stop and look. Robert Goetz and Alan Hargens did exactly that with the giraffe, and every answer they found showed another part fitted to the whole.

Practice answers

1. Seven each. The giraffe's are much longer.
2. Thick, tight skin and tough tissue underneath squeeze the legs like a compression stocking, and the leg arteries have thick walls, so fluid is not pushed out into the tissues.
3. They let blood flow only one way, toward the heart, which helps stop blood from rushing back down into the head when the giraffe lowers its head to drink.
4. 1.5 × 77 = 115.5 mmHg, about 116 mmHg.
5. (a) A straight line through zero: the pressure is directly proportional to the height. (b) 2.5 × 77 = 192.5, about 193 mmHg. (c) 1.2 × 77 = 92.4, about 92 mmHg.
6. (a) Independent: depth of the hole below the water surface. Dependent: distance the stream lands. (b) The deeper the hole, the farther the water squirts, because the pressure is greater with more water above it. (The distances grow a little less each time.) (c) The bottom hole, 20 cm deep, because it has the most liquid above it, just as the hooves are farthest below the heart.
7. It moves from 2 m above to 1 m below the heart, a change of 3 m. 3 × 77 = 231 mmHg. The pressure at the brain would rise by about 231 mmHg if nothing protected it, which is why vein valves and other controls matter.

8. Changes an animal gains during its life, such as stretched muscles, are not passed to its offspring through heredity. A weightlifter’s strong arms or a pianist’s skilled fingers are not inherited by their children; traits are passed on through what is in the parents’ cells from the start, not through use.
9. Sample design. Question: Do compression socks reduce ankle swelling during 2 hours of sitting? Independent variable: wearing compression socks or ordinary socks. Dependent variable: change in ankle circumference, measured with a tape measure at the same marked spot before and after. Keep the same: the same people, the same 2 hours of sitting, the same chair and room, the same time of day, the same drinks, the same measuring spot and person measuring. Control: the same person on a different day wearing ordinary socks. Test several volunteers and average the changes.
10. At its head, the problem is too little pressure: the heart must push blood about 2 m uphill, which takes about 154 mmHg, so the giraffe has a powerful heart with a thick-walled left ventricle that pumps at very high pressure. At its feet, the problem is too much pressure: gravity adds about 154 mmHg more, which could burst vessels or squeeze fluid into the tissues, so the giraffe has thick-walled leg arteries and tight, thick skin that acts like a compression stocking. (When it drinks, valves in the jugular veins help protect the head.)

Unit 7 review

Answer each question. Show your work for every calculation, include units, and use complete sentences for explanations. The test is worth 60 points.

1. (5 pts) Multiple choice. Fish take oxygen from (a) the oxygen atom in each water molecule, (b) oxygen gas dissolved in the water, (c) air they swallow at the surface every few seconds, (d) the food they eat. Explain your choice in one sentence.
2. (5 pts) Water enters a fish’s gills with 10.0 mg/L of dissolved oxygen and leaves with 2.5 mg/L. What percent of the oxygen did the fish remove? If it pumps 0.4 L of water per minute, how many milligrams of oxygen does it take in each minute?
3. (5 pts) Fresh water holds about 14.6 mg/L of oxygen at 0 °C and about 7.6 mg/L at 30 °C. Describe the pattern and explain why trout need cool streams.
4. (6 pts) Diagram item. Draw a gill lamella as two side-by-side channels. Label the water flow and the blood flow with arrows going the correct directions, and write sample oxygen levels (in percent) at four points along each channel that show countercurrent exchange working. Explain in one sentence why oxygen keeps moving into the blood.
5. (5 pts) Put in order the path of one puff of air through a bird, and tell how many breaths it takes: lung, anterior air sacs, trachea (out), posterior air sacs, trachea (in).
6. (5 pts) Multiple choice. Which statement about birds is correct? (a) Oxygen enters the blood in the air sacs. (b) Bird lungs expand and shrink like balloons. (c) Air flows through bird lungs in one direction during both inhaling and exhaling. (d) Birds use a diaphragm to breathe.
7. (5 pts) Oxygen is 21% of the air. Air pressure is 101 kPa at sea level and about 47 kPa at 6,000 m. Find the oxygen pressure at each and the fraction left at 6,000 m.
8. (5 pts) Name the three body parts of an insect, tell where its six legs attach, and explain how oxygen reaches its muscle cells.
9. (6 pts) A caterpillar’s head is 0.60 mm wide and grows by a ratio of 1.4 at each molt. Find its width after two molts. Then explain why it grows in steps instead of a little each day.
10. (5 pts) A column of blood 1 m tall presses with about 77 mmHg. A giraffe’s brain is 2.0 m above its heart and its hooves are 2.0 m below. (a) How much pressure does the heart need just to lift blood up to the brain? (b) If the average pressure at its heart is 200 mmHg, estimate the average artery pressure at its hooves.
11. (8 pts) Short answer. (a) How many neck vertebrae does a giraffe have? (b) Name two features of a giraffe’s body that help it handle high blood pressure in its legs or changes in pressure when it lowers its head, and explain each. (c) Name one thing an experiment would need to be a fair test of whether compression socks reduce ankle swelling.

Unit project: four ways to breathe: a comparative physiology field guide

Make a four-page illustrated field guide (or a large folded poster) with one page each for the fish, the bird, the insect and the giraffe. On each page, draw a labeled diagram of how that

animal gets oxygen to its cells or moves its blood (gills with countercurrent arrows, the bird's two-breath path, the insect's spiracles and tracheae, the giraffe's pressure from head to hoof), write a short paragraph naming the problem the animal faces and how its body solves it, and include one calculation from the unit with its work shown.

Add a closing page with a comparison table (columns: Animal, Breathing organ, Which way air or water flows, One special feature) and attach the data table and graph from one of your four experiments, with a short conclusion.

- **Accuracy**: Diagrams and labels are scientifically correct: arrows go the right way, parts are named correctly, and the comparison table is complete.
- **Calculations and data**: Each page has a correct calculation with units, and the attached experiment has a complete data table, a labeled graph and a conclusion that matches the data.
- **Explanation**: Each paragraph clearly states the problem the animal faces (little oxygen in water, flight, small body, long neck) and how its body is designed to solve it.
- **Care and craftsmanship**: The guide is neat, colorful, carefully drawn like a naturalist's notebook, and easy for another student to read.

Answer key

1. (b). Fish cannot split water molecules; their gills take in the oxygen gas (O_2) dissolved in the water.
2. Removed: 10.0 − 2.5 = 7.5 mg/L; 7.5 ÷ 10.0 = 75%. Per minute: 7.5 mg/L × 0.4 L = 3.0 mg of oxygen.
3. Cold water holds about twice as much dissolved oxygen as warm water; as temperature rises, dissolved oxygen falls. Trout are active fish that need a lot of oxygen, and warm water does not hold enough, so they live in cool, well-oxygenated streams.
4. Water and blood arrows point in opposite directions. Sample water values 100, 70, 40, 15 (in its direction of flow) and blood values beside them 90, 60, 30, 5 (blood entering at 5 beside water at 15 and leaving at 90 beside water at 100). At every point the water has more oxygen than the blood next to it, so oxygen keeps diffusing into the blood along the whole lamella. (Award points for correct opposite arrows, labels, values where water is always higher, and the explanation.)
5. Trachea (in), posterior air sacs, lung, anterior air sacs, trachea (out). It takes two complete breaths (two inhales and two exhales).
6. (c). The air sacs pump air through small, stiff lungs in one direction; gas exchange happens in the lungs, and birds have no diaphragm.
7. Sea level: 0.21 × 101 = 21.2 kPa. 6,000 m: 0.21 × 47 = 9.9 kPa. Fraction: 9.9 ÷ 21.2 = 0.47, a little less than half.
8. Head, thorax and abdomen; the six legs attach to the thorax. Air enters through spiracles on the sides of the thorax and abdomen and travels through branching tracheae directly to the cells, so the blood does not need to carry much oxygen.
9. 0.60 × 1.4 = 0.84 mm; 0.84 × 1.4 = 1.176, about 1.18 mm. Its hard exoskeleton cannot stretch, so it grows only when it molts, sheds the old exoskeleton, and expands the new soft one before it hardens.

10. (a) 2.0 × 77 = 154 mmHg. (b) Going down adds the same amount: 200 + 154 = 354 mmHg at the hooves.
11. (a) Seven, the same as a person. (b) Any two: thick, tight leg skin and tissue that squeeze like a compression stocking and keep fluid from leaking out (prevents edema); thick-walled leg arteries that withstand the high pressure; valves in the jugular veins that stop blood from rushing back to the head when it drinks; a powerful heart with a thick left ventricle that pumps blood up to the brain. (c) Any one: a control (same person with ordinary socks), the same sitting time, the same measuring spot and method, several volunteers averaged.

UNIT 8

Dissection Lab

Pictures in a book can show you where the organs go, but nothing teaches anatomy like the real thing. In this unit you will open a preserved earthworm and a frog (or work through a virtual frog dissection), take apart an owl pellet bone by bone, and finish with a timed lab practical like the ones in high-school and college biology. You will see with your own eyes how cells, tissues and organs are built into whole animals, and how each animal is fitted to its own way of life.

You will watch Charles Darwin test his pet earthworms with a bassoon and a piano for his 1881 worm book, stand beside Luigi Galvani in Bologna as a frog's leg kicks at the touch of a scalpel, time Gilbert White's barn owls with a pocket watch in Selborne, and sit with young Samuel Scudder as Louis Agassiz tells him, again and again, to look at his fish. Along the way you will measure, count segments, calculate heart rates and organ percents, key out skulls, find the minimum number of animals in a pellet and graph an owl's diet.

Dissection is serious work. You will use sharp tools and preserved specimens with care, wear goggles and gloves, and treat every animal with respect, remembering that each one is a creature God made "after his kind." Families who prefer not to do a real dissection can use a virtual one for Lesson 8.2 and still complete every measurement and drawing.

BEFORE YOU BEGIN

- Knows the levels of organization (cell, tissue, organ, organ system) and the main organelles of animal cells (Unit 2)
- Can use a microscope, find total magnification and estimate size from the field of view (Unit 1)
- Knows how fish, birds and insects breathe and how their bodies are built (Unit 7)
- Can calculate a percent, an average and a rate, and draw a bar graph with labeled axes

MASTERY CHECKLIST

- I can dissect an earthworm safely and identify its segments, setae, clitellum, digestive organs, aortic arches, blood vessels and nerve cord.
- I can identify the external features, mouth parts and main internal organs of a frog and explain how its heart and breathing differ from mine.
- I can sort the bones in an owl pellet, key out the prey skulls, find the minimum number of animals and graph the owl's diet.
- I can compare digestion, circulation, breathing, nervous control and support in an earthworm, a frog and an owl and its prey.
- I can calculate real sizes from scale bars and magnification, heart rates from short counts, and percents from class data.
- I can pass a timed lab practical by naming real structures and their jobs.

OPTIONAL BOOKS AND RESOURCES

- **Carolina Biological Supply** (https://www.carolina.com/): A long-established science supplier that sells preserved earthworms and frogs, heat-sterilized owl pellets, dissecting kits, trays and bone charts. Order specimens a few weeks before Lesson 8.1.
- **Home Science Tools** (https://www.homesciencetools.com/): A supplier aimed at homeschool families, with single preserved specimens, owl pellets, dissection kits and free how-to articles on earthworm, frog and owl pellet dissections.
- **Charles Darwin's *The Formation of Vegetable Mould, through the Action of Worms* (free at Project Gutenberg)** (https://www.gutenberg.org/ebooks/search/?query=vegetable+mould+darwin): Free. Read aloud the first chapter, where Darwin describes testing his worms with light, sound and the piano (Lesson 8.1). Older students may enjoy the chapter on how worms drag leaves into their burrows.

8.1 Earthworm Dissection ★

OBJECTIVES

- Describe how Charles Darwin studied earthworms by patient observation and simple experiments, and explain what worms do for the soil.
- Identify the external and internal parts of an earthworm (segments, setae, clitellum, pharynx, crop, gizzard, intestine, aortic arches, dorsal and ventral blood vessels, brain and ventral nerve cord) and state the job of each.
- Calculate population density from a soil sample, map organs by segment number, and read data from an earthworm choice experiment.

Warm-up. Lay a ruler beside your own forearm and count how many centimeters it is from wrist to elbow. Now imagine your body were built in rings like a stack of coins, each ring with its own muscles and its own little set of organs. Write two things that would be easier for a body built that way and two things that would be harder. Keep your list; you will check it after the dissection.

Video. Coming soon. Earthworm anatomy and earthworm dissection walkthrough, how earthworms move and make soil (search biology classroom dissection channels, Carolina Biological, Home Science Tools, Smithsonian, Homeschool Pop (Alternate), Moody Science classics, nature footage of worms surfacing and burrowing).

KEY TERMS

segment
One of the ring-shaped sections that make up an earthworm’s body. Earthworms belong to the phylum Annelida, from a Latin word meaning “little rings.”

setae (singular seta)
Tiny stiff bristles on the underside and sides of most segments. Earthworms have four pairs per segment and use them to grip the soil while they crawl.

clitellum
The thick, smooth band nearer the front end of an adult earthworm (about segments 32 to 37 in the common nightcrawler) that makes the cocoon in which eggs develop.

gizzard
A thick, muscular part of the digestive tube that grinds food with the help of swallowed sand grains.

aortic arches
Five pairs of muscular blood vessels near the front of the earthworm that pump blood from the dorsal vessel to the ventral vessel. They are often called the worm’s “hearts.”

closed circulatory system
A system in which blood always stays inside blood vessels as it travels through the body, as in earthworms and people.

CORE IDEAS

The story. In October 1881, an old naturalist in the village of Downe, in Kent, England, published a small book with a long title: *The Formation of Vegetable Mould, through the Action of Worms, with Observations on their Habits*. The author was Charles Darwin. For about forty years he had watched the earthworms in the fields and lawns around Down House. He kept worms in pots of soil in his study and tested them like a patient detective. Could they hear? His son played the bassoon at them, Darwin whistled and shouted, and the worms did not stir. But when the pots were set on top of a piano and a note was struck, the worms quickly pulled back into their burrows. They could not hear sound in the air, but they felt the vibration through the solid piano. Darwin shone lights on them at night and found that the front end of the worm was the most sensitive to light, even though worms have no eyes.

How they figured it out. Darwin wanted to know how much work worms really do, so he measured. He collected and weighed the little heaps of soil, called **castings**, that worms leave on the surface after passing earth through their bodies. Years after chalk and cinders had been spread over one of his fields, a trench was dug, and the white layer lay in a neat line about 18 centimeters (7 inches) below the turf. The worms had slowly carried fine soil up from below and dropped it on top. Darwin found the same quiet burying at work on fallen stones and on the floors of old Roman buildings in England. He closed his book by saying he doubted whether many other animals had played so important a part in the history of the world as these lowly creatures. (Darwin is best known for a different book, whose theory of origins you will weigh in Grade 8. Here we admire what anyone can check: his careful, repeatable observations.)

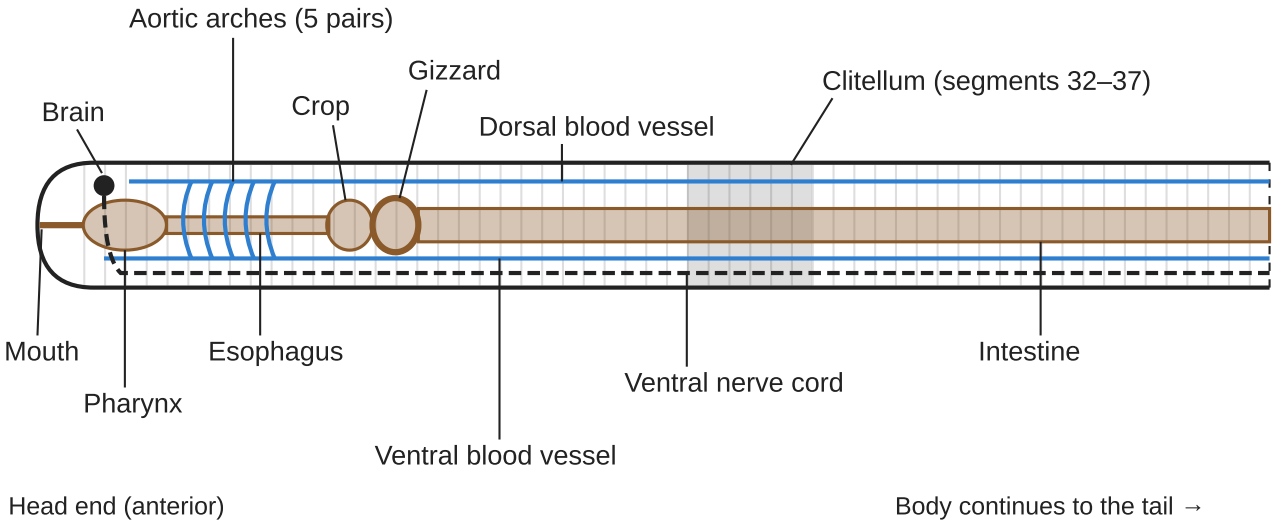
The big idea: the outside. An earthworm is an **annelid**, a segmented worm. A common nightcrawler (*Lumbricus terrestris*) has roughly 100 to 150 **segments**. Its body has bilateral symmetry: a left and right side that match, an *anterior* (front) end with the mouth and a *posterior* (back) end with the anus, a darker rounded *dorsal* (back) side and a paler, flatter *ventral* (belly) side. The thick band called the **clitellum** sits nearer the front, which is the quickest way to tell head from tail. Run a finger gently backward along the belly and you can feel the **setae**. The worm has no lungs. Oxygen passes in through its thin, moist skin, which is why a worm left on a dry sidewalk soon dies. The worm crawls by squeezing two sets of muscles in turn: circular muscles make a segment long and thin, and lengthwise muscles make it short and fat, while the setae hold it in place.

The big idea: the inside. The digestive tube runs straight from end to end. Soil and bits of dead leaves enter the **mouth**, are sucked in by the muscular **pharynx**, pass down the narrow **esophagus**, are stored in the thin-walled **crop** and ground up in the thick **gizzard**. The long **intestine** digests and absorbs the food; a fold along its top, the typhlosole, gives it extra surface. Blood stays inside vessels, so the worm has a **closed circulatory system**. It flows forward in the dorsal blood vessel, down through five pairs of **aortic arches** near the esophagus, and back along the ventral blood vessel. A small brain sits above the pharynx, and a **ventral nerve cord** runs along the belly side to the tail. Tiny tubes in nearly every

segment remove wastes. Each earthworm has both male and female parts, but two worms still pair up, and afterward the clitellum forms a cocoon that holds the eggs.

Try it and see. In a dissection you open a preserved worm along its dorsal side, pin back the body wall and look at each organ in place. Because the organs sit in particular segments, you can make a segment map: in a nightcrawler the aortic arches lie in about segments 7 to 11, the crop and gizzard in about segments 15 to 18, and the clitellum in about 32 to 37. Count carefully, and your own worm should come close.

God’s handiwork. Darwin called worms lowly, and in a way they are: no eyes, no ears, no bones. Yet they plow the soil without a plow, mix fallen leaves into the earth and open tiny tunnels that let air and rain reach the roots of plants. When God put Adam in the garden “to dress it and to keep it,” He had already filled the ground with these quiet helpers. A good gardener learns to care for them.



The front end of an earthworm, side view. Digestive tube in brown, blood vessels in blue, nerve cord dashed. The body continues to the anus at the tail end.

PICTURE STUDY



Chaetopoda, Plate 96 from Kunstformen der Natur (Art Forms in Nature), Ernst Haeckel (drawings); lithograph by Adolf Giltsch, 1904 (plates issued 1899–1904) · Color lithograph in a printed book · Published in Leipzig, Germany; copies in natural history libraries, and scanned copies online

Ernst Haeckel was a German zoologist who spent his life drawing tiny sea creatures, and his book *Art Forms in Nature* gathered one hundred of his most beautiful plates. This one shows **polychaetes**, the bristle worms of the sea. Their name means “many bristles.” They are annelids, like the earthworm. Earthworms belong to a group called *oligochaetes*, “few bristles,” because they have only small setae instead of the long tufts you see here.

You have seen Haeckel’s plates of diatoms, ciliates and mushrooms earlier in this course. Compare how he drew those with how he drew these worms.

Look closely

- Find a worm whose segments you can count. How does Haeckel show where one segment ends and the next begins?
- Find the bristles and the flaps along the sides. How are they like and unlike the earthworm’s tiny setae?
- Look at the heads. Which worms have tentacles or feathery fans? What might those be used for in the water?
- Notice the symmetry. Does each worm have matching left and right sides?

Talk about it

1. Why do you think a sea worm needs long bristles and flaps while an earthworm in a burrow has only short setae?

2. Haeckel arranged real animals into a pattern that looks almost like a design on a carpet. Does making a science picture beautiful help you look at it more carefully?

> **Safety first.** Use only preserved specimens bought from a biological supply company. Wear safety goggles and nitrile gloves, work in a well-ventilated room, and keep food and drinks out of the work area. Scissors and scalpels are sharp: cut away from your fingers, keep the blade low and shallow, and have an adult nearby. Rinse a preserved specimen in water before you begin, and wash your hands well afterward even though you wore gloves. Wrap leftover specimen parts in newspaper, seal them in a bag and put them in the outdoor trash. If you watch live worms, keep them moist and cool, use only a dim lamp, handle them gently and return them to the soil where you found them.

EXPERIMENT: EARTHWORM DISSECTION AND SEGMENT MAP

You will dissect a preserved earthworm the way biology students do, find its organs in place and record in which segments each one lies. Then you will compare your segment map with the textbook pattern.

Question: In which segments are the aortic arches, crop, gizzard and clitellum found, and does my worm match the usual pattern? **Prediction:** Write the segment numbers you expect. **What we measure:** the segment number where each organ begins and ends, the total number of segments and the length of the worm in centimeters. **What we keep the same:** we always count from the mouth (segment 1) backward, using the same hand lens and probe. **Record:** a labeled drawing and a segment table.

You will need

- Preserved earthworm (nightcrawler size) from a biological supply company
- Dissecting tray with a wax or foam bottom (a foam meat tray or a piece of thick cardboard will do)
- About 15 T-pins or long straight pins
- Fine dissecting scissors, a dissecting probe or blunt needle, forceps (tweezers) and a hand lens
- Metric ruler, paper towels and a small cup of water with a dropper
- Safety goggles and nitrile gloves
- Lab notebook and pencil

Steps

1. Rinse the worm in water and blot it. Measure its length in centimeters and record it.
2. Find the mouth, the anus and the clitellum. Decide which side is dorsal (darker and rounder) and which is ventral (flatter and paler). Use the hand lens to find the setae on the ventral side.
3. Count the segments from the mouth to the front edge of the clitellum, then count the segments of the clitellum. If you have time, count the whole worm. Record the numbers.

4. Lay the worm dorsal side up in the tray. Put one pin through the head end and one through the tail end, angled outward.
5. Starting just behind the clitellum, slide the tip of the scissors under the skin, slightly to one side of the dark dorsal blood vessel. Point the tip upward and make a shallow cut forward all the way to the mouth. Cut only the body wall, not the organs beneath.
6. With the probe, gently tear the thin walls (septa) between segments and spread the body wall open. Pin the flaps back at an angle every few segments. Add a few drops of water to keep the organs moist.
7. Find, from front to back: the pharynx, esophagus, aortic arches wrapped around the esophagus, crop, gizzard and intestine. Find the dorsal blood vessel on top of the gut.
8. For each organ, count the segment number where it begins and ends and record it in a table with columns: Organ, Segments, Color and texture, Job.
9. Gently lift the gut to one side to see the white ventral nerve cord running along the floor of the body. Find the small brain just above the pharynx.
10. Draw your dissection in your notebook, label at least ten parts, and write the magnification as “life size” or “×2” if you drew it larger.
11. Compare your table with the lesson: aortic arches about 7 to 11, crop and gizzard about 15 to 18, clitellum about 32 to 37. Write two sentences on how close your worm came and why worms might differ.

Tips

- Parent note: the lesson mentions that the clitellum forms an egg cocoon; nothing more about reproduction is needed for this dissection.
- Keep the scissors tip pointed up as you cut. Most ruined dissections come from cutting too deep.
- The crop is thin and soft; the gizzard just behind it is firm and feels gritty. Squeeze each gently with forceps to tell them apart.
- If a real dissection is not right for your family, use a good virtual earthworm dissection and do the same counting and drawing from it.

Check your work

- I wore goggles and gloves and cut away from my fingers.
- My drawing has at least ten correct labels and shows dorsal and ventral sides.
- My table gives the segment numbers for at least five organs.
- I compared my results with the usual pattern and gave a reason for any difference.

NATURE NOTEBOOK

1. Draw the outside of an earthworm from the side, life size, and label the mouth, anus, clitellum, segments, setae, dorsal side and ventral side.
2. Draw the digestive tube as a straight line of shapes from mouth to anus, and under each part write one word for its job (suck, carry, store, grind, absorb).

3. After a rain, find an earthworm outdoors. Record the date, weather, soil and where you found it, sketch it, measure its length without hurting it, and return it to damp soil.

From history. A hundred years before Darwin's book, the English country clergyman Gilbert White of Selborne, in Hampshire, wrote to a friend in 1777 that "earth-worms, though in appearance a small and despicable link in the chain of Nature, yet, if lost, would make a lamentable chasm." He noticed that worms loosen the soil so rain can soak in, drag straws and leaves into the ground and throw up heaps of fine earth. His letter was printed in *The Natural History of Selborne* (1789), one of the most loved nature books ever written in English. Darwin had read it as a boy.

WORKED EXAMPLES

Example 1. A student digs up a square of pasture 50 cm by 50 cm to the depth of a spade and sorts through the soil. She finds 14 earthworms. Estimate the number of earthworms per square meter and per hectare (10,000 m²).

1. Area of the sample: 0.50 m × 0.50 m = 0.25 m².
2. Worms per square meter: 14 ÷ 0.25 = 56 worms per m².
3. Worms per hectare: 56 × 10,000 = 560,000 worms.
4. This is an estimate. Taking several samples in different spots and averaging them would make it more reliable.

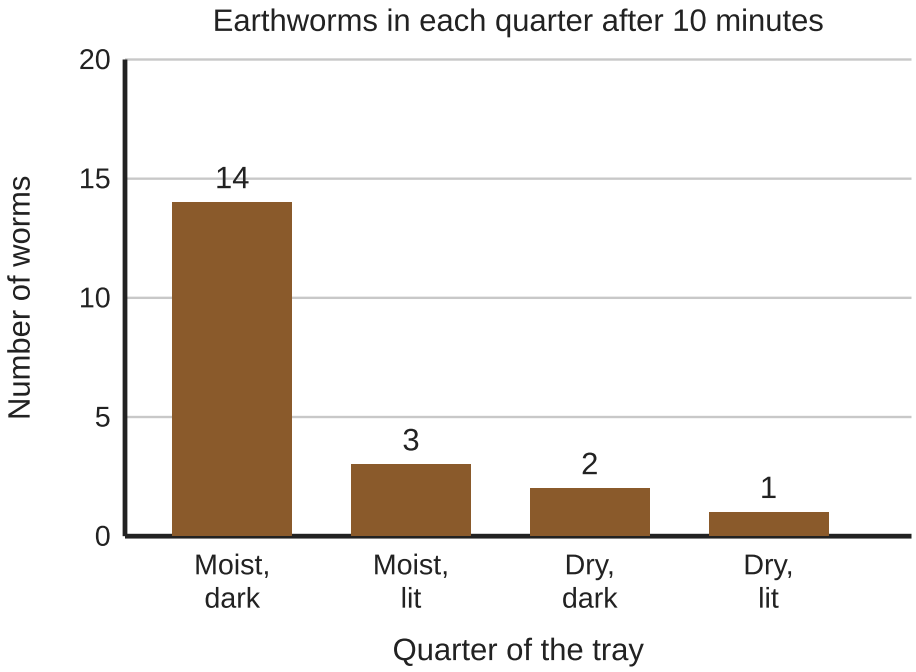
Answer: About 56 earthworms per square meter, or about 560,000 per hectare.

Example 2. A preserved worm is 18 cm long and has 144 segments. The clitellum begins at segment 32. (a) What fraction and percent of the segments lie in front of the clitellum? (b) Using the average segment length, estimate the distance from the mouth to the front of the clitellum.

1. Segments in front of the clitellum: segments 1 through 31, so 31 segments.
2. Fraction: 31 ÷ 144 ≈ 0.215, or about 21.5%.
3. Average segment length: 18 cm ÷ 144 = 0.125 cm = 1.25 mm.
4. Estimated distance: 31 × 1.25 mm ≈ 39 mm, or about 3.9 cm.
5. Real segments are not all the same length, so a ruler check on the actual worm is the final word.

Answer: (a) 31/144, about 21.5% of the segments. (b) About 3.9 cm from the mouth to the clitellum.

Example 3. In a choice experiment, 20 earthworms were placed in the center of a tray divided into four equal quarters: moist and dark, moist and lit, dry and dark, dry and lit (a dim lamp, not hot). After 10 minutes the worms were counted. Use the bar graph to find the percent in the moist, dark quarter, the percent in moist soil overall and the percent in the dark overall. What do the data suggest?



Where 20 earthworms went after 10 minutes in a four-way choice tray (class data).

1. Read the bars: moist and dark 14, moist and lit 3, dry and dark 2, dry and lit 1. Check: 14 + 3 + 2 + 1 = 20.
2. Moist and dark: 14 ÷ 20 × 100 = 70%.
3. All moist soil: (14 + 3) ÷ 20 × 100 = 85%.
4. All dark soil: (14 + 2) ÷ 20 × 100 = 80%.
5. If the worms moved at random, about 25% (5 worms) would end up in each quarter. Both moisture and darkness drew far more than that.

Answer: 70% chose the moist, dark quarter; 85% were in moist soil and 80% in the dark. The worms strongly preferred moist, dark places, which fits a skin that must stay damp and a body that avoids light.

PRACTICE

1. EASY What does the name Annelida mean, and what feature of the earthworm does it describe?
2. EASY Put these parts of the earthworm’s digestive tube in order from front to back: intestine, crop, mouth, gizzard, esophagus, pharynx, anus.

3. EASY How does an earthworm get oxygen, and why must its skin stay moist?
4. MEDIUM You are handed a preserved worm with no label. Describe how to find (a) the front end and (b) the dorsal side.
5. MEDIUM A worm 20 cm long has 160 segments. What is the average length of one segment in millimeters?
6. MEDIUM Data reading. Pots of the same soil were given different numbers of worms. After 2 weeks the castings on top of each pot were collected and weighed: 0 worms, 0 g; 5 worms, 11 g; 10 worms, 23 g; 20 worms, 45 g. (a) Describe the relationship. (b) Predict the mass of castings for 15 worms. (c) Which conclusion is best supported? A. Each worm makes about 2 g of castings in 2 weeks. B. Castings come from the soil, not the worms. C. More worms make less castings.
7. MEDIUM Data reading. Worms counted in equal soil samples at different soil moisture levels: 10%, 2 worms; 20%, 9 worms; 30%, 18 worms; 40%, 21 worms; 60% (waterlogged), 6 worms. (a) At which moisture were the most worms found? (b) Describe the trend from 10% to 40%. (c) Suggest a reason for the drop at 60%.
8. HARD Explain why the gizzard has thick, muscular walls and why the intestine has a fold called the typhlosole.
9. HARD Experiment design. A student wants to know whether earthworms prefer soil with chopped leaves mixed in over plain soil. Write the question, the independent and dependent variables, three things to keep the same, how many worms and trials to use, and one rule for caring for the worms.
10. CHALLENGE Darwin’s worms ignored a loud bassoon but pulled back when a note was struck on the piano they were sitting on. What did this show? How could he be sure it was not the sight or sound of the player that made them move?

COMMON MISTAKES

- Thinking an earthworm has five real hearts like ours. It has five pairs of muscular aortic arches that pump blood, but no chambered heart.
- Thinking a worm cut in half becomes two worms. If the worm is cut behind the clitellum, the front part can sometimes heal and grow a short new tail, but the cut-off tail end does not grow a new head and dies.
- Thinking the clitellum is in the middle or near the tail. It is closer to the head end, and that is the key to telling front from back.

Make it a game · Segment Street. Tape a line of 40 index cards on the floor and number them 1 to 40 as an earthworm’s segments. One player calls out an organ (pharynx, aortic arches, crop, gizzard, intestine, clitellum, brain). The other must walk to the right segment number and say the organ’s job and which body system it belongs to. A correct answer scores a point. Then switch, and finish with a peristalsis race: crawl across the room by stretching long and thin, then pulling short and fat, like a worm.

Scripture. “And the LORD God took the man, and put him into the garden of Eden to dress it and to keep it.” (Genesis 2:15, KJV)

God gave people the work of tending the earth, and He gave them helpers in the soil. Every earthworm is a tiny gardener, plowing and feeding the ground. Caring for the soil and its creatures is part of our calling to dress and keep the world.

Practice answers

1. It means “little rings.” It describes the earthworm’s body, which is built of many ring-shaped segments.
2. Mouth, pharynx, esophagus, crop, gizzard, intestine, anus.
3. Oxygen passes into its blood through its thin skin; it has no lungs. Oxygen must dissolve in a film of moisture on the skin to pass through, so a dried-out worm cannot breathe.
4. (a) The clitellum is closer to the front end, and the mouth is at that end. (b) The dorsal side is darker and more rounded (the dorsal blood vessel shows through as a dark line); the ventral side is flatter and paler, and you can feel the setae there.
5. 20 cm = 200 mm. 200 mm ÷ 160 = 1.25 mm per segment.
6. (a) As the number of worms increases, the mass of castings increases in nearly a straight line (roughly doubling when the worms double). (b) About 34 g (halfway between 23 g and 45 g). (c) A. For example, 45 g ÷ 20 worms ≈ 2.3 g and 23 g ÷ 10 ≈ 2.3 g per worm. B is wrong because the pot with no worms made no castings.
7. (a) 40%. (b) The number of worms rose as the soil got wetter, from 2 to 21. (c) Waterlogged soil has its air spaces filled with water, so there is little oxygen for the worms to take in through their skin; many leave or come to the surface.
8. Earthworms have no teeth. The thick muscular gizzard squeezes food against swallowed sand grains to grind it into small bits. The typhlosole folds the intestine wall inward, giving it more surface area so it can digest and absorb more food along the same length of gut.
9. Question: Do earthworms choose soil with chopped leaves over plain soil? Independent variable: leaves or no leaves in each half of a container. Dependent variable: number of worms in each half after a set time (for example, 1 hour). Keep the same: soil type, moisture, temperature, light (both halves dark), and the size of each half. Use at least 10 worms and repeat 3 times, switching which side has the leaves to rule out a side preference. Care: keep the soil moist and cool, handle gently and return the worms to the garden afterward.
10. It showed that worms do not detect sound waves in the air but do sense vibrations passing through a solid. To be sure, he could play the same note on the piano with the pots set on a separate table nearby (same sound in the air, no vibration through the pot); if the worms reacted only when the pots touched the piano, vibration through the solid was the cause. Keeping the player hidden and the light the same would rule out sight.

8.2 Frog Dissection (Real or Virtual)

OBJECTIVES

- Tell how Luigi Galvani’s frog experiments in Bologna led to the discovery that nerves carry electrical signals.
- Identify the external features, mouth structures and main internal organs of a frog, and explain how its three-chambered heart and its skin breathing differ from ours.
- Calculate a heart rate from a short count, find an organ’s percent of body mass and read a graph of heart rate against temperature for an ectotherm.

Warm-up. Put two fingers on the side of your neck and count your pulse for 15 seconds. Multiply by 4 to get beats per minute. Now do 20 jumping jacks and count again. Write both numbers. Predict: would a frog’s heart rate change if the frog got colder or warmer? Write your guess and one reason.

Video. Coming soon. Frog dissection walkthrough and frog anatomy, plus Galvani and animal electricity (search biology classroom dissection channels, Carolina Biological, Home Science Tools, virtual frog dissection demonstrations, Smithsonian, Moody Science classics, nature footage of frogs feeding and calling).

KEY TERMS

tympanum
The round eardrum on the outside of a frog’s head, just behind each eye.

nictitating membrane
A clear third eyelid that slides up over a frog’s eye to protect it and keep it moist, especially under water.

atrium and ventricle
The chambers of the heart. An atrium (plural atria) receives blood coming in; a ventricle pumps blood out. A frog has two atria and one ventricle.

cloaca
A shared chamber at the end of the frog’s gut into which the digestive, urinary and reproductive systems all empty before leaving the body.

cutaneous respiration
Breathing through the skin. Oxygen passes into a frog’s blood through its thin, moist, blood-rich skin.

ectotherm
An animal whose body temperature rises and falls with its surroundings, such as a frog, fish or earthworm. Its body processes speed up when it is warm and slow down when it is cold.

CORE IDEAS

The story. In the 1780s, in Bologna, Italy, a professor of anatomy named Luigi Galvani was studying the nerves and muscles of frogs. One day a prepared frog lay on a table near an electrical machine, the kind that made sparks when its glass wheel was turned. As someone touched a nerve in the frog's leg with the tip of a metal scalpel, a spark jumped from the machine, and the dead frog's leg kicked hard, as if alive. Galvani set out to find out why. He hung frog legs by brass hooks on an iron railing outside and watched them twitch during thunderstorms, and later found that they twitched even on calm days when the brass hook was pressed against the iron. In 1791 he published his results in a Latin book on the powers of electricity in muscle movement.

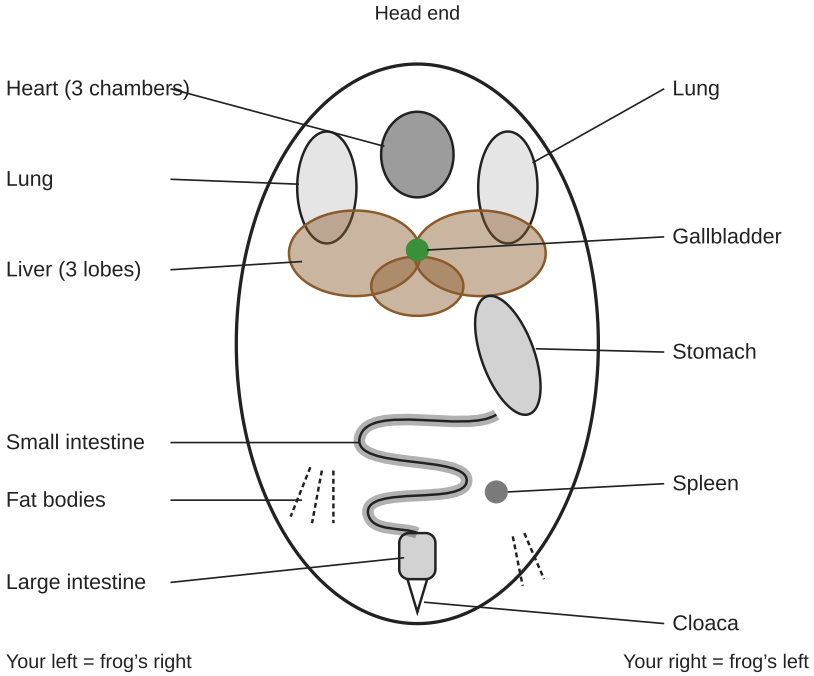
How they figured it out. Galvani believed the frog's own body held "animal electricity" that flowed along its nerves. Another Italian scientist, Alessandro Volta, disagreed. He thought the electricity came from the two different metals touching the wet frog. To prove it, Volta stacked disks of two metals separated by cardboard soaked in salt water, and in 1800 he announced the first battery. Each man was partly right. Two metals and a salty liquid really do make a current, and nerves really do carry tiny electrical signals. Today we know that a nerve impulse is electrical. Frogs became the favorite animal of early physiology labs because their muscles and nerves are large, simple to reach and keep working for a while after death.

The big idea: outside and the mouth. A frog is an **amphibian**, a vertebrate that usually begins life in water as a tadpole and lives as an adult on land and in water. Outside, look for the bulging eyes with their clear **nictitating membrane**, the nostrils, and the round **tympanum** behind each eye. The front feet have four toes and the long, powerful hind legs have five webbed toes. Open the mouth wide. The sticky tongue is attached at the *front* of the mouth, so it can flip out and snap back. Tiny teeth line the upper jaw, and two small patches of vomerine teeth on the roof of the mouth help grip prey; frogs do not chew but swallow whole. You will also find the internal nostrils on the roof, the openings of the Eustachian tubes at the back corners, the slit-shaped **glottis** leading to the lungs and the wider opening of the esophagus. A frog even pulls its eyeballs down into its head to help push food down its throat.

The big idea: inside. Open the belly and the organs are packed in order. At the top lies the small **heart**, with two atria and one ventricle, so oxygen-rich blood from the lungs and oxygen-poor blood from the body partly mix in the single ventricle. Beside it are two spongy, balloon-like **lungs**. The large brown **liver**, with three lobes, covers much of the front of the body cavity, and a small greenish gallbladder sits beneath it. The **stomach** curves on the frog's left side and leads to the coiled small intestine, held in loops by a thin sheet called the mesentery, and then to the short large intestine and the **cloaca**. Look also for the pancreas, the small round red spleen, the dark kidneys against the back wall and yellow, finger-like fat bodies that store energy for winter. In spring a female may be full of tiny black-and-white eggs, and a male has two small yellowish testes near the kidneys. A frog breathes with its lungs, through the lining of its mouth and through its moist skin. In cold water during winter it can get enough oxygen through its skin alone.

Try it and see. You may dissect a preserved frog or use a good virtual dissection; families choose differently, and both teach the same structures. Either way, work in order: outside, mouth, then the body cavity from front to back. Name each organ, say its job and say which body system it belongs to. Compare each one with the matching organ in your own body. Remember that the frog's left is on your right when it lies on its back facing you.

God's handiwork. A frog has a heart, lungs, liver, stomach, intestines and kidneys arranged much like ours, yet each is fitted to its own life: a three-chambered heart that works well for a creature that can slow down in cold mud for a whole winter, and skin that breathes. David looked at his own body and wrote that he was "fearfully and wonderfully made." Looking closely at a frog teaches us the same thing.



Main organs of a frog, ventral view (frog on its back). The frog's left side is on your right.

PICTURE STUDY



Plate 3 from Historia naturalis ranarum nostratium (Natural History of Our Native Frogs), August Johann Rösel von Rosenhof, 1758 · Hand-colored copper engravings in a printed book · Published in Nuremberg, Germany; copies in natural history libraries, and scanned copies online

August Johann Rösel von Rosenhof was a miniature painter and engraver in Nuremberg who loved to study small animals. After years of drawing insects, he spent much of his later life on a whole book about the frogs and toads of his own country. He drew them life size from living animals and showed them from different sides, along with their eggs and tadpoles. Each plate was colored by hand.

Naturalists have praised the book ever since for its accuracy. Rösel drew what he saw, not what he expected to see, which is exactly what you must do at the dissecting tray.

Look closely

- Find the tympanum behind the eye. How large is it compared with the eye?
- Look at the hind feet. Count the toes and look for the webbing between them.
- Look at the skin pattern. How might these colors help the frog in the grass or at the edge of a pond?
- If the plate shows eggs or tadpoles, put the stages in order from youngest to oldest.

Talk about it

1. Why do you think Rösel drew the frogs life size and from more than one side?
2. Rösel published his frog book about a generation before Galvani’s experiments. What could a careful drawing teach that an experiment could not, and the other way around?

> **Safety first.** For a real dissection, use only a preserved frog from a biological supply company. Wear safety goggles and nitrile gloves, work in a well-ventilated room, and keep food and drinks away. Rinse the specimen in water before you start. Scissors and scalpels are sharp: cut away from your fingers, lift the skin with forceps before cutting, and keep an adult nearby. Wash your hands well afterward. Wrap the remains in newspaper, seal them in a bag and put them in the outdoor trash. Never collect or harm wild frogs for this lesson.

EXPERIMENT: FROG DISSECTION: ORGAN MAP AND MEASUREMENTS

You will dissect a preserved frog (or work through a virtual dissection), map its organs, and take measurements that let you compare the frog with yourself.

Question: Is a frog's small intestine longer than its body, and how large is its liver compared with the whole frog? **Prediction:** Write your guesses as numbers. **What we measure:** snout-to-vent length (tip of the nose to the cloaca opening), the length of the uncoiled small intestine and, if you have a kitchen scale reading to 0.1 g, the masses of the whole frog and of the liver. **What we keep the same:** the same ruler and scale, and each measurement taken twice and averaged. **Record:** a labeled drawing and a data table.

You will need

- Preserved frog from a biological supply company, or a computer with a virtual frog dissection
- Dissecting tray, about 10 T-pins, fine scissors, forceps, a probe and a hand lens
- Metric ruler, a length of string and (optional) a kitchen scale that reads to 0.1 g
- Paper towels and a cup of water
- Safety goggles and nitrile gloves
- Lab notebook, pencil and colored pencils

Steps

1. Rinse and blot the frog. Weigh it if you have a scale. Measure the snout-to-vent length twice and record the average.
2. Outside: find and sketch the eyes, nictitating membrane, nostrils, tympanum, front feet (4 toes) and hind feet (5 webbed toes). Feel the skin.
3. Mouth: cut the corners of the jaw hinge so the mouth opens wide. Find the tongue (attached at the front), upper-jaw teeth, vomerine teeth, internal nostrils, Eustachian tube openings, glottis and esophagus. Slide a probe into each opening to see where it leads.
4. Lay the frog on its back and pin its four feet to the tray. Lift the belly skin with forceps and make a shallow cut up the middle from just above the cloaca to the jaw. Make short side cuts at the top and bottom, and pin the skin flaps back.
5. Lift the thin muscle wall underneath and cut it the same way. Near the front legs you will meet the breastbone; cut carefully through it to one side and pin the flaps open.

6. Find, in order: heart, lungs, liver (count its lobes) and gallbladder. Lift the liver to see the stomach on the frog’s left side. Follow the small intestine and its mesentery, the spleen, large intestine and cloaca. Find the fat bodies, and the kidneys against the back wall.
7. Draw and label your dissection in color with at least twelve parts. Next to each organ, write its job and its body system.
8. Cut the small intestine free where it leaves the stomach and where it joins the large intestine. Gently uncoil it along the ruler, or lay string along it and then measure the string. Record its length.
9. If you have a scale, remove the liver and weigh it. Calculate the liver’s percent of body mass and the ratio of intestine length to snout-to-vent length.
10. Write a conclusion: Was your prediction right? How does the frog’s heart differ from yours? Which organs looked most like the ones in the pictures of a human body?

Tips

- Parent note: a frog dissected in spring may contain eggs or visible reproductive organs, which the lesson names in one plain sentence. Preview the body cavity first if you wish.
- Cut skin and muscle separately and always lift with forceps before cutting, so the scissors never plunge into the organs.
- In a virtual dissection, do every measurement the program allows and use its scale bar to measure the rest from the screen.
- The heart of a preserved frog is small and firm. Use the hand lens to find where the two atria sit above the single ventricle.

Check your work

- I worked safely with goggles and gloves, or completed the full virtual dissection.
- My drawing has at least twelve correct labels.
- My data table has the snout-to-vent length, intestine length and their ratio (and liver percent if I weighed it).
- My conclusion compares the frog’s heart and breathing with mine.

NATURE NOTEBOOK

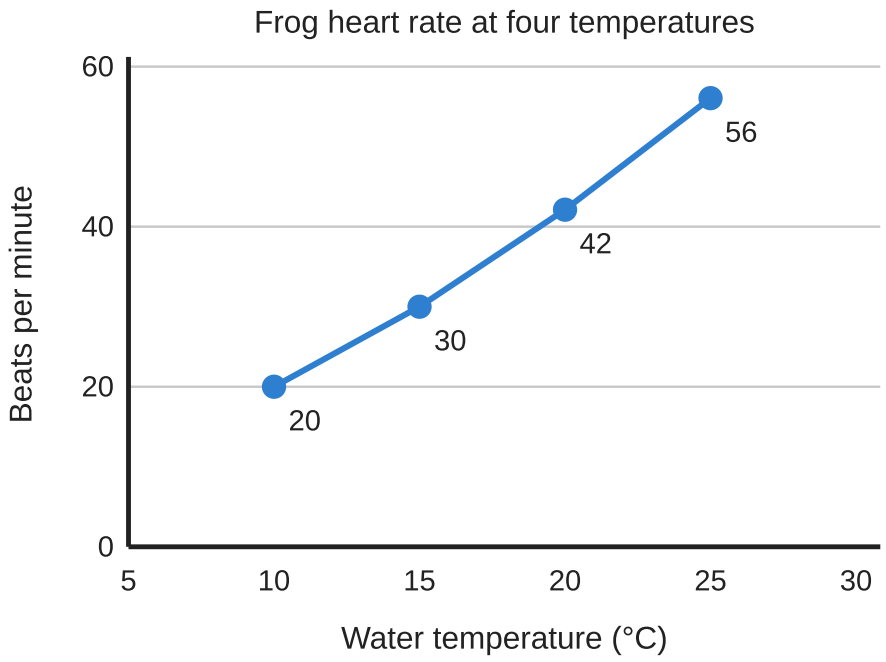
1. Draw the open mouth of a frog as if looking in from the front, and label the tongue, vomerine teeth, internal nostrils, Eustachian tube openings, glottis and esophagus.
2. Make a two-column chart, “Frog” and “Me,” comparing heart chambers, ways of breathing, skin and how food leaves the body.
3. Sketch the four stages of a frog’s life (egg, tadpole, tadpole with legs, young frog) from a pond visit, a book or a video, with the date and a note on what changes at each stage.

> **From history.** Galvani’s name lives on in everyday English. To *galvanize* people means to jolt them into action, the way the spark jolted the frog’s leg, and galvanized steel, iron coated with zinc to keep it from rusting, carries his name as well. Volta is remembered

too: the unit of electrical push, the *volt*, is named for him. Volta described his new battery in a letter to Sir Joseph Banks, president of the Royal Society of London, in 1800.

WORKED EXAMPLES

Example 1. In a virtual frog-heart lab, a student counts 11 beats in 15 seconds at 20 °C. (a) What is the heart rate in beats per minute? (b) The graph shows the heart rate at four temperatures. By how many beats per minute does the rate change for each 1 °C rise between 10 °C and 25 °C?



Frog heart rate at four temperatures (virtual lab data).

1. (a) There are four 15-second periods in a minute: 11 × 4 = 44 beats per minute. The graph shows 42, close to this count.
2. (b) Read the graph: 20 beats per minute at 10 °C and 56 at 25 °C.
3. Change in rate: 56 − 20 = 36 beats per minute.
4. Change in temperature: 25 − 10 = 15 °C.
5. Rate of change: 36 ÷ 15 = 2.4 beats per minute for each degree.

Answer: (a) 44 beats per minute. (b) About 2.4 beats per minute faster for each 1 °C warmer. The frog is an ectotherm, so its heart speeds up as it warms.

Example 2. A preserved frog has a mass of 45.0 g. Its liver has a mass of 2.2 g. What percent of the frog's mass is liver? An adult person of 60 kg has a liver of about 1.5 kg. Compare.

1. Frog: 2.2 g ÷ 45.0 g × 100 ≈ 4.9%.
2. Person: 1.5 kg ÷ 60 kg × 100 = 2.5%.
3. Ratio: 4.9 ÷ 2.5 ≈ 2. Compared with its body size, this frog's liver is about twice as large as a person's.
4. A frog stores food energy in its liver (and in its fat bodies) to last through a winter without eating.

Answer: The liver is about 4.9% of the frog's mass, roughly twice the share of a person's liver (about 2.5%).

Example 3. A student measures a frog's snout-to-vent length as 8.8 cm and 9.2 cm, and its uncoiled small intestine as 17.5 cm and 18.5 cm. Find each average and the ratio of intestine length to body length.

1. Body length: (8.8 + 9.2) ÷ 2 = 9.0 cm.
2. Intestine length: (17.5 + 18.5) ÷ 2 = 18.0 cm.
3. Ratio: 18.0 ÷ 9.0 = 2.0, or 2 : 1.
4. The intestine is twice as long as the body, so it must be coiled to fit. A plant-eating tadpole has an even longer, more tightly coiled intestine, which shortens as it changes into a meat-eating frog.

Answer: Body 9.0 cm, intestine 18.0 cm; the intestine is 2 times the body length (2 : 1).

PRACTICE

1. EASY What did Luigi Galvani observe about frog legs, and what did he think caused it?
2. EASY Where is a frog's tongue attached, and why does that help it catch insects?
3. EASY Name the three ways a frog takes in oxygen.
4. MEDIUM How many chambers does a frog's heart have, and what are they? How is this different from a human heart?
5. MEDIUM A frog's heart beats 9 times in 20 seconds. What is its rate in beats per minute?
6. MEDIUM Data reading. Using the heart-rate graph (10 °C: 20, 15 °C: 30, 20 °C: 42, 25 °C: 56 beats per minute): (a) Between which two temperatures did the rate rise the most? (b) Estimate the rate at 12.5 °C. (c) Which statement is supported? A. A frog's heart rate stays the same at all temperatures. B. A frog's heart rate rises as its surroundings warm. C. A frog keeps its body at 37 °C.
7. MEDIUM Data reading. Sample data for a frog resting in water: at 5 °C, 70% of its oxygen comes through the skin and 30% through the lungs; at 25 °C, 30% comes through the

skin and 70% through the lungs. (a) Describe how the share through the skin changes as the water warms. (b) Suggest why a frog can stay under water all winter but must come up to breathe in summer.

8. HARD A frog has a mass of 38 g and its liver is 1.9 g. What percent of its mass is liver? Give one reason a frog might need a large liver.
9. HARD Experiment design. A homeschool co-op wants to know whether students learn frog anatomy as well from a virtual dissection as from a real one. Design a fair test: give the question, the independent and dependent variables, a way to make the two groups fair, and three things to keep the same.
10. CHALLENGE Galvani said the electricity was in the frog; Volta said it came from the two metals. Explain how each man was partly right, and what Volta's battery proved.

COMMON MISTAKES

- Thinking a frog's heart is the same as ours. A frog has three chambers (two atria, one ventricle); people, other mammals and birds have four.
- Thinking a frog drinks water through its mouth. Frogs take in water mainly through their skin, which is also why polluted or dry surroundings harm them quickly.
- Mixing up left and right in a dissection. When the frog lies on its back facing you, its left side is on your right, so its stomach appears on your right.

Make it a game · Organ Draw. Write the names of fifteen frog structures on cards (tympanum, nictitating membrane, vomerine teeth, glottis, heart, lungs, liver, gallbladder, stomach, small intestine, mesentery, spleen, fat bodies, kidney, cloaca). Shuffle. A player draws a card and has 20 seconds to give its job and its body system. Correct answers score 1 point; naming the matching organ in the human body (or saying there is none) scores a bonus point. Highest score after three rounds wins.

Scripture. "I will praise thee; for I am fearfully and wonderfully made: marvellous are thy works; and that my soul knoweth right well." (Psalm 139:14, KJV)

When you see a frog's heart, lungs and liver, you are seeing a small version of the organs God placed in your own body. Studying them carefully should lead us to the same praise David gave.

Practice answers

1. A dead frog's leg twitched when its nerve was touched with metal while an electrical machine sparked nearby (and later when brass and iron touched it). He thought the frog's body had its own "animal electricity" that flowed through its nerves.
2. At the front of the mouth. The free back end can be flipped far out of the mouth and snapped back with the prey stuck to it.
3. Through its lungs, through the moist lining of its mouth and through its moist skin (cutaneous respiration).
4. Three: two atria and one ventricle. A human heart has four chambers (two atria and two ventricles), which keeps oxygen-rich and oxygen-poor blood fully separate; in the frog they

partly mix in the single ventricle.

5. There are three 20-second periods in a minute: 9 × 3 = 27 beats per minute.
6. (a) Between 20 °C and 25 °C (a rise of 14 beats per minute, compared with 10 and 12). (b) About 25 beats per minute (halfway between 20 and 30). (c) B.
7. (a) It falls from 70% to 30% as the water warms. (b) In cold water the frog’s body processes are slow and it needs little oxygen, so the skin can supply most of it. In warm water it is more active and needs much more oxygen than its skin can take in, so it must use its lungs at the surface.
8. 1.9 ÷ 38 × 100 = 5%. The liver stores food energy (and the frog also stores fat in its fat bodies) to last through months of winter when it does not eat; the liver also makes bile for digestion.
9. Question: Do students who do a virtual frog dissection score as well on an anatomy quiz as students who do a real dissection? Independent variable: type of dissection (virtual or real). Dependent variable: score on the same identification quiz. Fair groups: assign students to groups at random (for example by drawing names), with similar ages and past grades in each group. Keep the same: the lesson time, the list of structures to find, the teacher’s instructions, the quiz and when it is given (for example, one week later). Use as many students as possible in each group.
10. Volta showed that two different metals with a salty liquid between them make an electric current even with no frog at all; his stacked-disk battery (1800) proved it. Galvani was right that nerves and muscles work by electrical signals; later scientists measured these tiny currents in living nerves. In his metal-hook experiments the metals supplied the current, and the frog’s nerve carried it and made the muscle contract.

8.3 Owl Pellet Review

OBJECTIVES

- Explain how an owl forms and casts a pellet, and why owl pellets preserve whole bones that scientists can use to study an owl's diet.
- Sort bones from a pellet by type, identify prey skulls with a dichotomous key, and find the smallest number of animals the bones could come from.
- Pool class data into percents and a bar graph, and use a food chain and the 10 percent rule to explain an owl's place in its ecosystem.

Warm-up. Eat a small handful of sunflower seeds in the shell, or picture doing it: you keep the seed and spit out the shell. Make a list of three foods where we leave behind a part we cannot digest (shells, peels, pits, bones). Then guess: an owl swallows a mouse whole. What do you think happens to the fur and bones?

Video. Coming soon. Barn owl hunting at night and casting a pellet, owl pellet dissection and bone identification (search nature footage of barn owls, Cornell Lab of Ornithology, Smithsonian, Home Science Tools, Carolina Biological, Homeschool Pop (Alternate), Science Max).

KEY TERMS

owl pellet
A compact bundle of fur, bones, teeth, feathers and other hard parts that an owl cannot digest, formed in its gizzard and coughed up through its mouth.

regurgitate
To bring material back up from the stomach and out through the mouth. Owls regurgitate pellets; this is called casting.

gizzard
The muscular second part of a bird's stomach. In an owl it holds back hard parts and squeezes them into a pellet.

incisor
A front cutting tooth. Rodents have one pair of large, curved, chisel-like incisors in each jaw that keep growing all their lives.

mandible
The lower jaw. In a mouse or vole it comes apart into a left and a right half, so two halves come from one animal.

food chain
A path showing who eats whom and how energy moves from producers (plants) to consumers, such as seeds to vole to owl.

CORE IDEAS

The story. In the village of Selborne, in Hampshire, England, the clergyman and naturalist Gilbert White watched a pair of “white owls,” the bird we call the barn owl, that nested every year under the eaves of the village church. About an hour before sunset, when the mice began to run, the owls flew out and hunted low along the hedges and meadows. White stood outside with his watch in his hand and timed them. He found that one or the other returned to the nest with a mouse about once every five minutes. He also wrote that owls, like hawks, cast up the bones, fur and feathers of what they eat. His notes were printed in *The Natural History of Selborne* in 1789. If you dissected an owl pellet in an earlier year, this lesson takes you further: you will measure, count, key out and graph like a field biologist.

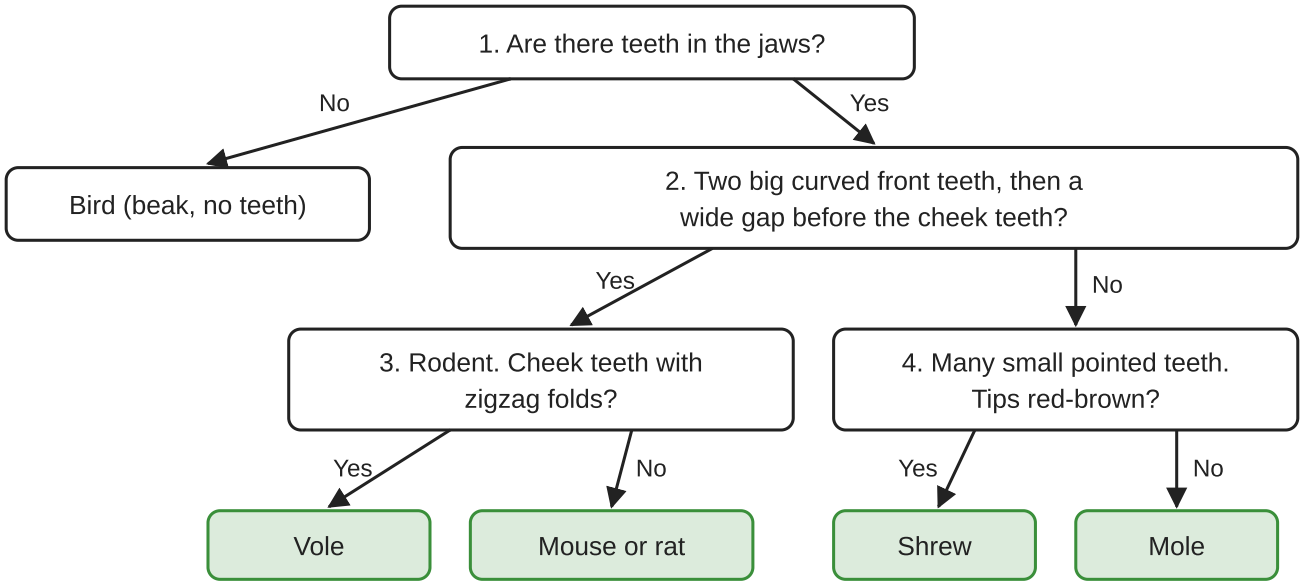
How they figured it out. An owl swallows small prey whole or in large pieces. It has no crop, so the food goes straight to the first part of its stomach, where digestive juices begin to work on the soft parts. The meal then passes to the muscular **gizzard**. Soft tissue is digested and moves on to the intestine, but the gizzard holds back fur, bones, teeth, claws, feathers and the hard wings of beetles and squeezes them into a tight, oval pellet. Some hours later the owl **regurgitates** the pellet, usually once or twice a day, and it usually cannot swallow its next meal until it does. Hawks and eagles also cast pellets, but their stronger stomach acid dissolves much of the bone. An owl’s digestion is gentler, so the bones come out nearly whole and clean. Because of this, biologists collect pellets under owl roosts to learn which small mammals live in an area without setting a single trap.

The big idea: reading the bones. A pellet holds skeletons in pieces. Sort them by type, just as you studied bones in earlier grades: the **skull**; the lower jaw halves, or **mandibles**; vertebrae; ribs; shoulder blades; the long bones of the front leg (humerus, radius and ulna) and back leg (femur, tibia and fibula); and the two halves of the hip, or pelvis. Skulls and jaws tell you what kind of animal it was. A bird has a beak and no teeth. A rodent such as a vole, mouse or rat has two large, curved, yellow-orange **incisors** at the front of each jaw, then a wide gap, then flat cheek teeth. Vole cheek teeth have zigzag folds on top; mouse and rat cheek teeth have rounded bumps. A shrew, a tiny insect eater, has a continuous row of many small pointed teeth, often with red-brown tips. To count animals, never count all the bones. Count the item that occurs once per animal (skulls) or in a known number (two mandibles, two femurs per animal) and take the largest result.

The big idea: energy. Owl pellets show a **food chain** you can hold in your hand: seeds and grass to vole to owl. Plants are producers, voles are first-level consumers and the owl is a second-level consumer and a predator. At each step only about a tenth of the food energy is passed on, because most is used for living, moving and keeping warm. That is why a meadow must hold a great many voles to feed one pair of owls, and why owls can keep rodent numbers in check. A pellet survey is a real census of the small animals of a field.

Try it and see. Use heat-sterilized pellets from a science supplier. Measure and weigh the pellet, soften it if you like, and tease it apart with a probe. Sort the bones onto a bone chart, key out each skull, and find the minimum number of animals. Then pool your results with your family or co-op and turn them into percents and a bar graph.

God’s handiwork. The psalmist watched night fall and saw the creatures of the dark go out to hunt. He said to the Lord, “Thou makest darkness, and it is night,” and the hunters “seek their meat from God.” The barn owl, with its silent feathers, its heart-shaped face that gathers sound to its ears and its gentle gizzard that saves every bone for us to read, is one of those night hunters, and a farmer’s good friend.



A dichotomous key for skulls found in owl pellets. Start at the top and answer each question about the teeth.

PICTURE STUDY



Barn Owl, Plate 171 from The Birds of America, John James Audubon; engraved by Robert Havell Jr., 1830s · Hand-colored engraving and aquatint · Published in London; original prints are held by several libraries and museums, and scanned copies are online

John James Audubon set out to paint every bird of America life size, and his great book *The Birds of America* was printed in London on enormous sheets, with each plate engraved by Robert Havell Jr. and colored by hand. Audubon drew from freshly collected birds wired into lifelike poses, and he liked to show them in action: hunting, feeding or caring for their young.

The barn owl is found on every continent except Antarctica. Audubon painted a pair of them, showing the soft, pale plumage and the flat facial disk that funnels sound to the owl's ears.

Look closely

- Find the heart-shaped facial disk. How might this shape help an owl hunting in the dark?
- Look at the feet and talons. How are they built for gripping prey?
- Compare the two owls. Are they posed the same way? What does each seem to be doing?
- Look at the colors of the back and wings. Where would these colors help an owl hide by day?

Talk about it

1. Audubon painted his birds life size. Why might that matter to a naturalist using the book?
2. After this lesson, what could you tell Audubon about the owl's diet that a painting cannot show?

> **Safety first.** Use only owl pellets that a science supplier has heat-sterilized. Do not dissect pellets you find outdoors, because they can carry bacteria such as Salmonella. Wear gloves or wash your hands well after handling pellets and bones, keep your hands away from your face, and do not eat or drink while you work. Cover the table with newspaper and wipe it down afterward. Probes and tweezers have sharp points; work slowly and keep them pointed away from you.

EXPERIMENT: PELLET TO PIE CHART: AN OWL DIET SURVEY

You will dissect one or more owl pellets, identify every animal inside, and combine your results with others to describe what the owls ate.

Question: What did this owl eat, and which prey was most common? **Prediction:** Write your guess with a reason. **What we measure:** pellet length, width and mass; the number of each kind of bone; the kind of animal for each skull; the minimum number of animals. **What we keep the same:** the same tools, bone chart and skull key for every pellet. **Record:** a data table, a bone board and a bar graph.

You will need

- 1 to 3 heat-sterilized owl pellets from a science supplier (barn owl pellets are most common)
- Bone sorting chart (many suppliers include one; or draw your own with spaces for skulls, jaws, vertebrae, ribs, shoulder blades, pelvis, front-leg and hind-leg bones)
- Wooden probe or toothpick, tweezers and a hand lens
- Metric ruler and a kitchen scale reading to 0.1 g (optional)
- Paper plate, newspaper, small cup of warm water (optional, to soften the pellet)
- Gloves, a sheet of dark card and white glue
- Lab notebook, graph paper and colored pencils

Steps

1. Measure the pellet’s length and width in millimeters and weigh it. Record what you see on the outside: fur, feathers, bits of bone.
2. Set the pellet on a paper plate. Gently pull it apart with the probe and tweezers. (If it is hard, soak it in warm water for a few minutes first.)
3. Separate every bone from the fur. Brush small bones clean with a dry toothbrush or the probe.
4. Lay each bone on the matching space of the bone chart: skulls, mandibles, vertebrae, ribs, shoulder blades, pelvis halves, front-leg bones, hind-leg bones.
5. Use the skull key to identify each skull: bird, vole, mouse or rat, shrew or mole. Look at the cheek teeth with the hand lens.
6. Count skulls, mandible halves and femurs. Find the minimum number of animals for each kind of prey, as in Worked Example 2.
7. Record your results in a table: Prey type, Skulls, Mandibles, Femurs, Minimum number.
8. Combine your table with the results of other pellets (from family members, a co-op or published class data). Find the percent of each prey type and draw a bar graph.
9. Glue one most-complete skeleton onto dark card in its natural shape and label at least ten bones.
10. Write a conclusion: What was the owl’s main food? What does that tell you about the habitat where it hunted?

Tips

- Skulls are fragile. Lift them with fingers or the flat of the tweezers, never by the teeth.
- Tiny bones hide in the fur. Before you throw the fur away, roll it between your fingers over a white plate.
- If a skull is broken, the lower jaws and their teeth can still tell you the kind of animal.
- Many pellet suppliers sell bone identification charts that match the pellets they sell.

Check your work

- I used a sterilized pellet and washed my hands afterward.
- I sorted every bone onto the chart and identified each skull with the key.
- I found the minimum number of animals and explained how.

- My bar graph has a title, labeled axes and the correct heights.
- My conclusion uses numbers from the data.

NATURE NOTEBOOK

1. Draw a vole skull from the side at twice life size, labeling the incisors, the gap, the cheek teeth, the eye socket and the mandible.
2. Draw a food chain from your pellet results with arrows showing the direction of energy: seeds or grass → vole → owl, and insects → shrew → owl.
3. Sketch a barn owl from Audubon's plate or a photograph and label the facial disk, eyes, beak, talons and soft flight feathers. Write one sentence about how each helps it hunt.

From history. Farmers have long put barn owls to work. In the oil palm plantations of Malaysia, growers began in the late twentieth century to set up nest boxes for barn owls to help control the rats that damage the palm fruit, and many plantations still use them. In parts of North America and Europe, farmers and vineyard owners put up owl boxes for the same reason. Pellets collected under the boxes let biologists check exactly what the owls are catching.

WORKED EXAMPLES

Example 1. Gilbert White timed his barn owls and found that one of the pair returned with a mouse about once every 5 minutes. At that rate, how many mice would the pair bring in during 1 hour? During a 2-hour evening hunt?

1. One hour has 60 minutes.
2. Trips in 1 hour: 60 ÷ 5 = 12.
3. Trips in 2 hours: 12 × 2 = 24.
4. This assumes the rate stays steady. Real hunting slows when prey is scarce or the young are full, so 24 is an upper estimate for that evening.

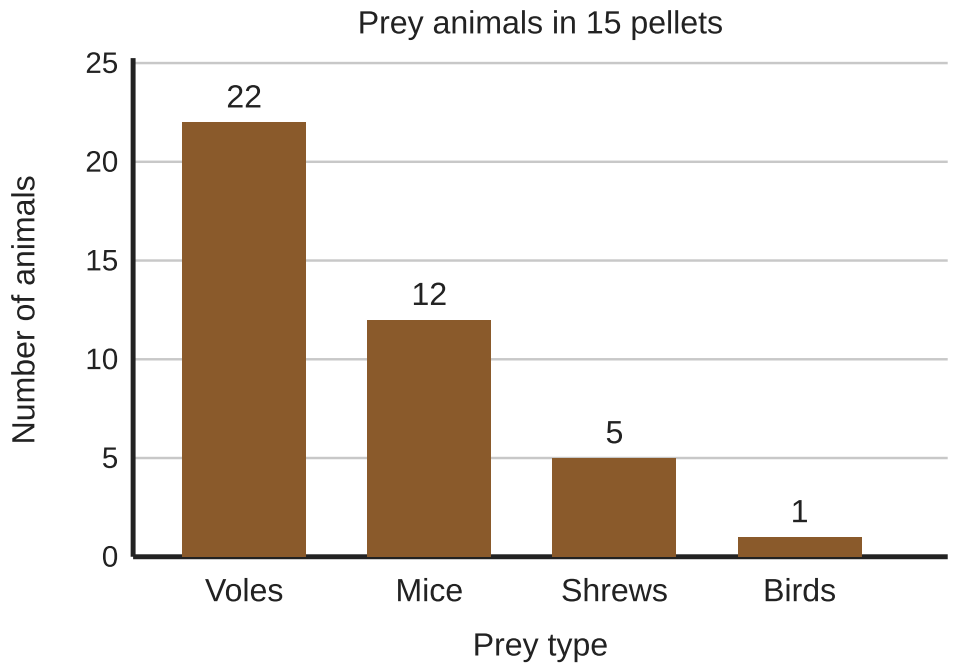
Answer: About 12 mice per hour and about 24 in a 2-hour hunt.

Example 2. A single pellet contains 3 skulls, 7 mandible halves and 9 femurs, all from voles. What is the smallest number of voles the owl could have eaten?

1. Each vole has 1 skull, 2 mandible halves and 2 femurs.
2. From skulls: 3 ÷ 1 = at least 3 voles.
3. From mandibles: 7 ÷ 2 = 3.5, and half a vole is not possible, so at least 4 voles.
4. From femurs: 9 ÷ 2 = 4.5, so at least 5 voles.
5. Take the largest: at least 5 voles. Some skulls must have been broken or lost.

Answer: At least 5 voles.

Example 3. A co-op pooled 15 pellets and identified 40 prey animals: 22 voles, 12 mice, 5 shrews and 1 bird. Find the percent of each prey type and describe the owls' diet.



Prey animals identified in 15 pooled owl pellets (co-op data).

1. Check the total: 22 + 12 + 5 + 1 = 40.
2. Voles: 22 ÷ 40 × 100 = 55%.
3. Mice: 12 ÷ 40 × 100 = 30%.
4. Shrews: 5 ÷ 40 × 100 = 12.5%.
5. Birds: 1 ÷ 40 × 100 = 2.5%. Check: 55 + 30 + 12.5 + 2.5 = 100%.
6. Average prey per pellet: 40 ÷ 15 ≈ 2.7 animals.

Answer: Voles 55%, mice 30%, shrews 12.5%, birds 2.5%. The owls fed mostly on rodents (85%), especially voles, which suggests they hunted over grassy fields where voles live.

PRACTICE

1. EASY Is an owl pellet the owl's droppings? Explain.
2. EASY Why do owl pellets contain nearly whole bones while hawk pellets contain few?
3. EASY Use the skull key: a small skull has a continuous row of many tiny pointed teeth with red-brown tips. What animal is it?
4. MEDIUM A skull has two big curved front teeth, then a gap, and cheek teeth with rounded bumps. Name the kind of animal and the step in the key that told you.
5. MEDIUM A pellet has 2 skulls, 6 mandible halves and 3 femurs from mice. What is the smallest number of mice? Show your reasoning.

6. MEDIUM Data reading. Pellets from two roosts: Roost A (beside a hayfield), 30 prey: 21 voles, 6 mice, 3 shrews. Roost B (beside a farmyard with grain bins), 30 prey: 6 voles, 3 shrews, 21 mice and rats. (a) What percent of Roost A's prey were voles? (b) Which roost's owls ate more mice and rats, and how many times as many? (c) What best explains the difference? A. The owls at B could not see voles. B. Each owl caught the prey that was most common where it hunted. C. Owls choose prey by color.
7. MEDIUM Data reading. Five pellets were weighed and dissected: 2.0 g, 1 prey; 3.8 g, 2 prey; 4.1 g, 2 prey; 6.2 g, 3 prey; 7.9 g, 4 prey. (a) Describe the relationship between pellet mass and number of prey. (b) Estimate the number of prey in a 5.9 g pellet. (c) Give one reason the relationship is not exact.
8. HARD The plants in a meadow capture 1,000,000 units of food energy in a season. Using the 10 percent rule, about how much reaches the voles, and how much reaches the owls that eat the voles? Why can a meadow hold far more voles than owls?
9. HARD Experiment design. A biologist wonders whether a pair of barn owls eats different prey in winter than in summer. Design a study using pellets: the question, what you will collect and when, the variables, what you keep the same and how you will show the results.
10. CHALLENGE A student counted every bone in a pellet (about 180 bones) and said, "There must be at least five mice, because one mouse has fewer than 180 bones." Explain why this method is unreliable and describe a better one.

COMMON MISTAKES

- Thinking owl pellets are droppings. They are regurgitated through the mouth from the gizzard.
- Counting every jaw or leg bone as a separate animal. Each animal has two mandible halves and two femurs, so divide by two and take the largest count.
- Thinking owls digest bones and fur. They cannot; the gizzard holds them back and packs them into the pellet.

> **Make it a game · Bone Detective.** A parent hides cut-out paper bones (skulls, mandibles, femurs, ribs and vertebrae) in a "pellet" of crumpled paper or yarn, choosing a secret number of voles, mice and shrews. Draw a red tip on shrew teeth and zigzags on vole teeth. The player unwraps the pellet, sorts the bones and must announce the minimum number of each prey animal within three minutes, explaining which bones they counted. A correct total scores 3 points; each correct prey type scores 1. Switch roles and play again.

> **Scripture.** "Thou makest darkness, and it is night: wherein all the beasts of the forest do creep forth. The young lions roar after their prey, and seek their meat from God." (Psalm 104:20–21, KJV)

God made the night as well as the day, and He made hunters to work in it. The barn owl, like the young lion, receives its food from His hand, and in doing so it keeps the numbers of mice and voles in balance.

Practice answers

1. No. A pellet is regurgitated through the mouth. It holds the hard parts (fur, bones, teeth, feathers) that the gizzard held back. Droppings leave through the other end and contain digested waste.
2. Owls have gentler stomach acid that does not dissolve much bone, so the bones are packed into the pellet nearly whole. Hawks' stronger acid digests most of the bone.
3. A shrew.
4. A mouse or rat (a rodent). Step 2 (big front teeth and a gap) shows it is a rodent; step 3 (rounded bumps, not zigzag folds) shows it is not a vole.
5. Skulls: at least 2. Mandibles: 6 ÷ 2 = 3. Femurs: 3 ÷ 2 = 1.5, so at least 2. The largest is 3, so at least 3 mice.
6. (a) 21 ÷ 30 × 100 = 70%. (b) Roost B: 21 ÷ 6 = 3.5 times as many. (c) B. Voles live in grassy fields, while mice and rats gather around stored grain.
7. (a) Heavier pellets held more prey, about 1 prey for every 2 g. (b) About 3 prey. (c) Prey animals differ in size (a rat is much larger than a shrew), and some bones and fur may be in a different pellet.
8. Voles: 10% of 1,000,000 = 100,000 units. Owls: 10% of 100,000 = 10,000 units. Most energy at each step is used for living, moving and keeping warm, so each level up has far less energy to support it, and so far fewer animals.
9. Question: Does the barn owls' diet change between summer and winter? Collect: all fresh pellets under the same roost every week for several weeks in summer and again in winter (sterilize or have them sterilized before handling). Independent variable: season. Dependent variable: percent of each prey type (and prey per pellet). Keep the same: the same roost and owls, the same key and counting method, the same number of weeks and, if possible, the same person identifying the skulls. Show results as side-by-side bar graphs of percent of each prey type for each season.
10. Bones break, small bones get lost or stay in the fur, and the number of bones in a mouse is hard to count exactly, so the total does not match whole animals. A better method counts bones that occur a known number of times in each animal (1 skull, 2 mandible halves, 2 femurs), divides, rounds up and takes the largest result as the minimum number of animals.

8.4 Lab Practical

OBJECTIVES

- Tell the story of Samuel Scudder’s fish and Louis Agassiz’s command to look, and explain why careful observation and drawing are at the heart of anatomy.
- Identify structures from the earthworm, frog and owl pellet at timed stations, and compare how the three animals carry out digestion, circulation, breathing, nervous control and support.
- Use scale bars, microscope field of view and data tables to calculate real sizes and compare body plans.

Warm-up. Set a timer for 2 minutes and look closely at a single leaf, coin or seashell without touching it. Then turn it over or put it away and write down everything you saw. Look again for 2 more minutes and add anything new to your list in a different color. How many new things did the second look reveal?

Video. Coming soon. How a biology lab practical works, comparative anatomy of earthworm, frog and owl pellet bones, and Louis Agassiz and the fish (search biology classroom review channels, Carolina Biological, Home Science Tools, Smithsonian, Harvard Museum of Natural History, Moody Science classics).

KEY TERMS

lab practical
A test in which you move from station to station and identify real specimens or structures, often with a time limit at each station.

comparative anatomy
The study of how the body structures of different kinds of animals are alike and how they differ.

levels of organization
The order in which a body is built: cells form tissues, tissues form organs, organs form organ systems and organ systems form an organism.

organ system
A group of organs that work together to do one main job, such as the digestive, circulatory, respiratory, nervous or skeletal system.

anatomical directions
Words for locating parts on an animal’s body: anterior (head end), posterior (tail end), dorsal (back side) and ventral (belly side).

hydrostatic skeleton
A body support made of fluid held under pressure inside a muscular body wall, as in the earthworm, instead of bones.

CORE IDEAS

The story. In the late 1850s, a young man named Samuel Scudder came to study natural history with Louis Agassiz, the Swiss-born professor at Harvard. Agassiz handed him a small preserved fish in a tin tray, told him to look at it, and left the room. Scudder looked for ten minutes and decided he had seen everything there was to see. But Agassiz did not come back. An hour passed, then three. Scudder grew bored, then curious. He counted scales, studied the fins and the teeth, and finally picked up a pencil and drew the fish, which made him notice things he had missed. When Agassiz returned, he listened and said Scudder had not looked carefully enough and must look again. The next morning Scudder had noticed something new: the fish's two sides matched, with paired organs on each. Agassiz was pleased, but all he said was "Oh, look at your fish!" and he left Scudder to look again. He kept him looking at the same fish for three days. Scudder became an expert on insects, and in 1874 he wrote that this was the best lesson in studying insects he ever had.

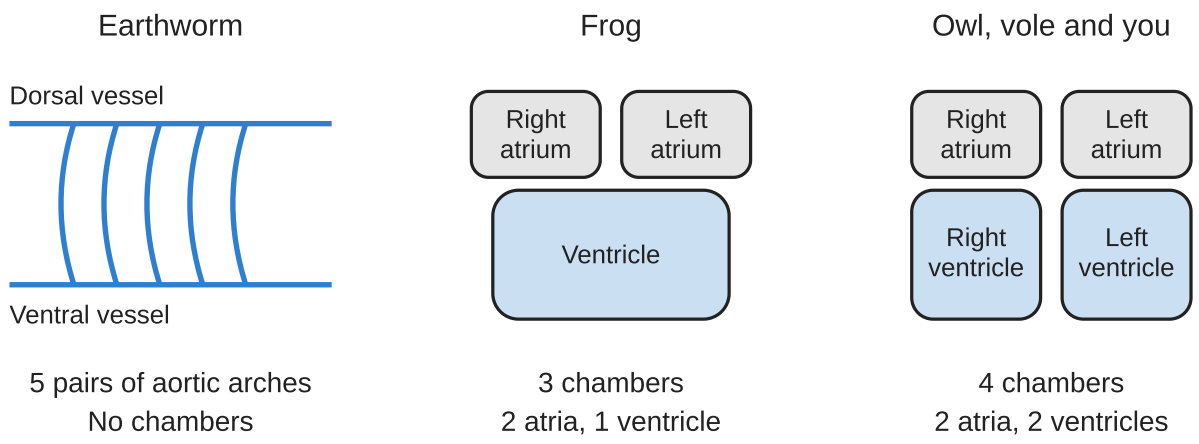
How they figured it out. Agassiz trained a whole generation of American naturalists this way: observe for a long time, draw what you see, then compare one animal with another. He founded Harvard's Museum of Comparative Zoology in 1859 so students could lay thousands of specimens side by side. Agassiz believed that the order he found in animal bodies reflected the thoughts of the Creator, and that the naturalist's work was to read that order patiently from the animals themselves. A **lab practical** tests exactly the skill he taught. It does not ask what the book said. It puts a pinned structure in front of you and asks what you see.

The big idea: comparing body plans. Every animal you dissected is built in the same **levels of organization**: cells, tissues, organs, organ systems, organism. All three are bilaterally symmetrical, with anterior, posterior, dorsal and ventral sides. But each **organ system** is fitted to a different way of life. *Digestion:* the earthworm has a straight tube with a crop to store and a gizzard to grind; the frog has a stomach, a coiled intestine and a large liver; the owl has a stomach and a gizzard that packs bones into pellets, and the voles it ate had chisel teeth for chewing seeds. *Circulation:* all three have closed systems, but the earthworm pumps with five pairs of aortic arches, the frog with a three-chambered heart, and the owl and its prey with four-chambered hearts. *Breathing:* the earthworm breathes only through its moist skin, the frog through lungs, skin and mouth lining, and the owl through lungs connected to air sacs, as you learned in Unit 7.

The big idea: control and support. Look at where the main nerve cord runs. In the earthworm it runs along the *ventral* side, below the gut. In the frog, the owl and the vole it runs along the *dorsal* side as a spinal cord protected inside the backbone, the vertebrae you sorted from the pellet. Support differs too. The earthworm has no bones; its fluid-filled body cavity, squeezed by circular and lengthwise muscles, works as a **hydrostatic skeleton**. The frog, owl and vole all have bony internal skeletons with a skull, backbone, limb girdles and limbs built on the same general plan. Seeing the same plan worked out in different ways is the heart of **comparative anatomy**.

Try it and see. In a practical, numbered pins or flags mark structures on specimens, photographs or bones set out at stations. You have a fixed time at each station (often 60 to 90 seconds) to write the name of the structure, and sometimes its job or the organ system it belongs to. When the timer sounds, everyone moves to the next station, and you cannot go back. Spelling counts, and so do details such as “left” or “ventral.” The best preparation is Agassiz’s: look at the real thing, draw it, say its name and job aloud, then cover the labels and test yourself.

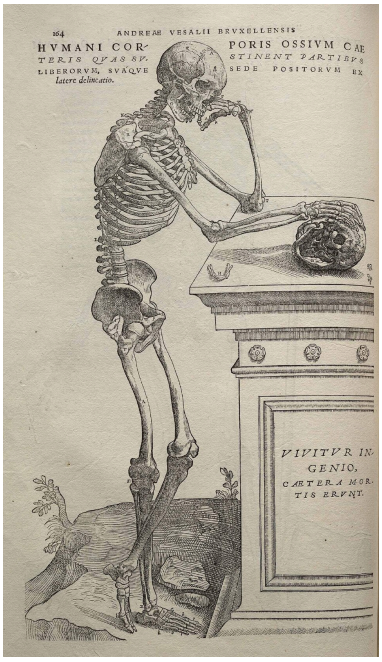
God’s handiwork. Job told his friends to ask the beasts and the fowls and the fishes, and they would teach. A worm, a frog and an owl’s dinner have taught you how bodies take in food and oxygen, move blood, sense the world and hold their shape. Every structure you named was there long before anyone gave it a name, and each one rewards a second, longer look.



All are closed systems. Hearts shown from the front: the animal’s right is on your left.

Three ways to pump blood in a closed circulatory system: aortic arches (earthworm), three chambers (frog) and four chambers (birds and mammals).

PICTURE STUDY



Skeleton Plate from De humani corporis fabrica (On the Fabric of the Human Body), Andreas Vesalius (author); woodcuts made in Venice, probably in the workshop of Titian and often credited to Jan Steven van Calcar, 1543 · Woodcut in a printed book · Published in Basel, Switzerland, by Johannes Oporinus; copies in many rare-book libraries, and scanned copies online

Andreas Vesalius was a young professor of anatomy at the University of Padua, in Italy. For centuries, teachers had read anatomy aloud from the books of the ancient physician Galen while an assistant did the cutting. Vesalius did his own dissections and checked every claim against what he could see, and he found that some of Galen's descriptions came from animals rather than from people. In 1543, when he was only 28, he published *De humani corporis fabrica*, with large, detailed woodcuts.

This plate shows the whole human skeleton in a calm, lifelike pose. It is a fitting picture for a lab practical: it rewards careful looking, and many of its bones have a match in the vole skeletons you sorted from the owl pellet.

Look closely

- Find the skull, the backbone, the ribs and the pelvis. Which of these did you also find in the owl pellet?
- Find the long bones of the arm and leg. How many bones are in the upper arm, and how many in the lower arm?
- Look at the skeleton's pose and the landscape. Why do you think the artist made a skeleton look thoughtful instead of stiff?
- Compare this skeleton with a vole skeleton from your bone chart. What is the same plan, and what is different?

Talk about it

1. Vesalius trusted what he saw over what the old books said. How is that like Agassiz's lesson with the fish?
2. Why would large, exact pictures be so important in a book about anatomy?

> **Safety first.** If you use preserved specimens saved from Lessons 8.1 and 8.2, keep them in a sealed, labeled container away from food, wear gloves and goggles when you handle them, and wash your hands afterward. Owl pellet bones should come from heat-sterilized pellets. Pins and probes at the stations have sharp points; set them with points down or stuck into the tray. Dispose of specimens in sealed bags in the outdoor trash when the practical is over.

EXPERIMENT: SET UP AND TAKE A LAB PRACTICAL

A parent (or a sibling or co-op partner) will set up timed stations using your specimens, photographs, drawings and pellet bones. You will take the practical, score it, and find which body system you know best and which needs more study.

Question: Which organ system can I identify most accurately: digestive, circulatory, respiratory, nervous or skeletal? **Prediction:** Write which you expect to score highest and lowest. **What we measure:** the percent correct for each organ system. **What we keep the same:** the same time at every station and the same scoring rules. **Record:** an answer sheet and a bar graph of percent correct by system.

You will need

- Specimens saved from Lessons 8.1 and 8.2 in sealed containers, or clear photographs or screenshots from a virtual dissection
- Bones and skulls from your owl pellet, and the skull key
- 15 to 20 small flags (toothpicks with numbered paper tags) or numbered sticky notes
- Timer, index cards and an answer sheet numbered 1 to 20
- Gloves, goggles and paper towels
- Graph paper and pencil

Steps

1. Helper: choose 15 to 20 structures, with at least three from each organ system: digestive, circulatory, respiratory, nervous and skeletal. Mix earthworm, frog and owl pellet items.
2. Helper: mark each structure with a numbered flag and set the stations around a table or room. At some stations add a second question card, such as "What is its job?" or "Which animal is this from?"
3. Student: number your answer sheet. Begin at any station. You have 90 seconds per station. Write the name of each flagged structure and answer any question card.
4. When the timer sounds, move to the next station. No going back.
5. Helper reads the answers aloud while the student marks each one right or wrong. Give half credit for the right structure misspelled.

6. Sort the questions by organ system and find the percent correct for each system.
7. Draw a bar graph of percent correct by organ system, with a title and labeled axes.
8. Write a conclusion: Which system was strongest and which weakest? Was your prediction right? For the weakest system, draw and label its structures in all three animals from memory, then check them.

Tips

- Read the flag carefully. Is it on the organ itself or on the vessel beside it?
- If you blank on a name, write a description and the job. Many teachers give partial credit for that.
- Use the anatomical direction words (dorsal, ventral, anterior, posterior) whenever they help pinpoint a structure.
- Keep preserved specimens wrapped in damp paper towels in a sealed, labeled bag in a cool place until the practical so they do not dry out.

Check your work

- I completed every station within the time limit.
- I scored my sheet honestly and found the percent for each organ system.
- My bar graph is labeled and matches my scores.
- I reviewed my weakest system by drawing it in all three animals.

NATURE NOTEBOOK

1. Make a large comparison table with three columns (Earthworm, Frog, Owl and its prey) and five rows (digestion, circulation, breathing, nerve cord, support). Fill in every box with words and a tiny sketch.
2. Choose one structure you missed on the practical. Look at it (or a photo) for five full minutes, draw it twice as large as life, and label everything you notice.
3. Draw a cross section of the earthworm and of the frog, as if each were cut in half across the middle, and show where the gut, main blood vessels and nerve cord lie in each.

> **From history.** In the summer of 1873, the last year of his life, Agassiz opened a summer school of natural history on Penikese Island, off the coast of Massachusetts, for teachers and students. His teaching is often summed up as a call to study nature itself rather than books about it, the same method he had used on Scudder. Many of the teachers who studied there carried hands-on nature study back to American schools.

WORKED EXAMPLES

Example 1. A student's drawing of a frog dissection has a scale bar 2.0 cm long labeled "5 mm." On the drawing, the liver measures 6.0 cm across. What is the magnification of the drawing, and how wide is the real liver?

1. Change the bar to the same units: 2.0 cm = 20 mm on the drawing stands for 5 mm in real life.
2. Magnification = drawing size ÷ real size = 20 mm ÷ 5 mm = 4, so the drawing is ×4.
3. Liver on the drawing: 6.0 cm = 60 mm.
4. Real width = 60 mm ÷ 4 = 15 mm.

Answer: The drawing is 4 times life size (×4), and the real liver is 15 mm (1.5 cm) across.

Example 2. Under a microscope at 40× total magnification (10× eyepiece and 4× objective), the field of view is 4.5 mm across. A slide of an earthworm cross section fills about two-thirds of the width of the field. (a) Estimate the diameter of the cross section. (b) The field of view at 100× is 1.8 mm. Will the whole cross section fit in view at 100×?

1. (a) Two-thirds of 4.5 mm: 4.5 × 2 ÷ 3 = 3.0 mm.
2. (b) Check the field at 100×: field width shrinks in proportion as magnification grows: 4.5 mm × 40 ÷ 100 = 1.8 mm, as stated.
3. Compare: 3.0 mm is larger than 1.8 mm.
4. So at 100× you will see only part of the section and must move the slide to look at the rest.

Answer: (a) About 3.0 mm across. (b) No. The 1.8 mm field is smaller than the 3.0 mm section, so only part of it fits.

Example 3. Sample measurements: earthworm, body 18 cm and gut 17 cm; adult frog, body (snout to vent) 9 cm and intestines 18 cm; vole, head and body 10 cm and intestines 55 cm. Find the ratio of gut length to body length for each animal and suggest what the pattern shows.

1. Earthworm: 17 ÷ 18 ≈ 0.94. The gut runs straight from end to end.
2. Frog: 18 ÷ 9 = 2.0.
3. Vole: 55 ÷ 10 = 5.5.
4. Order: earthworm 0.94, frog 2.0, vole 5.5.
5. The frog eats insects and other animals, which are easy to digest. The vole eats grass, seeds and roots, which are hard to digest, and its much longer gut gives the food more time and surface for digestion. The earthworm makes up for its short, straight gut with the folded typhlosole and by eating constantly.

Answer: Ratios: earthworm about 0.94, frog 2.0, vole 5.5. The plant-eating vole has by far the longest gut for its size, which fits a diet that is slow to digest.

PRACTICE

1. EASY Put these in order from smallest to largest: organ, cell, organism, tissue, organ system.
2. EASY In which animal does the main nerve cord run along the ventral side: the earthworm, the frog or the vole? Where does it run in the other two?
3. EASY Match each animal with the way it takes in oxygen: earthworm, frog, owl. Choices: lungs with air sacs; skin only; lungs, skin and mouth lining.
4. MEDIUM At a practical station, a flag marks a large, brown, three-lobed organ just behind the heart of a frog, with a small greenish sac under it. Name the organ and the sac, and give a job for each.
5. MEDIUM A drawing of an earthworm’s gizzard is labeled “×3” and measures 4.5 cm long. How long is the real gizzard, in millimeters?
6. MEDIUM Data reading. A co-op of 12 students took the same practical. Percent who answered each station correctly: liver 92%, aortic arches 42%, glottis 58%, gizzard 83%, mandible 75%, ventral nerve cord 50%, tympanum 92%, femur 67%. (a) Which station was hardest? (b) How many of the 12 students got the gizzard right? (c) Which statement is best supported? A. Structures that are large and easy to see were answered correctly more often than small or hidden ones. B. Bones were the hardest items. C. Every frog structure scored above 80%.
7. MEDIUM Data reading. Field of view on one microscope: 40×, 4.5 mm; 100×, 1.8 mm; 400×, 0.45 mm. (a) Describe the pattern. (b) Predict the field of view at 200×. (c) About how many cells 0.15 mm across would fit across the field at 400×?
8. HARD Both the earthworm and the frog have closed circulatory systems. Explain what that means and describe how blood is pumped in each.

9. HARD Experiment design. Agassiz told Scudder that drawing helps you see. Design an experiment to test whether students who draw and label a structure remember its name better than students who only look at a labeled picture for the same time. Give the question, the variables, how you make the groups fair and what you keep the same.
10. CHALLENGE Why do you think Agassiz made Scudder look at the fish for three days instead of simply telling him what to notice? Give two reasons, and connect one of them to the way a lab practical is designed.

COMMON MISTAKES

- Memorizing a list of names without knowing where each part lies or what it does. A practical asks you to find the structure on a real specimen, often with its job.
- Thinking all animals have the same organs in the same places. The earthworm's nerve cord is ventral and it has no bony skeleton or chambered heart; vertebrates have a dorsal spinal cord and a bony skeleton.
- Reading the size of a drawing as the size of the real structure. Always check the scale bar or the magnification (×2, ×4) and divide.

> **Make it a game · Speed Stations.** Make 20 cards, each with a sketch or photo of one structure from the unit on the front and its name, animal and organ system on the back. Lay them face up around the room. Set a timer for 15 seconds per card and walk the circuit, saying each name, animal and system aloud before the beep. Each correct card scores 1 point. Play again with 10 seconds per card, and try to beat your score.

> **Scripture.** "But ask now the beasts, and they shall teach thee; and the fowls of the air, and they shall tell thee: Or speak to the earth, and it shall teach thee: and the fishes of the sea shall declare unto thee." (Job 12:7–8, KJV)

Job said the creatures themselves would teach anyone willing to ask. In this unit an earthworm, a frog and an owl taught you how living bodies are put together. The next verse asks who does not know that the hand of the Lord has made all these.

Practice answers

1. Cell, tissue, organ, organ system, organism.
2. The earthworm. In the frog and the vole it runs along the dorsal side as a spinal cord inside the backbone.
3. Earthworm: skin only. Frog: lungs, skin and mouth lining. Owl: lungs with air sacs.
4. The liver (makes bile, stores food energy and cleans the blood) and the gallbladder (stores bile until it is sent to the small intestine to help digest fats).
5. 4.5 cm ÷ 3 = 1.5 cm = 15 mm.
6. (a) Aortic arches (42%). (b) 83% of 12 ≈ 10 students. (c) A. The liver and tympanum (large and obvious) scored 92%, while the aortic arches and nerve cord (small or hidden under other organs) scored lowest. B is wrong (the mandible and femur scored mid-range), and C is wrong (the glottis scored 58%).

7. (a) As magnification increases, the field of view shrinks in proportion: multiplying the magnification by a number divides the field width by the same number (40 × 4.5 = 100 × 1.8 = 400 × 0.45 = 180). (b) 180 ÷ 200 = 0.9 mm. (c) 0.45 ÷ 0.15 = 3 cells.
8. Closed means the blood always stays inside vessels. In the earthworm, blood is pumped forward in the dorsal vessel, down through five pairs of muscular aortic arches and back along the ventral vessel. In the frog, a three-chambered heart (two atria receive blood from the body and lungs, one ventricle pumps it out) pushes blood through arteries, capillaries and veins.
9. Question: Do students who draw and label structures remember their names better than students who only study a labeled picture? Independent variable: study method (drawing and labeling, or looking only). Dependent variable: score on the same identification quiz given later (for example, two days later). Fair groups: assign students at random to each group, with similar numbers and ages. Keep the same: the same structures and pictures, the same study time (for example, 10 minutes), the same quiz and the same time before the quiz. Repeat with more students or a second set of structures to check the result.
10. Reasons (any two): things you discover yourself are remembered far longer than things you are told; long looking trains the habit of noticing small details others miss; it teaches a scientist to trust evidence from the specimen over words from a book; drawing forces you to see shape and position exactly. Connection: a lab practical gives no word list or hints; it puts the real structure in front of you, so only a person who has truly looked at specimens can name it.

Unit 8 review

Answer each question. Show your work for calculations, include metric units, and use complete sentences for explanations. The test is worth 60 points.

1. (4 pts) Multiple choice. An earthworm takes in oxygen through its: (A) lungs; (B) gills; (C) moist skin; (D) aortic arches.
2. (5 pts) List the parts of the earthworm’s digestive tube in order from mouth to anus, and give the job of the crop and of the gizzard.
3. (8 pts) Diagram. Draw a side view of the front part of an earthworm, head end on the left. Label eight parts: mouth, pharynx, crop, gizzard, intestine, aortic arches, dorsal blood vessel and clitellum. Mark the dorsal and ventral sides.
4. (4 pts) Multiple choice. A frog’s heart has: (A) two chambers, one atrium and one ventricle; (B) three chambers, two atria and one ventricle; (C) four chambers, two atria and two ventricles; (D) five pairs of arches and no chambers.
5. (6 pts) Name, in order, four organs food passes through in a frog from the mouth to the outside, and name two organs that help digestion even though food does not pass through them.
6. (5 pts) What did Luigi Galvani observe in his frog experiments, and what do scientists now know nerves carry because of the work he began?
7. (6 pts) A frog’s heart beats 8 times in 15 seconds at 15 °C and 14 times in 15 seconds at 25 °C. (a) Find both heart rates in beats per minute. (b) Explain the difference using the word ectotherm.
8. (6 pts) An owl pellet contains 2 skulls, 5 mandible halves and 7 femurs, all from voles. What is the minimum number of voles? Show your reasoning.
9. (4 pts) Multiple choice. A small skull from a pellet has a continuous row of many tiny pointed teeth with red-brown tips. It came from a: (A) vole; (B) mouse; (C) bird; (D) shrew.
10. (6 pts) Data reading. Pooled pellets held 50 prey: 30 voles, 10 mice, 8 shrews and 2 birds. (a) Find the percent of each. (b) What percent were rodents? (c) In a food chain, what does the vole eat, and what is the owl’s role?
11. (6 pts) Compare the earthworm and the frog in two ways: (a) where the main nerve cord runs, and (b) what supports the body. Then (c) explain why Agassiz’s lesson with the fish is good advice for preparing for a lab practical.

Unit project: my comparative anatomy lab book

Assemble a lab book of the whole unit. Include your labeled earthworm drawing and segment table, your labeled frog drawing (real or virtual) with your measurements and calculations, your owl pellet bone board or a photo of it with your prey table and bar graph, and your lab practical answer sheet and bar graph of scores by organ system. Finish with a large two-page comparison chart of the earthworm, frog and owl and its prey for digestion, circulation, breathing, nerve cord and support, illustrated with small labeled sketches, and a paragraph explaining which animal’s body surprised you most and why.

- **Accuracy**: Every drawing is labeled correctly with anatomical names, and the comparison chart describes each organ system correctly for all three animals.
- **Data and math**: Measurements use metric units; segment counts, heart rates, organ percents, minimum numbers of prey and diet percents are calculated correctly; graphs have titles, labeled axes and correct bar heights.
- **Observation**: Drawings are made from the real specimen (or virtual dissection), show scale or magnification, and record details the student actually noticed, in the spirit of "Look at your fish!"
- **Safety and care**: The book records the safety steps followed in each lab, specimens were handled respectfully and disposed of properly, and the book is neat, dated and easy to follow.

Answer key

1. (C) Moist skin. Oxygen dissolves in the film of moisture and passes into the blood; the worm has no lungs or gills.
2. Mouth, pharynx, esophagus, crop, gizzard, intestine, anus. The crop stores food; the gizzard grinds it with muscle and swallowed sand grains.
3. From left to right along the gut: mouth, pharynx (bulb), narrow esophagus with the five pairs of aortic arches looping around it between the dorsal (top) and ventral (bottom) blood vessels, crop, thick gizzard, then the long intestine. The dorsal blood vessel runs along the top of the gut. The clitellum is a thick band farther back (about segments 32 to 37), still nearer the head than the tail. Dorsal is the top (back) side; ventral is the bottom (belly) side. One point per correct label (8 points); deduct 1 if dorsal and ventral are missing or reversed.
4. (B) Three chambers: two atria and one ventricle.
5. Passes through (in order): esophagus, stomach, small intestine, large intestine, cloaca (any four in the correct order). Help without food passing through: liver (makes bile), gallbladder (stores bile) and pancreas (makes digestive juices); any two.
6. A dead frog's leg twitched when its nerve was touched with metal while an electrical machine sparked, and also when brass and iron touched it. He concluded that electricity was at work in nerves and muscles. Scientists now know that nerves carry electrical signals (nerve impulses).
7. (a) $8 \times 4 = 32$ beats per minute at 15 °C; $14 \times 4 = 56$ beats per minute at 25 °C. (b) A frog is an ectotherm: its body temperature follows its surroundings, so when the water is warmer its body processes, including its heartbeat, speed up.
8. Each vole has 1 skull, 2 mandible halves and 2 femurs. Skulls: at least 2. Mandibles: $5 \div 2 = 2.5$, so at least 3. Femurs: $7 \div 2 = 3.5$, so at least 4. The largest result is 4, so at least 4 voles.
9. (D) Shrew.
10. (a) Voles 60%, mice 20%, shrews 16%, birds 4%. (b) Voles and mice: 80%. (c) Voles eat plants (grass, seeds, roots); the owl is a predator and a second-level consumer at the top of this chain.
11. (a) Earthworm: ventral nerve cord, below the gut. Frog: dorsal spinal cord inside the backbone. (b) Earthworm: a hydrostatic skeleton (fluid-filled body cavity squeezed by muscles), no bones. Frog: a bony internal skeleton. (c) A practical asks you to recognize real structures, not repeat words from a book. Looking long and carefully, and drawing what you see, trains you to notice and remember the details you will need to name at each station.

Bonus: Nature Lab

All year you have studied life up close: cells under the microscope, bacteria and yeast, molds and mushrooms, the lungs of birds and the gills of fish. This bonus unit takes you outdoors to practice the skills every field biologist uses. You will keep a nature journal like Gilbert White of Selborne, count plants and pill bugs in square-meter quadrats like the Nebraska prairie botanists, watch animals with an ethogram at a zoo or farm, read museum signs with care, and build a small labeled herbarium in the tradition of Ole Worm and Linnaeus.

These lessons are lighter and playful, but they are real science. Each one asks you to measure in metric units, record data with the date and place, calculate a rate, a mean, a percent or an estimate, and design a fair test. Each one also reminds you that microbes are everywhere you go, in puddle water, garden soil, barnyards and damp specimens. Do the lessons in any season and in any order, and come back to them in spring and fall to compare your notes.

Above all, this unit is an invitation to wonder. Job says to ask the beasts and the birds and they will teach you. Walk slowly, look closely, write it down, and take care of what you study.

BEFORE YOU BEGIN

- Can use a magnifying glass and a simple microscope (Unit 1)
- Can measure length in centimeters and meters and mass in grams
- Can find a mean and a percent, and read a bar graph
- Knows the handwashing and mold lessons from Units 4 and 6

MASTERY CHECKLIST

- I can keep a dated nature journal page with a measured sketch and observations labeled separately from inferences.
- I can measure my pace and use it to find distances and walking rates.
- I can survey a yard with quadrats and estimate a population from a mean and an area.
- I can record animal behavior with an ethogram and scan sampling and turn it into a time budget.
- I can tell observations from interpretations on a museum sign.
- I can collect, press and label plant specimens legally and responsibly.

OPTIONAL BOOKS AND RESOURCES

- **iNaturalist** (https://www.inaturalist.org/): Free. Begun as a joint project of the California Academy of Sciences and National Geographic, it is now an independent nonprofit. Photograph plants and animals on your walks and surveys and get help identifying them. A parent should set up and manage the account for students under 13; the companion app Seek works without an account.

- **Merlin Bird ID, from the Cornell Lab of Ornithology** (https://merlin.allaboutbirds.org/): Free app. Identifies birds by photo, by a few simple questions, or by recording their songs, which is a great help for the nature journal walk and the sit-spot test.
- **Smithsonian National Museum of Natural History** (https://naturalhistory.si.edu/): Free. If you cannot visit a museum in person, use the museum's online virtual tour for Lesson 9.3 and practice reading the signs in the halls of mammals and fossils.

9.1 Nature Journal Walk

OBJECTIVES

- Keep a dated nature journal page that records place, time, weather, a careful sketch with a scale bar, and written observations.
- Tell the difference between an observation and an inference, and turn an “I wonder” into a testable question.
- Measure your own pace length and use it to find distances and walking rates outdoors in meters.

Warm-up. Pick up one leaf, pebble or feather from a windowsill, yard or houseplant. Set a timer for 3 minutes and write down everything you can observe about it without naming it: size, color, texture, smell, edges, patterns. Then count your list. Most people find far more to write in the third minute than they expected.

Video. Coming soon. How to keep a nature journal for middle schoolers, with sketching tips, field notes and the “I notice, I wonder, it reminds me of” method (search nature journaling educators, Smithsonian, state park and extension channels, Homeschool Pop (Alternate), nature footage).

KEY TERMS

observation
Information gathered directly with the senses or instruments, such as “the leaf is 6 cm long” or “the bird has a red patch on its wing.”

inference
A reasoned explanation of what you observed, such as “the leaf has holes, so an insect probably chewed it.” An inference can be tested and may be wrong.

field notes
Written and drawn records made outdoors at the time of an observation, always with the date, time, place and conditions.

phenology
The study of the timing of yearly events in living things, such as the first leaves, first flowers, bird arrivals and leaf fall.

habitat
The place where a living thing finds the food, water, shelter and space it needs.

CORE IDEAS

The story. In the little English village of Selborne, in Hampshire, a country curate named Gilbert White kept a journal of the living things around his home for about twenty-five years, starting in 1768. Almost every day he wrote down the weather, the first swallow of spring,

the day a tree leafed out, and what the crickets, toads and tortoise in his garden were doing. Because he watched so patiently, he noticed things no one had written down before. He showed that three small, look-alike leaf warblers (the chiffchaff, the willow warbler and the wood warbler) were really three different kinds by listening to their very different songs, and he gave the first good description of the harvest mouse in Britain. His book, *The Natural History and Antiquities of Selborne* (1789), has hardly been out of print since.

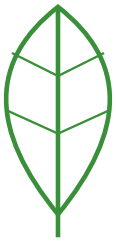
The big idea. A nature journal is a scientist's field notebook. Every page starts with the facts that make an observation useful later: **date, time, place and weather**. Then come careful sketches with a scale bar or a measurement, and words that keep two things apart. An **observation** is what you actually see, hear, smell, touch or measure ("the bark has diagonal cracks 2 mm deep"). An **inference** is your explanation of it ("a woodpecker made these holes"). Good naturalists write both but label them, because an inference can be tested and may turn out to be wrong. A simple pattern helps: *I notice* (observations), *I wonder* (questions), *It reminds me of* (connections). Over weeks and years a journal also records **phenology**, the timing of first flowers, nesting and leaf fall, which is exactly what White did. You can even bring home a spoonful of puddle or pond water in a clean jar and look at it under your microscope, as you did in Unit 1, to see the single-celled life that lives in that habitat.

God's handiwork. In a short note at the front of his book, White wrote that he hoped it might lead readers to pay closer attention to the wonders of Creation that people overlook because they seem common. Job says the same thing: ask the beasts and the birds, and they will teach you. A nature walk is not a race. When you stop, look, measure and draw, you begin to see order and variety the Creator built into one hedge, one lawn or one tree. Every carefully dated page also becomes a gift to the next scientist, who can compare your notes with their own.

Date: _____ Time: _____ Place: _____

Weather: _____ Temperature: ____ °C

Sketch (with scale bar)



1 cm

I notice...

I wonder...

It reminds me of...

Tally of kinds seen: plants ___ insects ___ birds ___ fungi ___

Follow-up question (O = observation, I = inference):

A useful journal page: the facts first, then a measured sketch, then observations kept separate from questions.

PICTURE STUDY



Young Hare, Albrecht Dürer, 1502 · Watercolor and bodycolor (opaque watercolor) on paper · Albertina, Vienna, Austria

Albrecht Dürer of Nuremberg, Germany, was one of the greatest artists of his time, and he loved to draw real things from life: a clump of grass, a stag beetle, a bird’s wing and this hare. He painted it hair by hair, using fine brushes and opaque white for the lightest

whiskers. Look closely at the eye and many viewers see the reflection of a window, a sign that Dürer was looking at a real animal in a real room.

Dürer was not making a field journal page, but he did the most important thing a field naturalist does. He looked longer and more carefully than anyone else, and he drew only what he saw.

Look closely

- Find the eye. What tiny shape is reflected in it, and what does that tell you about where the hare was?
- Look at the fur. Does it all go the same direction? Find places where the hairs change direction.
- Compare the ears, the back feet and the whiskers. Which part did Dürer paint most carefully?
- Notice the plain background. Why might a naturalist leave out the background when drawing a specimen?

Talk about it

1. What is one observation and one inference you can make about this hare from the painting alone?
2. How long do you think it took to paint every hair? What does that teach you about patience in science?
3. If you were adding this hare to a field journal, what facts would you need that the painting does not give you?

> **Safety first.** Walk with an adult or a buddy and tell someone your route. Wear closed shoes, long pants in tall grass, and check for ticks afterward. Learn to spot poison ivy (“leaves of three, let it be”) and stinging nettles before you go. Never touch or feed wild animals, never eat any wild plant, berry or mushroom, and stay well back from deep or fast water. Never look at the sun, even through binoculars or a magnifier. Wash your hands when you return, especially if you collected a water sample.

EXPERIMENT: THE SIT-SPOT TEST

Naturalists often say you see more by sitting still than by walking. You will test that claim with your journal.

Question: Do I notice more kinds of living things in 10 minutes sitting still or 10 minutes walking slowly? **Prediction:** Write yours with a reason. **What we change:** sitting or walking. **What we keep the same:** the general area, the length of time, the time of day and the weather (do both on the same outing). **What we measure:** the number of different kinds of living things you record (plants, insects, birds, fungi, other).

You will need

- Notebook or sketchbook with unlined pages, pencil and eraser
- Ruler or measuring tape (metric), a 10-meter measuring tape if you have one
- Magnifying glass, timer, outdoor thermometer if available
- Optional: clean small jar with a lid for a water sample, colored pencils, binoculars

Steps

1. Before you set out, measure your pace (see the worked example) and write it inside the cover of your journal.
2. At your first stop, write the header: date, time, place, weather and temperature in °C.
3. Sit in one spot for 10 minutes. Tally every different kind of living thing you notice, by sight or by sound, sorted into plants, insects and spiders, birds, fungi and other.
4. Then walk slowly for 10 minutes along a path nearby, counting your steps, and tally again on a fresh list.
5. Choose one thing from either list and make a careful sketch at least 8 cm tall with a scale bar or a measurement written beside it.
6. Fill in “I notice,” “I wonder” and “It reminds me of” for your sketch. Label each line O (observation) or I (inference).
7. At home, compare the two tallies in a small table and write whether your prediction was supported.
8. Turn one “I wonder” into a question you could test, naming what you would change and what you would measure.

Tips

- Sounds count. Many birds and insects are heard long before they are seen.
- Draw what is there, not what you expect. If a leaf has a bite out of it, draw the bite.
- One test on one day is a small sample. Repeat it on two more days before drawing a firm conclusion.

Check your work

- Every page I made has a date, time, place and weather.
- My sketch has a scale bar or a measurement.
- I labeled observations and inferences.
- I compared sitting and walking with a fair test and recorded my numbers.

NATURE NOTEBOOK

1. Copy the journal page layout from this lesson into the front of your notebook as a template, and fill in your first complete page.
2. Draw one leaf at life size, trace around a real one first, then add the vein pattern and a 1 cm scale bar. Write three observations and one inference about it.
3. Start a phenology list on the last page of your journal: date of the first dandelion, first robin, first leaves on one chosen tree, and keep adding to it all year.

From history. On the Lewis and Clark expedition (1804–1806), Meriwether Lewis kept careful journals describing plants and animals that scientists in the eastern United States had never seen. In the spring of 1805 the expedition sent a shipment back down the Missouri River to President Thomas Jefferson, including pressed plants, skins and a live prairie dog, which arrived alive in Washington. Many of Lewis's pressed plant specimens are still kept today at the Academy of Natural Sciences in Philadelphia.

WORKED EXAMPLES

Example 1. Kate lays out a measuring tape 20 meters long and walks beside it, counting 25 steps from start to end. Later she walks a loop trail and counts 650 steps, which takes her 26 minutes. (a) How long is one of her steps? (b) How long is the trail? (c) What was her walking rate in meters per minute?

1. (a) Step length = distance ÷ steps = 20 m ÷ 25 = 0.80 m per step.
2. (b) Trail length = 650 steps × 0.80 m = 520 m.
3. (c) Rate = distance ÷ time = 520 m ÷ 26 min = 20 m per minute.
4. Check: 20 m/min × 26 min = 520 m. ✓

Answer: (a) 0.80 m per step. (b) 520 m. (c) 20 m per minute.

PRACTICE

1. EASY Label each as an observation (O) or an inference (I): (a) The bark has three round holes about 1 cm wide. (b) A woodpecker made the holes. (c) The air temperature is 14 °C. (d) The robin is looking for worms.
2. MEDIUM Your pace is 0.70 m. You count 400 steps from your door to the creek. How far is the creek? If the walk took 8 minutes, what was your rate?
3. MEDIUM Data reading. A student watched the same flowering bush for 10 minutes at noon on five spring days and counted bee visits. 12 °C: 2 visits. 15 °C: 5. 18 °C: 9. 21 °C: 14. 24 °C: 15. (a) What is the independent variable? (b) Describe the trend. (c) Predict roughly how many visits on a 10 °C day, and explain.
4. HARD Experiment design. You wonder whether birds visit your feeder more in the early morning or in the late afternoon. Describe a fair test.
5. CHALLENGE Explain why Gilbert White's notes were valuable to scientists long after he died, and why a journal page with no date or place loses most of its value.

COMMON MISTAKES

- Writing inferences as if they were observations. "The bird is hungry" is an inference; "the bird pecked at seeds 12 times in one minute" is an observation.
- Drawing from memory or from a book picture instead of from the real object. A field sketch is evidence only if it shows what was really there.

- Leaving off the date, place, time or weather. Without them, even a beautiful page cannot be compared with any other observation.

Make it a game · Observation or Inference?. On your walk, take turns making statements about something you both can see. The other player calls out “Observation!” or “Inference!” A correct call scores a point. If it is an inference, the caller earns a bonus point by naming one observation that could test it. First to 10 points chooses the next stop.

Scripture. “But ask now the beasts, and they shall teach thee; and the fowls of the air, and they shall tell thee: Or speak to the earth, and it shall teach thee: and the fishes of the sea shall declare unto thee.” (Job 12:7–8, KJV)

Creation is a teacher, but only to those who stop and ask. A nature journal is a way of asking the beasts, the birds and the earth careful questions and writing down what they show us.

Practice answers

1. (a) O. (b) I. (c) O. (d) I (we see the robin pecking at the ground; “looking for worms” is our explanation).
2. 400 × 0.70 m = 280 m. Rate = 280 m ÷ 8 min = 35 m per minute.
3. (a) Air temperature. (b) Visits rose steadily as temperature rose from 12 °C to 21 °C, then nearly leveled off between 21 °C and 24 °C. (c) Fewer than 2, perhaps 0 or 1, because the trend shows fewer bees in cooler air (bees are less active when it is cold).
4. Independent variable: time of day (early morning versus late afternoon). Dependent variable: number of bird visits counted in a fixed watch time, such as 15 minutes. Keep the same: the feeder, the amount and kind of seed (filled to the same level), the watching spot, and the length of each watch. Repeat on at least five days, record the weather, then average the counts for each time of day and compare.
5. Because White recorded dates, places and weather for decades, later scientists could compare their own observations with his and see what had or had not changed, such as when birds arrived or trees leafed out. A page with no date or place cannot be compared with anything; it records what was seen but not when or where, so no one can use it as evidence.

9.2 Backyard Survey

OBJECTIVES

- Lay out square-meter quadrats and count the plants and small animals inside them to survey a yard, park or field.
- Estimate the size of a population from sample counts by finding a mean and scaling up to the whole area.
- Compare two habitats (such as sun and shade) fairly and explain why scientists use several samples instead of one.

> **Warm-up.** Toss a coin onto a patch of lawn or a rug with a pattern. Lay a sheet of notebook paper flat around where it landed and count every dandelion, clover leaf or pattern spot inside the paper. Toss again somewhere else and count again. Were the two counts the same? What would you need to do to estimate the total for the whole lawn or rug?

> **Video.** Coming soon. How to use a quadrat to survey plants and animals, and how to estimate population size from samples, for middle school (search university extension, field biology education channels, Smithsonian, Homeschool Pop (Alternate), Science Max, nature footage of soil and lawn invertebrates).

KEY TERMS

quadrat
A square frame of known size, often 1 m by 1 m, placed on the ground to mark an area where every living thing of a chosen kind is counted.

population
All the living things of one kind living in one area at the same time, such as all the dandelions in a lawn.

community
All the populations of different kinds living together in one area, such as the grasses, clovers, ants, worms and birds of a yard.

sample
A small part that is measured to learn about the whole. Several samples placed fairly give a better estimate than one.

species richness
The number of different kinds of living things found in an area.

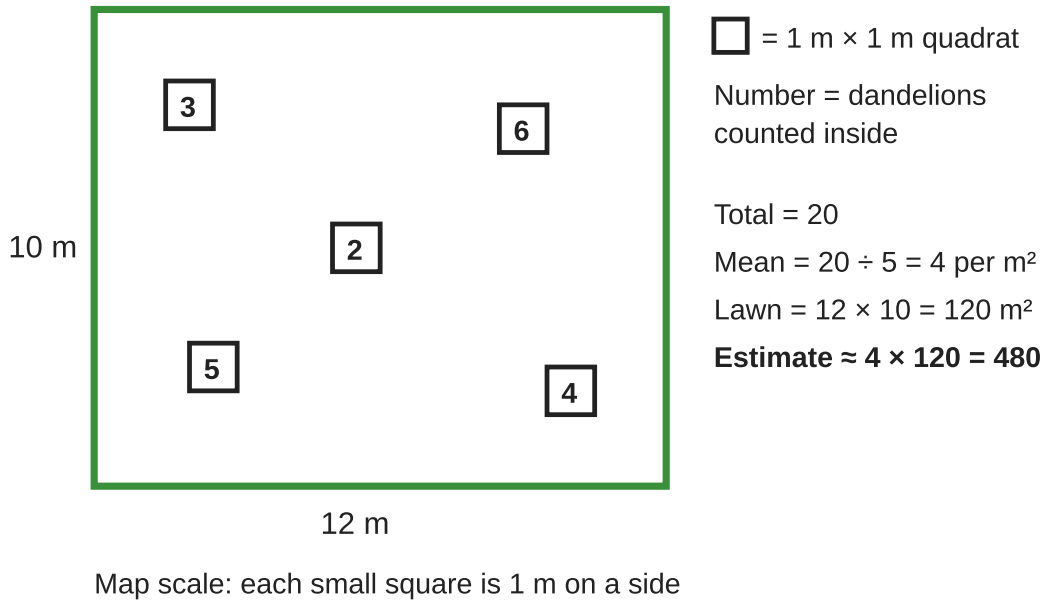
CORE IDEAS

The story. In the 1890s two young botanists at the University of Nebraska, Roscoe Pound and Frederic Clements, set out to describe the plants of the great prairie. They faced a

problem every naturalist meets: there were far too many plants to count. Their answer, published in 1898, was to mark off small squares of prairie and count every plant inside each square. From the squares they could compare one place with another in numbers instead of words like “common” or “rare.” The square plot, the **quadrat**, became one of the basic tools of ecology. Clements spent his life studying plant communities. Pound went on to become one of the most famous lawyers in America and the dean of Harvard Law School, yet ecologists still remember him for those prairie squares.

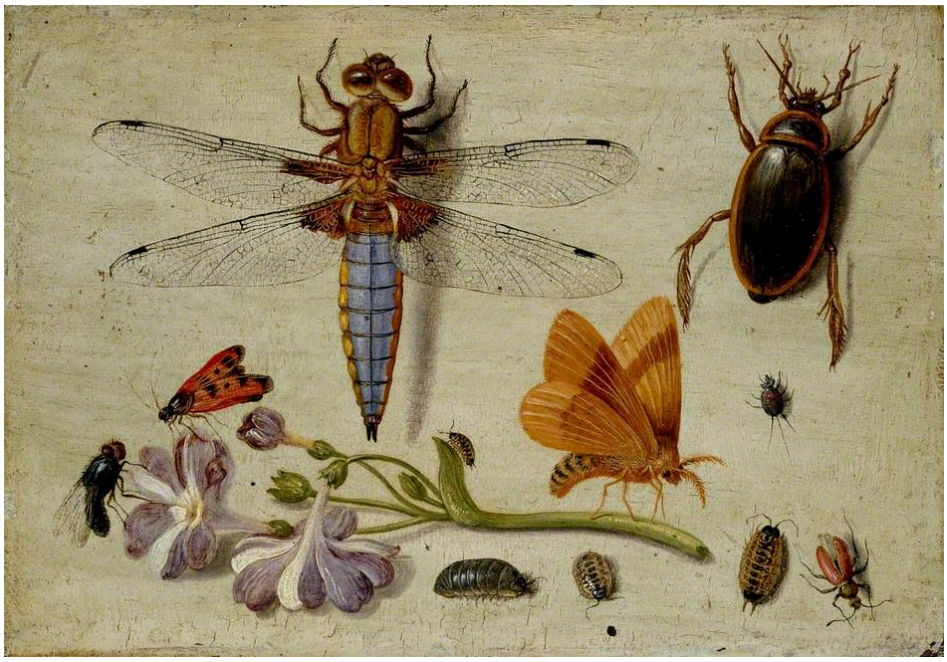
The big idea. Your yard holds a **community**: populations of grasses, clovers, dandelions, ants, earthworms, spiders, pill bugs and beetles, plus fungi and billions of bacteria in every handful of soil. You cannot count them all, so you **sample**. Place several 1 m by 1 m quadrats fairly (for example, by tossing a marker over your shoulder or using a grid), count one kind of living thing in each, and find the **mean** per square meter. Multiply by the total area to estimate the whole **population**. More quadrats give a more trustworthy estimate, because one square might land in an unusually thick or bare patch. To survey small animals, lay a flat board or a piece of cardboard on damp ground overnight and lift it in the morning: pill bugs, earthworms, slugs, beetles and centipedes gather in the cool, moist shelter. Count them, then set the board back gently.

Try it and see. Compare two habitats in the same yard, such as full sun and the shade under a tree. Count the **species richness** (how many different kinds) in each quadrat as well as the numbers of one chosen kind. Look for patterns: clover and moss often prefer different conditions, and the board in the shade may hide more animals than the one in the sun. Solomon told the lazy man to go to the ant and consider her ways. A survey is a way of considering: patiently counting, comparing and asking why the living things in one corner differ from those in another.



Five quadrats on a 12 m by 10 m lawn. The mean count per square meter, times the area, estimates the whole dandelion population.

PICTURE STUDY



A Cockchafer, Beetle, Woodlice and Other Insects, with a Sprig of Auricula, Jan van Kessel the Elder, mid-1600s · Oil on copper · Ashmolean Museum, University of Oxford, England

Jan van Kessel the Elder was a painter in Antwerp, in what is now Belgium, and a grandson of the famous painter Jan Brueghel the Elder. He painted many tiny pictures on thin sheets of copper, each filled with insects, spiders and other small creatures set out almost like specimens on a table. This panel is only about 12 centimeters wide.

Look closely and you will find woodlice, the same pill bugs and sow bugs you may find under your survey board. Van Kessel painted common creatures that most people stepped over without a glance, and he painted them with the same care other artists gave to kings and queens. That is the spirit of a backyard survey: small, common creatures are worth looking at closely.

Look closely

- Find the woodlice. How many legs and body segments can you see? Are they insects?
- Find the cockchafer, the large beetle. Where are its antennae, and what shape are they?
- Count how many different kinds of creatures are in the painting. That number is the picture’s species richness.
- Look at how the creatures are spread across the panel. Did the painter arrange them like a survey, a collection or a real scene?

Talk about it

1. Why do you think a painter would spend so much care on creatures most people ignore?

2. If you turned over a board in your yard, which creatures in this painting might you find?
3. How is a painting like this one different from a real survey record? What would a scientist want to know that the painting does not tell?

> **Safety first.** Work with an adult. Wear gloves when lifting boards, rocks or leaf litter, and lift them toward you so anything underneath scurries away from you. In areas with venomous snakes, scorpions or black widow spiders, let an adult lift the board with a stick. Do not handle stinging insects, and avoid ant mounds. Learn to recognize poison ivy. Do not survey near roads. Wash your hands after touching soil. Put every board and rock back gently where it was.

EXPERIMENT: SUN VERSUS SHADE SURVEY

You will survey two parts of the same yard or park and compare their plant and animal communities.

Question: Does a sunny patch of lawn have more kinds of plants (and more small animals under a board) than a shady patch? **Prediction:** Write yours with a reason. **What we change:** sun or shade. **What we keep the same:** quadrat size, number of quadrats, the day, the way quadrats are placed, and the size of the boards. **What we measure:** number of kinds of plants per quadrat, count of one chosen plant (dandelion or clover) per quadrat, and number of animals under each board.

You will need

- 4 sticks or tent pegs and 4.5 m of string (to make a 1 m by 1 m quadrat with a little extra for tying), or a hula hoop of known size
- Meter stick or measuring tape
- 2 flat boards or pieces of thick cardboard about 30 cm by 30 cm
- Gloves, magnifying glass, notebook, pencil, a simple plant or bug field guide or app
- Optional: soil thermometer

Steps

1. Measure the length and width of your survey area in meters and draw a map of it in your notebook.
2. Mark a sunny zone and a shady zone. The evening before, lay one board flat on the ground in each zone.
3. Make your quadrat: push in 4 sticks at the corners of a 1 m square and tie string around them.
4. Place 5 quadrats in each zone without choosing “good” spots: for example, stand in the middle, close your eyes and toss a marker behind you.
5. In each quadrat, count the different kinds of plants (give each unknown kind a nickname such as “fuzzy leaf”) and count every dandelion or clover plant. Record soil temperature if you can.

6. In the morning, lift each board and count the animals under it by kind. Put the board back gently.
7. Find the mean count per square meter for each zone. Multiply by the area of each zone to estimate the dandelion or clover population.
8. Make a bar graph comparing sun and shade, and write whether your prediction was supported.

Tips

- Pill bugs and sow bugs are not insects. Count their legs: they have 14 (seven pairs), so they are crustaceans, cousins of shrimp.
- If you cannot name a plant, sketch a leaf and give it a nickname. Consistent nicknames work fine for counting kinds.
- Take a spoonful of soil from each zone into a sealed bag. Smell it: the earthy smell comes partly from soil bacteria called actinomycetes.

Check your work

- I placed my quadrats fairly and used the same number in each zone.
- I recorded counts in a table and calculated a mean for each zone.
- I estimated a population for each zone from the mean and the area.
- I compared the two zones with a graph and a written conclusion.

NATURE NOTEBOOK

1. Draw a map of your survey area to scale (for example, 1 cm on paper = 1 m on the ground) and mark where each quadrat fell.
2. Draw one animal you found under a board, as Hooke drew his flea: large, with every leg and body segment counted and labeled.
3. Make a two-column list, Sun and Shade, of every kind of living thing you found in each. Circle the ones found in both.

> **From history.** In *On the Origin of Species* (1859), Charles Darwin described a small experiment in his own garden at Down House in Kent. He dug and cleared a plot of ground about 3 feet by 2 feet (roughly 90 cm by 60 cm) and marked every seedling of the native weeds as it came up. Out of 357 seedlings, 295 were destroyed, mostly by slugs and insects. It was a backyard survey in miniature, and it showed how many young plants never survive to grow up.

WORKED EXAMPLES

Example 1. A lawn is 12 m wide and 10 m long. A student places five 1 m by 1 m quadrats and counts dandelions: 3, 6, 2, 5 and 4. Estimate the number of dandelions in the whole lawn.

(Use the map or drawing "The five sample quadrats." shown earlier in this lesson.)

1. Total counted: 3 + 6 + 2 + 5 + 4 = 20 dandelions.
2. Total area sampled: 5 quadrats × 1 m² = 5 m².
3. Mean density: 20 ÷ 5 m² = 4 dandelions per m².
4. Area of the lawn: 12 m × 10 m = 120 m².
5. Estimate: 4 per m² × 120 m² = 480 dandelions.

Answer: About 480 dandelions (an estimate, since only 5 of 120 square meters were counted).

PRACTICE

1. EASY What is the difference between a population and a community? Give an example of each from a backyard.
2. MEDIUM A pasture is 50 m by 40 m. Four 1 m² quadrats contain 7, 9, 5 and 11 clover plants. Estimate the clover population.
3. MEDIUM Data reading. A survey found these results. Sunny zone: mean 6 kinds of plants per quadrat, 1 animal under the board, soil 24 °C. Shady zone: mean 4 kinds of plants per quadrat, 17 animals under the board, soil 17 °C. (a) Which zone had greater plant species richness? (b) Which zone had more animals under the board? (c) Suggest one reason for the difference in animals, using the data.
4. HARD Experiment design. You wonder whether leaving grass clippings on the lawn changes the number of earthworms. Design a fair test.
5. CHALLENGE A student places one quadrat, right in a thick clover patch, counts 40 clover plants and says the 100 m² lawn has 4,000 clover plants. Explain two problems with this method and how to fix them.

COMMON MISTAKES

- Choosing where to put quadrats. Placing them on the "interesting" spots makes the estimate too high; place them randomly or on a grid.
- Thinking pill bugs, spiders and centipedes are insects. Insects have six legs and three body parts; pill bugs are crustaceans, spiders are arachnids and centipedes are myriapods.
- Treating an estimate as an exact count. A quadrat survey gives an estimate; more quadrats make it more trustworthy but never perfect.

Make it a game · Quadrat Guess. Each player looks at the same 1 m² quadrat for 15 seconds and secretly writes a guess for how many plants (or clover leaves, or ants) are inside. Then count together. The closest guess scores 2 points and anyone within 10 percent scores 1. Play five rounds in different spots and see whose guesses improve.

Scripture. “Go to the ant, thou sluggard; consider her ways, and be wise:” (Proverbs 6:6, KJV)

Solomon sends us to the smallest creatures of the yard to learn. To consider means to look carefully and think, which is exactly what a survey asks of you.

Practice answers

1. A population is all the living things of one kind in an area (all the clover plants in the yard). A community is all the populations of different kinds living together there (the clover, grass, ants, worms and robins of the yard).
2. Mean = (7 + 9 + 5 + 11) ÷ 4 = 32 ÷ 4 = 8 per m². Area = 50 × 40 = 2,000 m². Estimate = 8 × 2,000 = 16,000 clover plants.
3. (a) The sunny zone (6 kinds versus 4). (b) The shady zone (17 versus 1). (c) The shady soil was 7 °C cooler, and probably damper; pill bugs, worms and slugs dry out easily and gather in cool, moist places.
4. Choose two similar patches of the same lawn (same sun, soil and slope). Independent variable: clippings left on one patch, removed from the other, for several weeks. Dependent variable: number of earthworms counted in several 1 m² quadrats per patch (for example, by counting worms that come up after soaking the soil, or under boards). Keep the same: mowing height, watering, quadrat size, number of quadrats, and the day and time of counting. Repeat counts and compare means.
5. First, one quadrat is too small a sample; one unusual square can throw off the whole estimate. Second, the quadrat was placed in a chosen spot (a thick patch), which biases the count upward. Fix: use several quadrats (at least five) placed randomly or on an even grid, find the mean, and then multiply by the area.

9.3 Zoo, Farm or Museum Visit

OBJECTIVES

- Observe one animal at a zoo or farm with an ethogram, using scan sampling to record its behavior every 30 seconds.
- Turn behavior counts into a time budget in percent and display it on a bar graph.
- Read a natural history museum sign carefully, separating what is observed from how it is interpreted.

Warm-up. Watch a pet, a backyard bird or a squirrel from a window for 5 minutes. Every 30 seconds, write one word for what it is doing at that instant (eating, resting, walking, grooming, looking). At the end, count your words. Which behavior did you write most often? Was it what you expected?

Video. Coming soon. How scientists observe animal behavior with an ethogram and scan sampling, plus a virtual tour of a natural history museum hall of mammals and fossils (search Smithsonian National Museum of Natural History, zoo education channels, university animal science and extension channels, Homeschool Pop (Alternate), nature footage).

KEY TERMS

behavior
Anything an animal does in response to its surroundings or its body’s needs, such as eating, resting, grooming or calling.

ethogram
A list of an animal’s behaviors, each with a short, clear definition and a code, used to record behavior the same way every time.

scan sampling
Recording what an animal is doing at fixed moments, such as every 30 seconds, instead of trying to write down everything continuously.

time budget
How an animal divides its time among different behaviors, usually shown as percents.

domestic animal
An animal kept and bred by people for many generations, such as cattle, sheep, goats, chickens, horses and dogs.

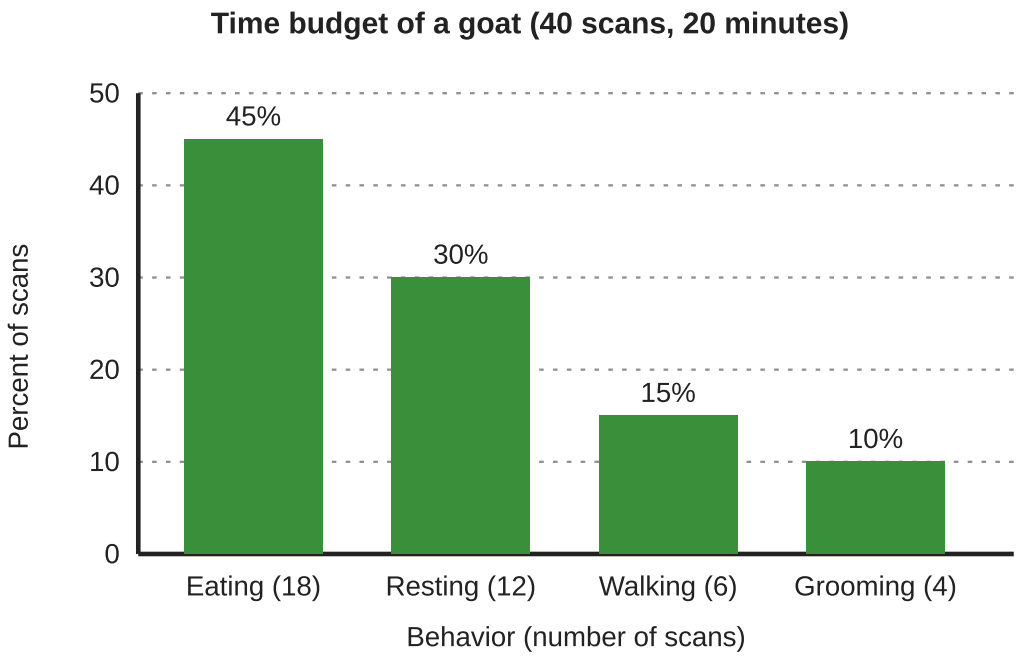
CORE IDEAS

The story. In 1801 the Philadelphia painter and naturalist Charles Willson Peale heard that farmers near Newburgh, New York, had found enormous bones in a waterlogged marl pit on John Masten’s farm. Peale bought the rights to dig, and with a great wooden wheel turned by

men walking inside it, he pumped the pit dry and lifted out the bones of a mastodon, a huge elephant-like animal. He took them home to Philadelphia, rebuilt the skeleton and put it on display in his museum, one of the first natural history museums in America. Peale did something new there too: he mounted birds and animals in cases with painted backgrounds showing their habitats, and he arranged them in the order of Linnaeus's classification so visitors could learn as they walked. At age 81 he painted himself lifting a curtain to invite you in.

The big idea. A zoo, a farm and a museum are three outdoor classrooms. At a zoo or farm you can study **behavior** the way field biologists do. First make an **ethogram**, a short list of behaviors with clear definitions ("Eating: food in mouth or chewing"). Then use **scan sampling**: every 30 seconds, glance at one animal and record what it is doing at that instant. After 20 minutes you have 40 records you can turn into a **time budget**. Farm animals are especially rewarding. Cattle, sheep and goats are **ruminants** that swallow grass, bring it back up as cud and chew it again, so you will often see them lying down with their jaws moving. If your family keeps chickens or goats, compare their time budgets with the matching animals in the Gardening & Animals course. At a natural history museum, practice reading the signs. Separate what is **observed** (the bones, their size, where they were dug up) from what is **interpreted** (how old they are, how the animal lived).

Try it and see. Every place you visit is also a place full of microbes. Farm animals carry bacteria in their guts that help them digest grass, but some of those bacteria, such as certain kinds of *E. coli*, can make people very sick. That is why farms and petting zoos post handwashing stations by the exits, and why the lesson of Semmelweis from Unit 4 matters on every field trip: wash with soap and running water after touching animals, fences or straw, and before eating anything. A righteous man regards the life of his beast, and a wise visitor respects both the animals and the unseen life that comes with them.



A time budget from scan sampling: each bar is the share of 40 scans spent on one behavior.

⏳ Thinking About Time

What the sign says. What the sign says: a museum sign beside a mastodon skeleton will usually call it an Ice Age animal of the Pleistocene epoch and say that the last American mastodons died out about 10,000 to 11,000 years ago.

What we observe. What we observe: mastodon bones and teeth are often dug from bogs, swamps and marl pits only a few meters below the surface, many still made of real bone rather than turned fully to stone, and sometimes with chewed twigs and plant remains from the animal's meals nearby. Their teeth have cone-shaped cusps suited to browsing on twigs and leaves.

How Christians read it. How Christians read it: many Christians read the mastodon as part of the elephant kind that came off Noah's Ark, spreading over the earth in the centuries after the Flood. They see the Ice Age as a single period after the Flood, when warm oceans and cooler, ash-darkened skies brought heavy snows, and they place the mastodon's dying out within the last few thousand years. Both readings agree on the bones; they differ on the dates assigned to them.

Try it. At a museum, copy one sign word for word, then underline the observations in one color and the interpretations in another.

Genesis 8:19

PICTURE STUDY



The Artist in His Museum, Charles Willson Peale, 1822 · Oil on canvas · Pennsylvania Academy of the Fine Arts, Philadelphia

Charles Willson Peale was a painter who fought in the American Revolution, painted George Washington from life several times, and then spent much of his life building a museum of natural history in Philadelphia. He painted this large self-portrait at age 81, at the request of the museum's trustees. He shows himself lifting a curtain like a showman, inviting you in to see the order of God's creatures on display.

Everything in the picture tells a part of Peale's story: his painting tools, his taxidermy tools, his rows of birds arranged by Linnaeus's system, and the mastodon bones he dug up in 1801.

Look closely

- Find Peale's palette and brushes, and then the tools for preparing animal skins. Why would he show both?
- Look down the long gallery. How are the bird cases arranged? What kind of order does it suggest?
- Find the mastodon bones near the edge of the curtain. How large do they look next to the people?
- Find the woman with her hands raised. What do you think she has just seen?

Talk about it

1. Why do you think Peale painted himself lifting a curtain instead of sitting still for a portrait?
2. What does a good museum do that a book or a video cannot?

3. If you made your own museum case, what would you put in it and how would you arrange it?

> **Safety first.** Stay behind all zoo barriers and never climb, lean over or reach through them. At a farm, ask the farmer before touching any animal, approach calmly from the side, never stand directly behind a horse or cow, and keep fingers flat when offering food. Do not eat, drink or put fingers in your mouth in animal areas. Wash hands with soap and running water after touching animals, fences, straw or manure; hand sanitizer alone is not enough. Wear closed shoes. In museums, touch only exhibits marked for touching.

EXPERIMENT: MY ETHOGRAM STUDY

You will study one animal the way a field biologist does, and then test whether its behavior changes at different times.

Question: Does my animal spend more of its time eating in the morning or in the afternoon? **Prediction:** Write yours with a reason. **What we change:** time of day. **What we keep the same:** the same animal (or same group), the same ethogram, a 30-second scan interval and 20-minute sessions. **What we measure:** the number of scans of each behavior, turned into percents. *Museum option:* if you visit a museum instead, choose one skeleton, measure or estimate three of its bones, sketch it, and compare it with a living animal of a similar kind, while copying and marking one sign as in the time note.

You will need

- Notebook with a ruled scan sheet (40 rows, one for each 30 seconds)
- Watch or timer that can beep every 30 seconds
- Pencil, clipboard
- Optional: binoculars, a camera for sketching later, graph paper

Steps

1. Choose one animal you can see clearly for 20 minutes: a goat, cow, sheep, chicken, horse, zoo giraffe or zoo meerkat.
2. Watch it for 5 minutes without writing. Then write an ethogram of 4 to 6 behaviors, each with a one-line definition and a one-letter code (E = eating, R = resting, W = walking, G = grooming, O = other).
3. Start the timer. Every 30 seconds, look at the animal and write the code for what it is doing at that instant. Do 40 scans (20 minutes).
4. If possible, repeat with the same animal at a different time of day.
5. Count the scans for each behavior and divide by 40, then multiply by 100 to get percents.
6. Make a bar graph of each time budget.
7. Write a conclusion: was your prediction supported? What else, besides time of day, might have changed the results (weather, feeding time, visitors)?
8. Before leaving, wash your hands with soap and water.

Tips

- Clear definitions matter. Decide ahead of time whether chewing cud while lying down counts as “eating” or “resting,” and stick to it.
- If the animal is out of sight at a scan, write “X” and leave it out of the total.
- Zoo keepers and farmers love good questions. Ask when the animals are fed and how much they eat in a day.

Check your work

- My ethogram has clear definitions and codes.
- I recorded 40 scans at even intervals.
- I turned counts into percents that add to 100 (or very close).
- I made a bar graph and wrote a conclusion that mentions other things that could affect the results.

NATURE NOTEBOOK

1. Draw your animal in three different behaviors from your ethogram, each labeled with its code.
2. At a museum, sketch one skeleton and label at least five bones you recognize (skull, spine, ribs, upper leg bone, shoulder blade).
3. Copy one museum sign word for word and underline the observations and the interpretations in two colors.

> **From history.** The Zoological Society of London, founded in 1826 by Sir Stamford Raffles and others, opened its gardens in Regent’s Park in 1828 for scientific study, and opened them to the paying public in 1847. Later the short word “zoo,” from “zoological garden,” came into everyday use. In the United States, the Philadelphia Zoo was chartered in 1859 and opened its gates in 1874, after the delay of the Civil War, in the same city where Peale’s museum had once taught Philadelphians about animals.

WORKED EXAMPLES

Example 1. A student scan-sampled a goat every 30 seconds for 20 minutes. Results: eating 18 scans, resting 12, walking 6, grooming 4. (a) How many scans were made? (b) Find the time budget in percent. (c) About how many minutes did the goat spend eating?

(Use the map or drawing "The goat's time budget." shown earlier in this lesson.)

1. (a) 20 minutes × 2 scans per minute = 40 scans. Check: 18 + 12 + 6 + 4 = 40. ✓
2. (b) Eating: 18 ÷ 40 = 0.45 = 45%. Resting: 12 ÷ 40 = 30%. Walking: 6 ÷ 40 = 15%. Grooming: 4 ÷ 40 = 10%. Total = 100%. ✓
3. (c) 45% of 20 minutes = 0.45 × 20 = 9 minutes.

Answer: (a) 40 scans. (b) Eating 45%, resting 30%, walking 15%, grooming 10%. (c) About 9 minutes.

PRACTICE

1. EASY What is an ethogram, and why must each behavior have a clear definition?
2. MEDIUM Data reading. A student scan-sampled a cow once a minute for 60 minutes: grazing 24, lying and chewing cud 18, standing and chewing cud 6, walking 6, drinking 3, other 3. (a) What percent of the time was the cow grazing? (b) What percent of the time was it chewing cud, lying or standing? (c) Which behavior shows that cattle are ruminants?
3. MEDIUM Suppose a museum sign says: "This mastodon skeleton was found in a bog in 1845. It stood about 3 meters tall at the shoulder. Mastodons lived during the Ice Age and died out about 10,000 years ago." List the observations and the interpretations.
4. HARD Experiment design. A zoo keeper wonders whether hiding food in puzzle feeders makes the meerkats spend more time active. Design a fair study using scan sampling.
5. CHALLENGE Explain why a farm or petting zoo tells visitors to wash with soap and water instead of only using hand sanitizer, and connect this to what you learned about germs in Unit 4.

COMMON MISTAKES

- Recording what the animal was doing "most of the time" in each interval. In scan sampling you record only what it is doing at the exact moment of the scan.
- Reading every sentence on a museum sign as an observation. Measurements and find spots are observed; ages and life stories are interpretations based on assumptions.
- Thinking a cow lying down and chewing is just resting. It is ruminating, an important part of how it digests grass.

Make it a game · Freeze-Frame Scan. One player acts like an animal (a goat, chicken or cow) and switches behaviors whenever they like. Every 30 seconds a timer beeps and the other players write the behavior code at that instant. After 10 scans, compare sheets.

Each scan everyone agrees on earns a point for the whole group; disagreements show which definitions need to be clearer.

Scripture. “A righteous man regardeth the life of his beast: but the tender mercies of the wicked are cruel.” (Proverbs 12:10, KJV)

Good farmers and good zoo keepers watch their animals closely, which is exactly what an ethogram teaches you to do. Careful observation is a way of caring well for the creatures God has put in our charge.

Practice answers

1. An ethogram is a list of an animal’s behaviors with definitions and codes. Clear definitions make sure every scan is recorded the same way, by the same person on different days or by different observers, so the data can be compared.
2. (a) 24 ÷ 60 = 40%. (b) (18 + 6) ÷ 60 = 24 ÷ 60 = 40%. (c) Chewing cud: bringing swallowed food back up and chewing it again is what ruminants do.
3. Observations: the skeleton was found in a bog in 1845; it stands about 3 m at the shoulder (measured from the bones). Interpretations: that mastodons lived during “the Ice Age” as the museum dates it, and that they died out about 10,000 years ago (dates assigned by a method and its assumptions).
4. Independent variable: food in puzzle feeders versus food in an open bowl. Dependent variable: percent of scans in which the meerkats are active (foraging, digging, walking). Keep the same: the same group of meerkats, the same amount and kind of food, the same time of day, the same scan interval and session length, and similar weather. Alternate the two conditions over several days (at least five sessions each) and compare the mean percent active.
5. Animals and their surroundings carry bacteria such as certain *E. coli* that can make people sick. Washing with soap and running water physically removes dirt, manure and the germs in it, while sanitizer works poorly on visibly dirty hands. Like Semmelweis’s handwashing, it removes invisible microbes before they reach the mouth.

9.4 Specimen Collection

OBJECTIVES

- Collect, press, dry and mount plant specimens responsibly, following the laws and the one-in-twenty rule.
- Write a complete collection label and explain why a specimen without data has little scientific value.
- Measure the loss of water as a specimen dries and calculate the percent water in fresh leaves.

Warm-up. Find five small natural objects in your house or yard (a leaf, a seed, a shell, a pebble, a pine cone). Put them in a row and write one label for each with three facts: what it is, where it was found, and when. Which object was hardest to label? Which fact did you not know?

Video. Coming soon. How to press and mount plant specimens for a herbarium, and a behind-the-scenes look at a natural history museum's specimen collections (search Smithsonian National Museum of Natural History, university herbarium channels, botanical gardens, state extension services, Homeschool Pop (Alternate)).

KEY TERMS

specimen
A real organism, or part of one, that is collected, preserved and kept for study, such as a pressed plant, a shell or a pinned insect.

herbarium
A collection of pressed, dried plant specimens mounted on sheets and filed in order, used for identifying and studying plants.

collection label
The record kept with a specimen: scientific name, place, date, habitat and collector. Without it, a specimen is nearly useless to science.

scientific name
The two-part Latin name of a species, genus first and capitalized, species second in lowercase, both in italics, such as *Taraxacum officinale* (common dandelion).

type specimen
The particular specimen that a scientist used when first naming and describing a species, kept as the standard for that name.

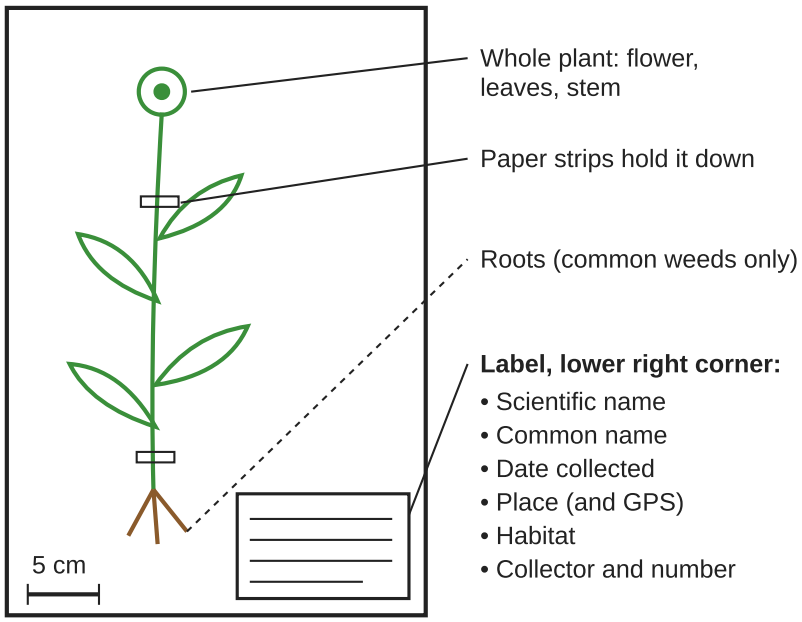
CORE IDEAS

The story. In Copenhagen in the 1600s, a Danish physician and professor named Ole Worm filled a room of his house with shells, minerals, skulls, horns, stuffed animals and dried

plants. Visitors came from far away to see it. Worm did not keep his objects only as curiosities. He studied them and tested old stories against them. Merchants sold long, spiral "unicorn horns" as costly medicine, but in 1638 Worm showed that these horns were really the tusks of the narwhal, a small whale of the Arctic seas. After his death in 1654, a catalog of his collection called *Museum Wormianum* was printed in 1655, and its engraved front picture became one of the most famous images of an early natural history collection. His specimens went to the collection of King Frederick III of Denmark.

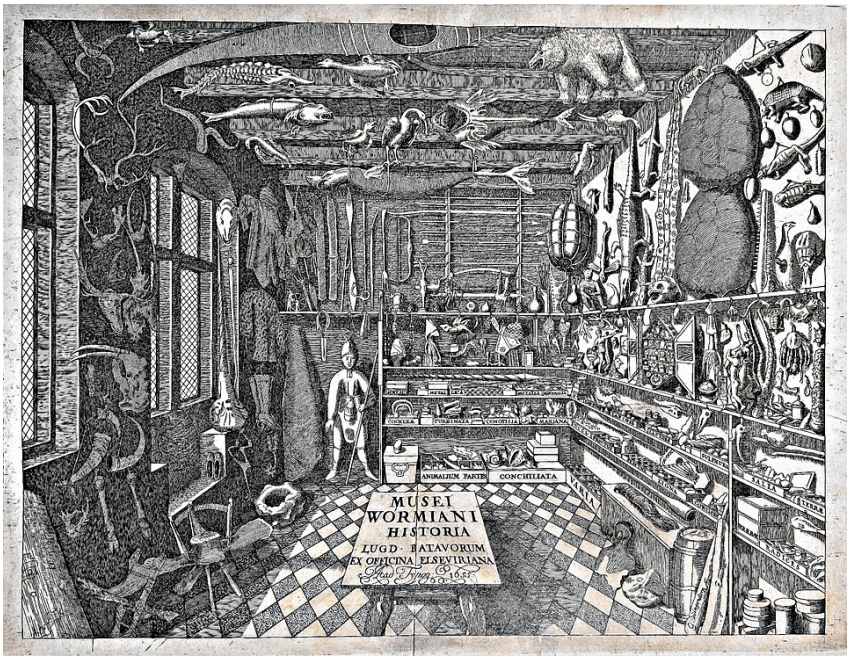
The big idea. A real **specimen** is evidence. Anyone can come back to it years later, look again, measure again or even study its cells. That is why the most important part of a specimen is its **collection label**: scientific name, exact place, date, habitat and collector. Since Linnaeus published *Species Plantarum* in 1753, every plant species has had a two-part **scientific name**, and the specimen first used to name a species, the **type specimen**, is guarded in a museum or **herbarium**. Plants are the easiest specimens to collect at home: press them flat between sheets of newspaper with weight on top, change the paper every day or two, and they dry in about a week. Drying matters for the reasons you learned in Units 3 and 6. A damp specimen is food for mold and bacteria. A fresh leaf is mostly water held in its cells' vacuoles, and when the water is gone the microbes cannot grow. Museums also freeze new specimens for several days to kill tiny beetles that would otherwise eat them.

God's handiwork. Solomon "spake of trees, from the cedar tree that is in Lebanon even unto the hyssop that springeth out of the wall," and of beasts, birds, creeping things and fishes. Collecting is part of that same work of naming and knowing. It is also a matter of stewardship. Good collectors follow the law and the land owner's wishes, take only common plants, and use the **one-in-twenty rule**: collect a plant only if at least twenty more of its kind grow in that spot, and never take more than one in twenty. Many things are better photographed than taken.



A mounted herbarium specimen. The label in the lower right corner holds the data that make it useful to science.

PICTURE STUDY



Frontispiece of Museum Wormianum, Unknown engraver, for Ole Worm's catalog, 1655 · Engraving, printed as the front picture of the book *Museum Wormianum* · Printed in Leiden, in the Netherlands; copies are kept in many rare-book libraries

This engraving shows Ole Worm's collection room in Copenhagen as it looked to visitors. It is drawn as if one wall of the room had been taken away so you can see inside. Every surface is used: the ceiling, the walls and long rows of shelves and boxes.

Rooms like this were sometimes called cabinets of curiosities. In time they grew into the natural history museums you can visit today, where specimens are sorted by kind and every one carries a label.

Look closely

- Look up at the ceiling. What animals can you find hanging there?
- Find the skulls, antlers and horns on the walls. Do any look like they belong to animals you know?
- Look at the boxes on the shelves. How did Worm keep small objects in order?
- Find a place in the room where you think Worm would sit to study. What clues did you use?

Talk about it

1. How is Worm’s room like a modern museum, and how is it different?
2. Why might a collection that is sorted and labeled be more useful than one that is simply crowded with wonders?
3. If you started a collection room in your house, what three kinds of things would you collect, and how would you sort them?

> **Safety first.** Collect only with permission of the land owner, and never in national parks or other protected areas where collecting is forbidden. Never collect protected or rare plants. In the United States it is against federal law to keep the feathers, nests or eggs of most native wild birds, even ones you find on the ground, so photograph and draw them instead. Learn to recognize poison ivy, poison oak and stinging nettles, and wear gloves. Never taste any plant, berry or mushroom you collect. Use scissors or pruners carefully, with an adult. Wash your hands after handling specimens.

EXPERIMENT: MY HERBARIUM AND THE DRYING TEST

You will make a small herbarium of at least five plants and, along the way, measure how much of a fresh leaf is water.

Question: What percent of a fresh leaf’s mass is water, and does it differ between a thin leaf and a thick, waxy one? **Prediction:** Write yours with a reason. **What we change:** the kind of leaf (for example, a thin maple or grass leaf versus a thick waxy leaf such as a succulent, holly or ivy). **What we keep the same:** the press, the paper, the weight and the room. **What we measure:** mass in grams at the start and every day until it stops changing.

You will need

- Newspaper, sheets of cardboard cut to about 30 cm by 45 cm, and heavy books or two boards and straps for a press
- Kitchen scale that reads to 0.1 g
- Scissors or small pruners (with an adult), a sealable bag for carrying plants home

- Heavy white paper or card stock for mounting, white glue or narrow strips of paper tape
- Pencil, notebook, a regional plant field guide or app

Steps

1. On a walk, choose at least five common plants. Before cutting, count: collect only if at least twenty of that kind grow there. Take a small whole plant (with roots, if it is a common weed) or a branch with leaves and flowers or fruit.
2. In your notebook, write field data on the spot: date, place (address or GPS), habitat (lawn, woods edge, fencerow) and a number for each specimen.
3. At home, weigh 10 g or more of fresh leaves of each kind for the drying test, and record the mass.
4. Lay each plant flat in a folded sheet of newspaper, spreading the leaves so some show their undersides. Stack the newspapers between cardboard sheets and put heavy books on top.
5. Change the newspapers every day or two. Weigh the test leaves each day until the mass stops changing.
6. When the plants are dry and stiff (about a week), mount each on card stock with dabs of glue or paper strips. Write the label in the lower right corner.
7. Calculate the percent water in each kind of leaf and compare.
8. Store finished sheets flat in a folder in a dry place, sorted by family or by your own key.

Tips

- Pick on a dry day, after the dew is gone; wet plants mold in the press.
- Use a pencil or waterproof ink on labels. Some inks fade or run.
- Thick, juicy stems can be sliced in half lengthwise before pressing so they dry faster.

Check your work

- I followed the one-in-twenty rule and collected only where I had permission.
- Each of my five sheets has a complete label.
- I recorded masses every day and calculated percent water.
- My specimens are flat, dry and free of mold.

NATURE NOTEBOOK

1. Before pressing one plant, draw it at life size from the side, so you have a record of its shape and color before drying changes it.
2. Copy the label form from this lesson and fill it in for each of your specimens.
3. Write the scientific names of five common plants or animals near your home correctly (genus capitalized, species lowercase, both underlined or in italics) with their common names.

From history. After Linnaeus died in 1778, his collections of plants, insects, shells and books were sold in 1784 to a young English naturalist, James Edward Smith, and shipped to London. In 1788 Smith and friends founded the Linnean Society of London, which still keeps the Linnaean collections. Scientists continue to examine those pressed plants, many of them type specimens, to settle exactly which plant Linnaeus meant by a name.

WORKED EXAMPLES

Example 1. A student weighs fresh maple leaves at 12.0 g. After a week in the press their mass stops changing at 3.0 g. (a) How much water was lost? (b) What percent of the fresh mass was water? (c) What percent was dry material?

(Use the map or drawing "After drying, the specimen is mounted and labeled." shown earlier in this lesson.)

1. (a) Water lost = fresh mass − dry mass = 12.0 g − 3.0 g = 9.0 g.
2. (b) Percent water = 9.0 g ÷ 12.0 g × 100 = 75%.
3. (c) Percent dry material = 3.0 g ÷ 12.0 g × 100 = 25%. Check: 75% + 25% = 100%. ✓

Answer: (a) 9.0 g of water. (b) 75% water. (c) 25% dry material.

PRACTICE

1. EASY Which name is written correctly? (a) taraxacum officinale (b) *Taraxacum Officinale* (c) *Taraxacum officinale* (d) Officinale taraxacum
2. MEDIUM Data reading. Mass of a sample of thick, waxy evergreen leaves in the press: day 0, 10.0 g; day 1, 8.6 g; day 2, 7.4 g; day 3, 6.5 g; day 5, 5.6 g; day 7, 5.5 g; day 9, 5.5 g. (a) On which day was the sample first fully dry? (b) What percent of the fresh mass was water? (c) The maple leaves in the worked example were 75% water. Which kind of leaf held more water?
3. MEDIUM You find a perfect blue jay feather on the sidewalk. What should you do with it for your collection, and why?
4. HARD Experiment design. You wonder whether changing the newspaper every day instead of every third day helps pressed flowers keep their color. Design a fair test.
5. CHALLENGE Two specimens of the same plant sit in a museum drawer. One is beautiful but has no label. The other is broken but labeled "Old field edge, Lancaster County, Pennsylvania, 12 June 1902, collector J. Smith." Which is more valuable to a scientist, and why?

COMMON MISTAKES

- Thinking the specimen matters more than the label. A specimen with no place, date and collector cannot be used as evidence.

- Thinking “it was lying on the ground, so I can keep it.” Feathers, nests and eggs of most native wild birds are protected by law, and collecting is forbidden in national parks.
- Thinking a dried plant has lost its cells. Pressed plants keep their cell walls, and scientists can still study their cells and even their DNA a century later; the water is what is gone.

Make it a game · Label Detective. A parent writes six specimen labels on cards, each missing one piece of information (no date, no place, no habitat, a misspelled scientific name, no collector, or a capital letter on the species name). Players race to find what is missing or wrong on each card. A correct find scores a point, and a full fix of the label scores a bonus point.

Scripture. “And he spake of trees, from the cedar tree that is in Lebanon even unto the hyssop that springeth out of the wall: he spake also of beasts, and of fowl, and of creeping things, and of fishes.” (1 Kings 4:33, KJV)

God gave Solomon wisdom to speak of the whole range of living things, from the tallest tree to a little plant in a crack in the wall. A careful collection, labeled and sorted, is a small way of following his example.

Practice answers

1. (c). The genus is capitalized, the species is lowercase, and both are in italics.
2. (a) Day 7, when the mass stopped changing (5.5 g on days 7 and 9). (b) Water lost = 10.0 − 5.5 = 4.5 g; 4.5 ÷ 10.0 × 100 = 45%. (c) The maple leaves (75% versus 45%).
3. Photograph it and sketch it with a scale bar and label data, but leave it. In the United States, federal law makes it illegal to keep feathers of most native wild birds, including blue jays, even if found on the ground.
4. Use several flowers of the same kind, picked at the same time and of similar size and freshness. Independent variable: how often the newspaper is changed (daily versus every third day). Keep the same: the press, the weight, the paper type, the room and the total drying time. Dependent variable: flower color after drying, rated on a 1 to 5 scale by someone who does not know which group is which, or compared with a paint color chart. Use at least five flowers per group, also note any mold, and compare averages.
5. The broken, labeled one. Its label tells where and when the plant grew and in what habitat, so a scientist can compare it with plants growing there today, map where the species lived, or check an identification. The unlabeled specimen cannot be tied to any place or time, so it is almost useless as evidence.

Unit 9 review

Answer each question. Show your work for calculations, use metric units, and write complete sentences for explanations. The review is worth 60 points.

1. (10 pts) (a) Multiple choice: Which is an observation? A. The fox was hunting mice. B. The tracks are 5 cm long with four toe pads. C. The tree died from disease. D. The bird is happy. (b) Write one inference that could follow from your answer to (a), and name one way to test it.
2. (10 pts) Ben walks beside a 15 m tape and takes 20 steps. He then walks a trail in 840 steps, which takes 21 minutes. Find (a) his step length, (b) the trail length, and (c) his walking rate in meters per minute.
3. (10 pts) A field is 30 m by 20 m. Five 1 m² quadrats contain 2, 5, 3, 6 and 4 thistle plants. (a) Find the mean per square meter. (b) Estimate the thistle population. (c) Give one reason the estimate might be wrong and one way to improve it.
4. (10 pts) A chicken was scan-sampled every 30 seconds for 10 minutes: pecking 10 scans, walking 5, dust bathing 3, preening 2. (a) How many scans were made, and what percent was pecking? (b) A museum sign reads: “This mastodon tusk, 2.5 m long, was found in a swamp in Michigan. Mastodons lived in the Ice Age and died out about 11,000 years ago.” Name one observation and one interpretation on the sign.
5. (10 pts) Diagram item. Draw a herbarium sheet with a pressed plant on it. Show where the label goes and list the six pieces of information the label must contain. Add a scale bar.
6. (10 pts) (a) Fresh leaves weigh 8.0 g. When fully dry they weigh 2.0 g. What percent of the fresh mass was water? (b) Multiple choice: Which may you collect for your specimen collection? A. A hawk feather found in a field. B. A wildflower from a national park. C. A dandelion from your own yard, where hundreds grow. D. A rare orchid with only three plants on the hillside. (c) Explain why a specimen must be dried quickly.

Unit project: my nature lab field book

Assemble a field book that shows a season of real outdoor science. Include at least three dated nature journal pages with measured sketches; your sun-versus-shade quadrat survey with a map, data table, population estimate and bar graph; your ethogram study with its time budget graph (or your museum sketch and marked sign); and at least five mounted, fully labeled herbarium specimens with your drying-test data.

Finish with a one-page reflection: which observation surprised you most, which question you would test next, and one way your work this unit showed care for the creatures God has made.

- **Records**: Every page and specimen carries a date, place and conditions; observations and inferences are labeled separately.
- **Data and math**: Pace, mean, population estimate, time budget percents and percent water are calculated correctly with metric units and clear graphs.

- **Fair testing**: Each study names what was changed, what was kept the same and what was measured, and its conclusion matches the data.
- **Care and stewardship**: Sketches and specimens are neat and accurate; collecting followed the law, permission and the one-in-twenty rule; animals were observed without disturbing them.

Answer key

1. (a) B. (b) Example: “A fox or dog made these tracks.” Test: compare the track size and toe-pad pattern with a field guide, or set up a trail camera at the spot.
2. (a) 15 m ÷ 20 = 0.75 m per step. (b) 840 × 0.75 m = 630 m. (c) 630 m ÷ 21 min = 30 m per minute.
3. (a) (2 + 5 + 3 + 6 + 4) ÷ 5 = 20 ÷ 5 = 4 per m^2. (b) Area = 30 × 20 = 600 m^2; 4 × 600 = 2,400 thistles. (c) Five quadrats is a small sample and may have landed on unusually thick or thin patches; use more quadrats placed randomly or on a grid.
4. (a) 10 min × 2 = 20 scans; pecking 10 ÷ 20 = 50%. (b) Observation: the tusk is 2.5 m long, or it was found in a Michigan swamp. Interpretation: that mastodons lived “in the Ice Age” and “died out about 11,000 years ago” (a date assigned by the museum’s methods and assumptions).
5. The drawing shows a whole flattened plant held down with paper strips, a label box in the lower right corner, and a scale bar. The label lists: scientific name, common name, date collected, place (with GPS if possible), habitat, and collector’s name and number.
6. (a) Water = 8.0 − 2.0 = 6.0 g; 6.0 ÷ 8.0 × 100 = 75%. (b) C. (Hawk feathers are protected by federal law, national parks forbid collecting, and the orchid fails the one-in-twenty rule.) (c) A damp specimen is food for mold and bacteria, which would rot it; removing the water stops microbes from growing.