Spring 2026

Idaho State University

Human Anatomy & Physiology I Laboratory

BIOL 2227L

Academic Year 2025-2026

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Prepared by:

Idaho State University

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Please note that this manual is a work in progress and was compiled specifically for the ISU Department of Biological Sciences. It changes each semester/session. It is a free and unpublished manual that has not seen reviewers or editors; there are errors.

ROAR

Laboratory Policies (https://www.isu.edu/biology/biolab/student-behavior/laboratory-rules/)

1. **No** food or drink allowed.
2. Coats, bags, purses, backpacks, etc. must be stored on the shelves provided on the side of the room. Do NOT keep them next to you at your workstation. Only your binder with syllabus, task sheets, pencil, colored pencils, and mini-stapler should be with you at the workstation.
3. Standard lab attire is required:
   - work shirt that covers the upper torso and arms,
   - lower body clothing that covers the leg to the knee (e.g., pants, skirt, coveralls, lab coat) and fully protects exposed skin
   - shoes that have a closed toe AND heel (i.e. NO flip flops or sandals).
4. Long hair must be restrained for safety reasons (i.e., braided, tied in a pony tail, etc.).
5. Notify your instructor of unsafe conditions such as broken glassware or water on the floor.
6. Never pour chemical reagents down the sink drain unless instructed to do so.
7. Participation in lab is required. If the lab instructor observes that a student is not actively participating in the completion of the task sheet, the student will receive a zero on the task sheet.
8. **No** mobile electronic devices are allowed in the lab (smartphones, tablets, smartwatches, smart glasses, ear pieces, and laptops). If a student has one on his/her person OR anywhere near their seat they will receive a zero on their quiz and/or task sheet. They must be placed in your bag or coat and stored on the shelves provided.
9. **No** use of generative artificial intelligence (AI) allowed in the lab or on lab assignments (except during Lab 1). If a student uses AI, they will receive a zero on their quiz and/or task sheet.
10. Random assigned seating is required during each lab.
11. Before and after each lab, wipe down all student benches, computer keyboard and mouse, microscope knobs and eyepieces, and all other equipment that is touched during lab with the correct cleaning supplies.
12. Instructors must also wipe down the instructor benches, computer keyboard and mouse, microscope knobs and eyepieces, projector remote, as well as any other equipment they use during lab the correct cleaning supplies.

Idaho State
UNIVERSITY

Welcome to Human Anatomy and Physiology I Lab (BIOL 2227L) for students majoring in the health professions. The laboratory is intended to provide you with opportunities to:

- ✓ Gain transferable skills.
- ✓ Familiarize yourself with the language of biology and science.
- ✓ Use and improve your critical reasoning, analytical, and communications skills.
- ✓ Partially satisfy Objective 5 of the General Education Requirements.

Learn your laboratory instructor's name and keep a record of his or her contact information:

Instructor | _____ Section | _____

E-mail Address | _____ Office hours | When: By Appointment Where: _____

Take the syllabus Google quiz (linked in Canvas), read about and acknowledge the following:

- Occupational Health & Safety
- Academic Integrity
- Attendance Policy

Attendance & Communication Policy (https://www.isu.edu/biology/biolab/student-behavior/attendance--communication/):

Attendance

For the first lab of the semester, it will be considered an UNEXCUSED ABSENCE if a student:

- ➢ Does not attend the laboratory for which they are registered,
- ➢ Registers after labs have begun, OR
- ➢ Attends a lab section for which they are not registered although they are registered for another section.

For the duration of the semester, students are expected to:

- ➢ Arrive on time,
- ➢ Stay for the entire lab,
- ➢ Attend the laboratory section for which they are enrolled, and
- ➢ Contact the lab instructor four business days BEFORE a lab is missed to request an excused absence. **Excused absences** are granted for university-sponsored extra-curricular activities **only** and the student must provide a note from their coach, trainer, etc. **Unexcused absences** cannot be made up.

Communication

When students communicate with their instructor using e-mail they are required to use the **ISU Academic Communication guidelines** (https://www.isu.edu/advising/atoz-index/academic-communication/) as well as specific rules 1-4 or the instructor is NOT required to respond. Instructors are also not required to respond to an e-mail if it is about an assignment that is due in 2 business days or less OR an absence that has already occurred or will occur in less than two business days.

1. Subject must include the course number (i.e. Biol 1101L) AND the section number for which you are registered (i.e. 01, 02 etc.),
2. A greeting that includes your instructor's name,
3. A concise paragraph that explains your situation and/or asks a question, and
4. A salutation that includes your full name AND Bengal ID number.

IMPORTANT NOTE: The **lab syllabus** and **entire manual** must be printed by the student before the first lab.

Disruptive Behavior (https://www.isu.edu/biology/biolab/student-behavior/disruptive-behavior/):

You are expected to behave in a congenial and respectful manner as you participate in all laboratory activities. Disruptive behavior will not be tolerated. This syllabus is your first warning. Examples of disruptive behavior include but are not limited to:

- ➢ Not following laboratory rules and safety.
- ➢ Behavior that impedes another student's ability to learn such as talking out of turn, texting, talking on your cell phone, etc.
- ➢ Disrespectful, rude, or threatening behavior directed toward a classmate or instructor.
- ➢ Cell phones or other electronic devices may not be used; they need to be turned off and placed with your personal belongings on the shelves provided. If others need to contact you at any time, give them the telephone number of the Biology Office (282-3765) or Public Safety (282-2515).
- ➢ Eating and drinking in the lab.
- ➢ Inappropriate use of any electronic devices.

Grades

Biol 2227L is a one-credit lab course for which a letter grade will be awarded. If you earn lower than a 60% AND you have missed more than 30% of the lab, you will receive an X in the lab.

Grade scale for BIOL 2227L (1 credit lab course)					
A	100-94%	B-	82-80%	D+	69-67%
A-	93-90%	C+	79-77%	D	66-63%
B+	89-87%	C	76-73%	D-	62-60%
B	86-83%	C-	72-70%	F	< 60%

- ☐ **Lab Notebook (pencil, mini-stapler, hole-punch are also needed):** You will bring a 3-ring binder to lab to create a laboratory notebook consisting of the lab syllabus, assigned journal article, handwritten terms and definitions, and all completed and to-be completed task sheets. Your lab instructor will review your notebook each week and points will be awarded; be sure to keep it up-to-date. You will turn in the completed notebook during Lab 15.
- ☐ **Pre-quizzes:** you will have a quiz during the first ten minutes of each lab using the pre-quiz sheet provide by your instructor. The quiz will cover the important terms and any information from the task sheet before completion. If you are late, you cannot take the pre-quiz.
- ☐ **Post-quizzes:** you will have a quiz during the last ten minutes of each lab using the post-quiz sheet bundle provide by your instructor. The quiz will cover the important terms and any information from the task sheet before AND after completion If you do not stay for the entire lab period and do not hand in your completed task sheet, you cannot take the post-quiz.
- ☐ **Task sheets:** Complete the lab task sheets using information found within the task sheets themselves, supplies offered in lab, and the course textbook. If you do not stay for the entire lab period and do not hand in your completed task sheet, you cannot take the post-quiz.

Graded items	Quantity	Points	
		Each	Total
Lab Notebook	15	5	75
Pre-Quizzes	15	10	150
Post-Quizzes	14	10	140
Task sheets	14	30	450
Extra points	Used to offset points missed due to unexcused absences	82	

- ☐ **Extra Points (Terms):** complete by the submission date and time using the assigned materials. Late submissions will not be accepted.
- ☐ Do not participate in academic dishonesty. Academic Integrity expected of all students. **Academic dishonesty** (https://www.isu.edu/biology/biolab/student-behavior/attendance--communication/) in any form is unacceptable. Academic dishonesty includes cheating (e.g. providing or using unauthorized materials) and plagiarism (e.g. representing another's work as your own). Penalties for cheating and plagiarism include reduction in grade for the assignment, grade of F in the course, and dismissal from the University.
- ☐ **ANY violation of laboratory rules and safety** (https://www.isu.edu/biology/biolab/instructor-training/lab-safety/) **will result in a zero for all lab grades on the day the violation occurred.**

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- ✓ Instructors must also wipe down the instructor benches, computer keyboard and mouse, microscope knobs and eyepieces, projector remote, as well as any other equipment they use during lab the correct cleaning supplies.

Academic Success / Reasonable Accommodations

Idaho State University is committed to providing equal opportunity in education for all students. If you have a diagnosed disability or if you believe you have a disability (physical, learning, hearing, vision, psychiatric) that might require reasonable accommodation in this course, please contact the Disability Services Center, Rendezvous Building, Room 125 (208-282-3599). It is the responsibility of students to contact instructors during the first week of each semester to discuss appropriate accommodations.

How to Succeed in this Course

- ✓ Take responsibility for your own education:
  - Always attend lecture and laboratory.
  - Take notes and review your notes after class each day so you are sure you understand them before lab. Check your notes for accuracy by comparing them to a colleague's or the textbook. If you have questions, ask your instructor.
  - Read the assigned materials before lab.
  - Review and think about what you have heard and read.
- ✓ Visit your laboratory instructor during his or her office hours and "talk biology." Learn about what they are doing in their research programs.

Schedule for BIOL 2227L (subject to change)**:**

DATES	LAB	TOPIC		DUE Dates
January 13 – 15	1[A]	Software Install, Generative AI, & Journal Articles *(terms due)*		• Task sheets and quizzes – DURING the lab in which they are assigned. • Extra points terms – BEFORE the lab for which they were assigned. • Lab Notebook – DURING each lab
January 20 – 22	2	Introduction: Microscopy, Cells, & Tissues *(terms due)*		
January 27 – 29	3	Unit **I**: Urinary System	Part 1. pH & Conductivity *(terms due)*	
February 3 – 5	4		Part 2. Macromolecules	
February 10 – 12	5		Part 3. Diffusion & Osmosis	
February 17 – 19	6	Unit **II**: Digestive System	Parts 1 & 2. Enzymes & Respiration *(terms due)*	
February 24 – 26	7		Part 3. Fermentation	
March 3 – 5	8	Unit **III**: Integumentary System	Part 1. Skin Vascularity *(terms due)*	
March 10 – 12	9	Unit **IV**: Endocrine System	Parts 1 & 2. DNA *(terms due)*	
March 17 – 19	10		Part 3. Molecular Analysis of Cancer	
March 31 – April 2	11		Parts 4 & 5. Cell Cycle	
April 7 – 9	12	Unit **V**: Immune & Lymphatic Systems	Parts 1-3. Meiosis & Simple Genetics *(terms due)*	
April 14 – 16	13		Parts 4-5. Population Genetics	
April 21 – 23	14	Unit **VI**: Nervous System	Part 1. Electrolytes *(terms due)*	
April 28 – 30	15		Part 2. Neuromuscular Reflexes	

[A]Bring your laptop to lab this week.

Journal Articles (JA)

Objectives

- Understand why digital object identifiers are used.
- Using a journal article, learn about the following aspects of scientific research; 1) scientific method, 2) experimental design, 3) technical & quantitative methods, 4) intellectual merit, and 5) broader impacts.
- Learn about the footprint of large language models and generative artificial intelligence.

Terms & Definitions

Axes of a graph:
A. x-axis is the horizontal axis of a graph; typically describes the predictor (independent) variable.
B. y-axis the vertical axis of a graph; typically describes the response (dependent) variable.

Biology - the study of life

Controlled experiment groupings:
A. Control - in a clinical trial, the group that does not receive the new treatment being studied. This group is compared to the group that receives the new treatment, to see if the new treatment works
B. Experimental - the sample in an experiment that is subjected to some type of variation that does not occur naturally.

Controls - constant and unchanging standards of comparison in scientific experimentation:
A. Negative - is not exposed to any treatment (experimental or otherwise) that is known to produce the expected effect.
B. Positive - is exposed to some other treatment that is known to produce the expected effect but not the experimental treatment.

Experimental design - the laying out of a detailed experimental plan in advance of doing the experiment. Well chosen experimental designs maximize the amount of "information" that can be obtained for a given amount of experimental effort.

Generative artificial intelligence (AI) - programming models that emulate the structure and characteristics of input data in order to generate derived synthetic content such as text, images, music, videos, code, and more, based on the input data and prompts; computational frameworks used to train machines to perform specific tasks by learning from data.

Genus - a taxonomic category ranking used in biological classification that is below family and above species.

Investigator error - is a type of systematic error caused by technical skills of the investigator and can result from measuring solutions inaccurately, not rinsing equipment well enough between tests (contamination), etc.

Graphics processing unit (GPU) - a specialized electronic circuit designed for digital image processing and to accelerate computer graphics. GPUs can easily handle data-intensive and computationally demanding tasks because they can rapidly perform vast numbers of calculations which has led to their adoption in artificial intelligence (AI), the training of neural networks, and cryptocurrency mining.

Large language model (LLM) - the foundation of AI. LLMs are trained on vast amounts of text to understand existing content and generate original content. They function as chatbots, responding to user prompts by processing natural language in a conversational, human-like way. They can perform a variety of language-based tasks, like generating, summarizing, and translating text.

Observational / measurement error – the difference between a measured value of a quantity and its true value and can be the result of systematic error and random error. Systematic error always occurs, with the same value, when we use the instrument in the same way and in the same case and can be reduced with standardized procedures. Random error varies from one observation to another:
A. Accuracy - how close or far off a given set of measurements (observations or readings) are to their true value, accurate if their average (mean) is close to the true value.
B. Precision - how close or dispersed the measurements are to each other describes random errors; standard deviation is relatively small.

Organism - a living thing that maintains an internal order that is separated from the environment; descended from a single-celled ancestor that appeared almost 4 billion years ago: Consist of one or more cells, Contain genetic information, Use genetic information to reproduce themselves, Are genetically related, Covert molecules obtained from their environment into new biological molecules, Extract energy from the environment and use it to do biological work, Can regulate and internal environment.

Reasoning - the process of thinking about something in order to make a decision; logical thinking:
A. Inductive - a logical process that argues from specific instances to a general conclusion; deriving a generalization from specific details.
B. Deductive - making a prediction about the outcome of a test; generating a specific expectation from a generalization.

Science - the observation, identification, experimental investigation, and theoretical explanation of natural phenomenon.

Scientific method - a hypothesis-prediction approach to acquiring scientific knowledge about the natural world.
A. Observation - a note, record, of an occurrence, or phenomenon. Observations may be made directly or indirectly using tools.
B. Question - address something that can ultimately be measured. This means that the question has to be answerable – one that can be used to propose a set of hypotheses that can be tested and a set of predictions against which one can compare the results from the study.
C. Hypothesis - a tentative statement, derived from inductive reasoning, that proposes a possible explanation to the question and states a generalized relationship between two variables.
D. Prediction - a specific statement, derived from deductive reasoning, about what will occur (i.e. the outcome or pattern that will be observed) in a particular research investigation (e.g., an experiment).

Specimen - something shown or examined as an example

Species - a group of related organisms that share a distinctive form in nature and (for sexually reproducing species) are capable of interbreeding.

Species name – a formal system of naming species of living things by giving each a name composed of two parts (binomial nomenclature) and are italicized. The two words are as follows:
A. generic name – identifies the genus to which the species belongs and is capitalized.
B. specific name or specific epithet – distinguishes the species within the genus and is not capitalized.

Statistics - a branch of mathematics that estimates the reliability of data by dealing with the collection, analysis, interpretation, presentation, and organization of data:

A. Data - a collection of discrete values that convey information, describing quantity, quality, fact, statistics, other basic units of meaning, or simply sequences of symbols that may be further interpreted.
B. Descriptive statistics - quantitatively describe or summarize features of a collection of information.
C. Mean - is a descriptive statistical measure that reports the central location in a sample of data. A central value of a discrete set of numbers: specifically, the sum of the values divided by the number of values.
D. Replication - the repetition of an experimental condition so that the variability associated with the phenomenon can be estimated
E. Standard deviation - a measure that is used to quantify the amount of variation.

Testable - possible to evaluate through observations of the measurable universe.

Theory - a broad explanation of some aspect of the natural world that is substantiated by a large body of evidence.

Variables - any characteristics, number, or quantity that can be measured or counted.

A. Predictor - causes or affects the response variable; are also known as explanatory or independent variables; are denoted by an X and are shown on the horizontal x-axis.
B. Response - influenced by the predictor variable; are also known as dependent variables; are denoted by a Y and are shown on the vertical y-axis.

Part 1. Software Install

☐ Bring your laptop and charging cord (please contact your instructor if you do not have a laptop).

☐ Load software onto your personal laptop:

- How to install Microsoft Office 365 Education.
- How to install Adobe Reader.

Part 2. Journal Article

Textbooks and websites are the most familiar form of educational media. University freshman and sophomore students use these for most of their coursework but as a student progresses to junior and senior level courses they are expected to rely on relevant research articles. Research articles in the sciences must be rigorously peer-reviewed against strict criteria if they are to be published in scientific journals. Students do not have the foundational or applied knowledge in their chosen field nor the quantitative background to accurately critique most articles. As a student progresses to their upper division courses, they will become more proficient at critically reading research articles but they have not acquired the advanced education and experience to be considered 'peers' of the principal investigators that conduct the research and publish the peer-reviewed articles.

Your lab instructor has chosen one journal article from a specific group of journals from a specific publication year that is an **experimental study done by the authors, NOT a review / note / comment.** Your entire lab section will be required to read the article and answer questions about the article **during lab 1**.

☐ Work as an individual.

☐ For questions 1-8 write your answer in the space provided on the last three pages.

A. DOI (https://www.doi.org/)

1. What is a DOI and what is its purpose?
2. What is the DOI of the article your instructor chose?

B. Questions

☐ Go to Biolab > Training > Learning Environment > Research > Scientific Method & Experimental Design (https://www.isu.edu/biology/biolab/instructor-training/learning-environment/research/#d.en.218210) and study the content at that web page.

☐ Skim the journal article your lab instructor chose and emailed to your ISU Gmail account.

☐ **Answer questions 3-7** concisely, thoughtfully, and in **complete sentences and paragraph form**:

- Use ChatGPT (https://chatgpt.com/) but you must edit (and understanding) the generative AI response in **YOUR OWN WORDS and handwriting**, no quoting. Be sure to check your spelling and grammar.
- If terms and acronyms are unfamiliar to you, include the definitions in your answer.
- Make sure you have completed **question 8**.

3. What is the **species** name of the model/study organism? If the study is looking at human disease choose the disease causing organism. If the study is on some aspect of human physiology or anatomy, the model organism is a human. You may need to find this information using another resource.
4. Intellectual merit and broader impact:
   A. Intellectual merit - Why is this research important enough to spend time and money doing it? Does it advance knowledge and understanding within its own field or across different fields?
   B. Broader impact - What were the economical, environmental, medical, health, or a combination of reasons that make this research valuable? Does it benefit society or advance desired societal outcomes?
5. What is the major question and/or hypothesis studied in the journal article?
6. Sampling for the study:
   A. Where did they sample?
   B. How did they sample?
   C. Number of samples taken?
7. Describe how the evidence supported/answered the major hypothesis/question?
8. What was the major conclusion of the study?
9. Reference your resources **in**cluding the article under study and the Generative AI program.

✓ Author, A. A., Author, B. B., & Author, C. C. (Year). Title of article. *Title of Journal*, *volume number*(issue number), pages. http://dx.doi.org/xx.xxx/yyyyy

✓ OpenAI. (20XX). ChatGPT X (Month Day version) [Large language model]. https://chatgpt.com/

Part 3. Calculate AI Footprint

Generative artificial intelligence (AI) has become a common part of daily life but behind this innovation is a growing environmental footprint. In 2023, data centers consumed 4.4% of U.S. electricity with the expectation that it could triple by 2028. The sustainability concerns associated with AI are not only due to its energy demands but also its high water usage, emissions, and e-waste. Initially, these concerns in computing were consumer-driven (such as increased battery life) but now the focus is shifting to environmental sustainability, carbon footprint reduction, and making AI models more energy efficient. AI, particularly large language models (LLMs), requires enormous computational resources. Training these models involves thousands of graphics processing units (GPUs) running continuously for months, leading to high electricity consumption. It is projected that by 2030–2035 data centers could account for 20% of global electricity use, putting an immense strain on power grids.

☐ Use the AI Environmental Footprint Estimator (https://sites.google.com/i-biology.net/ai-footprint-estimator/) to calculate the environmental impact of completing Part 2B of this task sheet using the ChatGPT LLMs and AI.

10. Fill in Table JA-1 with the environmental impact estimate from the eight text queries needed to complete Part 2B for:
    - you as an individual
    - you doing a similar text query daily per year
    - everyone in your section (~25)
    - everyone in the lab course (~250 people in the fall and ~150 in the spring)

Table JA-1. AI Environmental Footprint.

	Individual (n =1)	Individual per year (n =1)	Section (n=____)	Entire Lab Course (n=___)
Energy Consumption kWh				
CO_2 Emissions kg				
Water Usage L				
Smartphone charges				
Laptop hours				
Car equivalent km				
Trees needed for offset				

11. Using the Estimator website summarize the following questions:
    A. Why Does AI Have Such a Big Environmental Footprint?
    B. What might happen in the future?
    C. So what can we do about it?

Introduction

Objectives

- Learn about and practice microscopy.
- Learn about cells, tissues, organ systems, and symmetry.

Terms & Definitions

Animalia - a eukaryotic kingdom of the domain Eukarya that encompasses those organisms called animals. Animals are multicellular heterotrophs with cells that lack cell walls.

Autotroph - an organism that has a metabolic pathways that use energy either from inorganic molecules or light to make organic molecules.

Axes of anatomy - a hypothetical axis used to transect an anatomical entity in a straight line through space:

A. Anterior-posterior (AP) - extends longitudinally from head to tail.
B. Dorsal-ventral (DV) - ventral typically faces toward, and dorsal away, from a substrate (meaning towards the ground for land-dwelling organisms or towards the ocean or river/lake bottom for marine or aquatic organisms).
C. Left-right (LR) - to a plane running along the anterior-posterior midline.

Archaea - one of the three domains of life that encompasses those one-celled organisms called archaeans.

Bacteria - one of the three domains of life that encompasses those one-celled organisms called bacteria.

Biological classification - is a system for comparing and grouping organisms, and the naming of those groups.

Cell - the simplest unit of a living organism:

A. Cell wall - a relatively rigid, porous, structure located outside the plasma membrane of prokaryotic plant, fungal, and certain protists cells; provides support and protection.
B. Cilium(a) - membrane-bound organelle attached to the cell membrane that can be motile or non-motile.
C. Chloroplast - plastids found in plant and algal cells that carry out photosynthesis.
D. Cytoplasm - the region of the cell that is contained within the plasma membrane.
E. Cytoskeleton - in eukaryotes, a network of three different types of protein filaments in the cytosol called microtubules, intermediate filaments, and actin filaments.
F. Cytosol - the semifluid portion of the cytoplasm.
G. Endoplasmic reticulum: the transportation system of the eukaryotic cell, and has many other important functions such as protein folding. Smooth - lacks ribosomes and helps synthesize and concentrate various lipids, phospholipids as in plasma membranes, and steroids needed by the cell. Rough - studded with protein-manufacturing ribosome.
H. Flagellum(a) - relatively long cell appendages that facilitate cellular movement or the movement of extracellular fluid.
I. Golgi apparatus - a complex of vesicles and folded membranes within the cytoplasm of most eukaryotic cells, involved in secretion and intracellular transport.
J. Lysosome - a membrane-bound organelle found in many animal cells. They are spherical vesicles that contain hydrolytic enzymes that can break down many kinds of biomolecules. A lysosome has a specific composition, of both its membrane proteins, and its lumenal proteins.
K. Microtubule - protein structure that moves chromosomes around during mitosis and meiosis.
L. Mitochondrion(a) - organelle found in eukaryotic cells that supply most of a cell's ATP.
M. Nuclear envelope - a double-membrane structure that encloses the cell's nucleus.
N. Nucleolus - a prominent region in the nucleus of nondividing cells where ribosome assembly occurs.
O. Nucleus - cell structure that houses DNA; found in eukaryotes.
P. Organelle - a subcellular structure or membrane-bound compartment with its own unique structure and function.
Q. Peroxisomes - small, membrane-enclosed organelles found in the cytoplasm of virtually all eukaryotic cells that contain enzymes involved in a variety of metabolic reactions, including several aspects of energy metabolism.
R. Plasma (cell) membrane - the biomembrane that separates the internal contents of a cell from its external environment.
S. Pseudopod - a part that temporarily sticks out of the protoplasm (= the liquid inside a cell) of some organisms that have only one cell, used for movement and to get food
T. Ribosome - a structure composed of proteins and rRNA that provides the site where polypeptide synthesis occurs.
U. Vacuole - a space that contains air or liquid inside a living cell, often storing an important chemical or food substance.

Desmosomes - intercellular junctions/bridges that mediate cell–cell adhesion and anchor the intermediate filament network to the plasma membrane, providing mechanical resilience to tissues and act as mediators of cell signaling important for proper cell and tissue functions.

Domain - one of the three major categories of life; Bacteria, Archaea, and Eukarya.

Eukaryote - one of the three categories into which all forms of life can be placed. The distinguishing feature of eukaryotes is cell compartmentalization, including a cell nucleus; includes protists, fungi, plants, and animals.

Genus - a taxonomic category ranking used in biological classification that is below family and above species.

Germ layers - three primary germ layers in the very early embryo; cells or tissue of an embryo in early development:

A. Ectoderm - the outermost layer or the parts derived from this, which include the epidermis and nerve tissue.
B. Endoderm - the innermost layer or the parts derived from this, which include the lining of the gut and associated structures.
C. Mesoderm - the middle layer or the parts derived from this, which include the muscle, bone, and nervous tissue.

Habitat - place where an organism lives.

Heterotroph - organism that cannot produce their own organic molecules and thus must obtain organic food from other organisms.

Kingdom - a taxonomic group; the second largest after domain.

Life - a monophyletic group (refers to a group that consists of an ancestor and all of its descendants) that includes all known organisms. Characterized by a nucleic acid based genetic system (DNA or RNA), metabolism, and cellular structure. Some parasitic forms have secondarily lost some of these features and rely on the cellular environment of their host.

Microscope - a magnification tool that enables researchers to study very small structures and cells:

A. Depth of field - is determined by the distance from the nearest specimen plane in focus to that of the farthest plane also simultaneously in focus.
B. Magnification - the ratio between the size of an image produced by a microscope and its actual size.
C. Field of View - the visible area seen through the microscope when the specimen is in focus. The greater the magnification the smaller the view.
D. Focus - a specimen is in focus at the desired magnification when the image seen through the ocular lens is sharp and clear.
E. Objective lens - the primary optical system which produces a magnified image of the specimen. There are typically four objective lenses attached to the nosepiece with the magnification of each objective engraved on its side.
F. Ocular lens - the secondary optical system that you look through. The ocular lens further magnifies (10x) the image and brings the light rays to a focal point.
G. Resolution - point-to-point resolving power in the plane perpendicular and parallel to the optical axis. The ability to observe two adjacent objects as distinct from one another; a measure of clarity of an image

Multicellular - the condition of being composed of many coordinated cells.

Organ - a group of tissues with similar functions.

Organ system - a biological system consisting of a group of organs that work together to perform one or more functions. Humans have eleven organ systems: respiratory system, digestive system, circulatory system, urinary system, integumentary system, skeletal system, muscular system, endocrine system, lymphatic system, nervous system, and reproductive systems.

Organism - a living thing that maintains an internal order that is separated from the environment; descended from a single-celled ancestor that appeared almost 4 billion years ago: Consist of one or more cells, Contain genetic information, Use genetic information to reproduce themselves, Are genetically related, Covert molecules obtained from their environment into new biological molecules, Extract energy from the environment and use it to do biological work, Can regulate and internal environment.

Phospholipid bilayer - the basic framework of the cellular membrane, consisting of two layers of lipids.

Phylum - in taxonomy, a subdivision of a kingdom.

Plane (section) - a flat 2D plane intersecting an anatomical continuant (a thing that retains its identity even though its states and relations may change), dividing it into two adjacent portions:

A. Transverse (Synonyms: axial plane; axial section; cross section) - anatomical plane that divides body into anterior and posterior parts.
B. Frontal (Synonyms: horizontal anatomical plane; longitudinal section; horizontal section; coronal plane) - anatomical plane that divides bilateral body into dorsal and ventral parts.
C. Sagittal (Synonyms: left/right plane; sagittal section; longitudinal section; median plane) - anatomical plane that divides a bilateral body into left and right parts, not necessarily of even size.

Specimen - something shown or examined as an example

Species - a group of related organisms that share a distinctive form in nature and (for sexually reproducing species) are capable of interbreeding.

Species name – a formal system of naming species of living things by giving each a name composed of two parts (binomial nomenclature) and are italicized. The two words are as follows:

A. generic name – identifies the genus to which the species belongs and is capitalized.
B. specific name or specific epithet – distinguishes the species within the genus and is not capitalized.

Symmetry - the balanced distribution of duplicate body parts or shapes within the body of an organism; generally with respect to external appearance only:

A. Spherical - any plane that passes through the center of the object divides the form into two identical halves that are mirror images of each other.
B. Radial - all planes passing through a central axis divides the form into two identical halves that are mirror images of each other.
D. Biradial - a combination of both radial and bilateral symmetry. The organs are arranged radially and the body can be divided into two by a mid-longitudinal plane.
C. Bilateral - only one plane will divide an organism into roughly mirror image halves.

Unicellular - an organism that is composed of only one cell.

Tissues - the association of many cells of the same type:

A. Connective - the tissue that supports, protects, and gives structure to other tissues and organs in the body; develops from the mesoderm.
B. Epithelial - line the outer surfaces of organs and blood vessels throughout the body, as well as the inner surfaces of cavities in many internal organs; develops from the ectoderm or endoderm.
C. Muscle - a soft tissue that makes up the different types of muscle in animals, and gives the ability of muscle to contract; develops from the mesoderm.
D. Nervous - the main tissue component of the nervous system; develops from the ectoderm.

Name:_____ Team #:_____ Section #:_____

Part 1. Microscopy

A. Microscope Components

☐ Go to Biolab > Training > Technology > Microscopes (https://www.isu.edu/biology/biolab/instructor-training/technology/microscopes/) and study the parts of the microscope.

1. Look at a compound microscope and determine the magnification of each objective lens and the ocular lens then calculate the total magnification of an image viewed (Table Intro-1).

Table Intro-1. Compound microscope magnification.

Objective Lens Magnification		Ocular Lens Magnification	Total Magnification
Scanning			
Low power			
High power			
Oil immersion			

B. Viewing Specimens

☐ Obtain a thread slide and place it carefully into the stage clip. In order to view each thread in focus at greater magnification, you must focus downward to view the ones underneath and upward to view the ones that are above.

☐ What is the slide's ID number / letter?_____

2. Which thread is on top, which is on bottom, and which is in the middle at each objective?

Top 4X___10X ____40X ____ Middle 4X___10X ____40X ____ Bottom 4X___10X ____40X ____

3. When you adjust the fine focus knob, are all of the threads in focus at the same time?
_____

4. What happens to the depth of field when you increase to a higher magnification? Does it increase, decrease, or remain the same? _____

☐ Obtain an **e** slide and view it under the scanning (4X) objective. Make sure the e slide is on the stage with the e in its normal orientation.

5. Move the slide to the left. Draw an arrow to depict the direction the **e** moved?_____

6. Sketch the orientation of the **e** image you view using the microscope compared to the image seen unaided on the slide? _____

Part 2. Cells & Tissues

Morphology is defined as the structure or form of a body part or an entire organism. Functional morphology is the study of the design of tissues and organ systems, the principles of physics affecting animals, and the mechanisms of the body. Physiology is the study of how living organisms adjust to their environments and regulate critical functions at the system, organ, tissue, cellular, and molecular levels. Together the two related fields include a broad range of topics such as feeding mechanics, digestion, locomotion, muscle contraction, circulatory design, oxygen exchange and other topics focused on animal function. Most animals (excluding the sponges which have a cellular level of organization) are made from cells which are organized into tissues and these are themselves combined to form organs and systems. The bodies of animals with a tissue level of organization have four tissue types; **1)** epithelial tissues that form linings, coverings and glands, **2)** connective tissues for transport and support, **3)** muscle tissues for movement, and **4)** nervous tissues for carrying messages. Their functional morphology can be divided into eleven organ systems; **1)** integumentary, **2)** lymphatic, **3)** endocrine **4)** nervous **5)** urinary, **6)** circulatory, **7)** skeletal, **8) r**eproductive, **9)** respiratory, **10)** digestive and **11)** muscular.

A. Cell Components

7. In Figure Intro-1, label and color the listed subcellular structures and organelles:

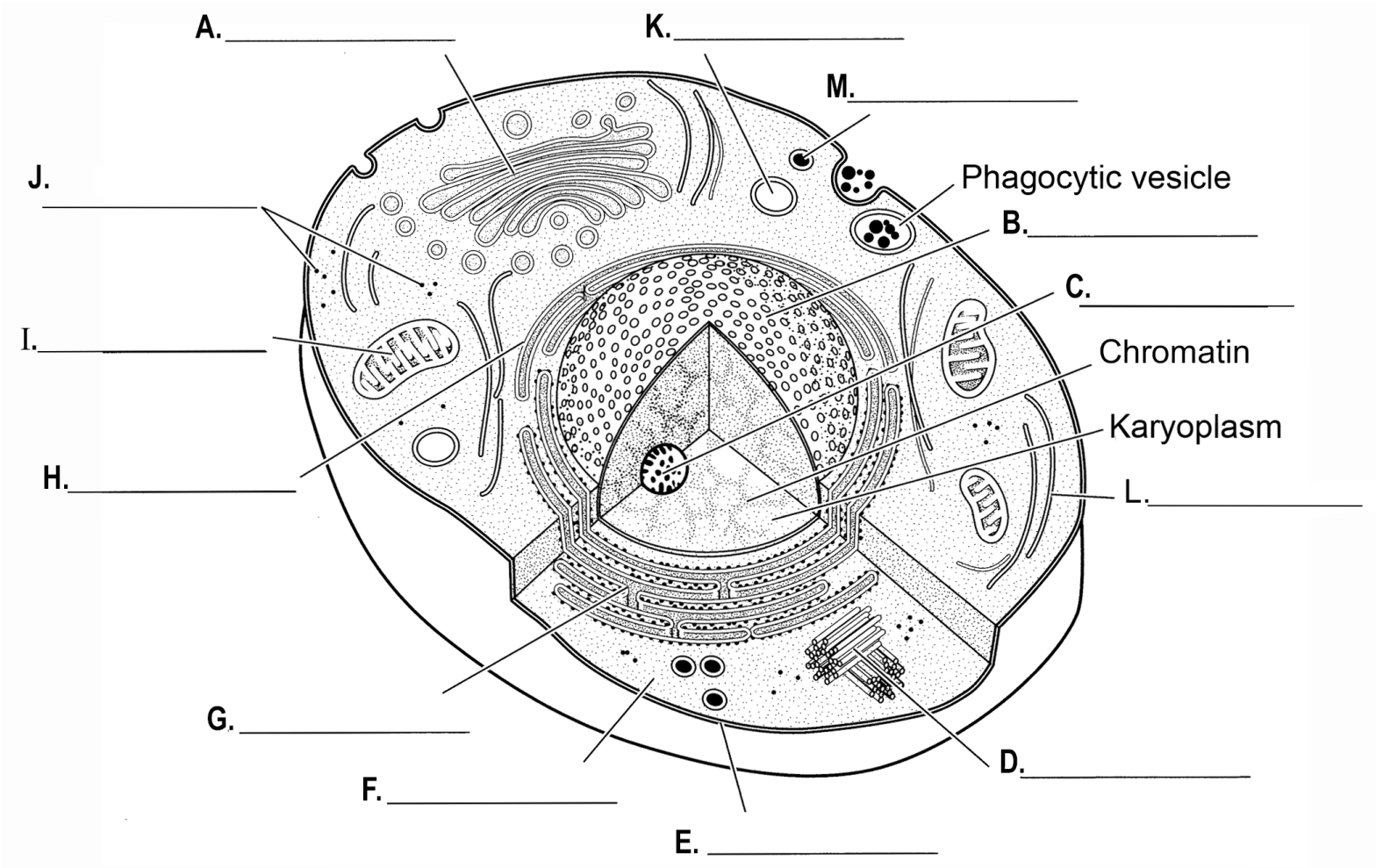


Figure Intro-1. Cell diagram, subcellular structures and organelles; A. Golgi apparatus (green), B. Nucleus (purple), C. Nucleolus (dark blue), D. Centrioles (brown), E. Plasma membrane, F. Cytoplasm (yellow), G. Rough endoplasmic reticulum (red), H Smooth endoplasmic reticulum (pink), I. Mitochondrion (orange), J. Free ribosomes. K. Lysosome, L. Cytoskeleton, and M. Peroxisome.

.☐ Make a wet mount (Figure. Intro-2) of each microbe suspension available in lab:

- Add a drop of suspension to the center of a slide.
- Place a cover slip on the slide at an angle so that it douches the drop.
- Slowly lower the raised end of the cover slip so that it forces the drop to flow away from the edge of the cover slip that is touching the slide.
- Place the wet mount on the stage of the microscope.
- Do not use the 100X objective.

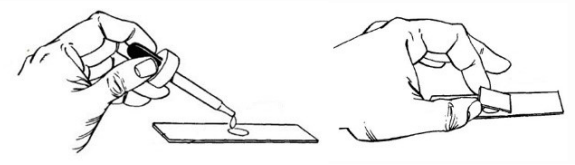
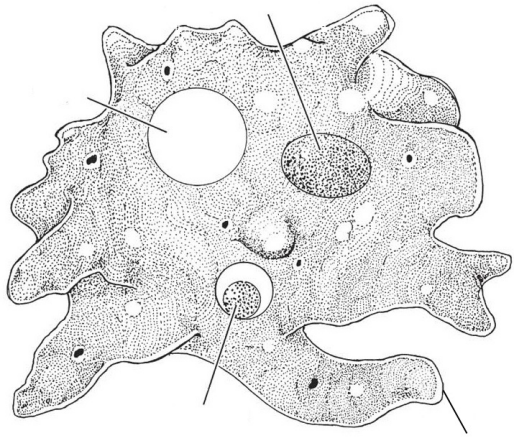


Figure Intro-2. Diagram demonstrating how to make a wet mount.

☐ View an *Amoeba* using a microscope:

- Focus on the specimen in suspension with the scanning objective lens (4X)
- Move to the higher objectives in order (4X to 10X to 40x), focusing on the specimen in the suspension at each magnification.
- Do not use the 100X objective.

8. Label the diagram accordingly.



Phylum:_____

Genus: _____

Habitat:_____

Autotroph and/or Heterotroph

Total Magnification:_____

9. Describe the movement of the cytoplasm in the cell and the formation of pseudopods.

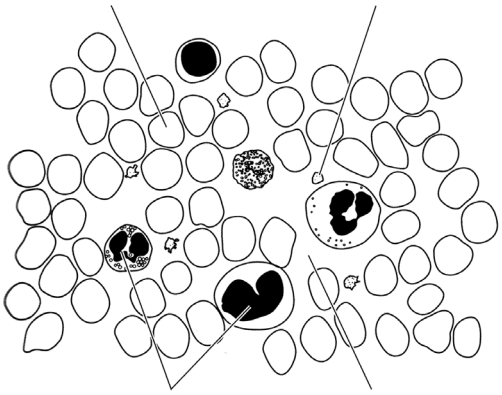
10. Match diagrams in Figure Intro-3 to the tissue slides you observed, color, label accordingly, and include total magnification.

Tissue Type:_____

Specimen: _____

Total Magnification: _____

A. _____ B._____



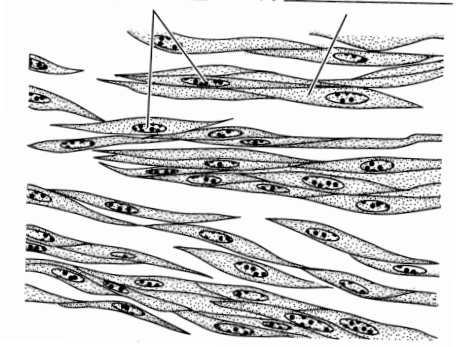
C. _____ D._____

Tissue Type:_____

Specimen: _____

Total Magnification: _____

E._____ F. _____

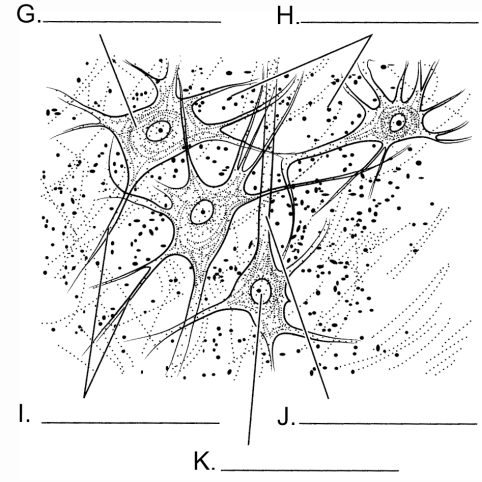


Tissue Type:_____

Specimen: _____

Total Magnification: _____

G._____ H._____



I. _____ J._____

K. _____

Tissue Type:_____

Specimen: _____

Total Magnification: _____

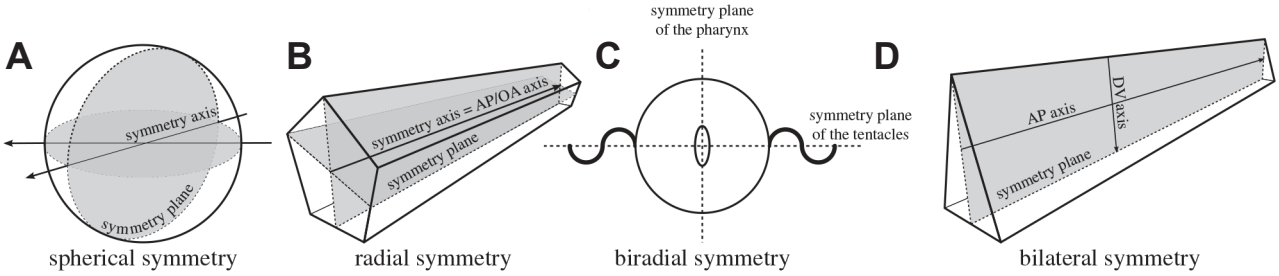
Top view

Side view

Figure Intro-3. Tissue types with the following structures; A. Erythrocyte (red), B. Platelet, C. Leukocytes (pale blue), D. Plasma (yellow), E. Nuclei, F. Cell, G. Nerve cell body (light purple), H. Glial cells, I. Dendrites, J. Axon, and K. Nucleus of nerve cell body (dark purple).

Part 4. Symmetry, Axes, Planes, & Sections

Symmetry (Figure Intro-4) in biology is the balanced distribution of duplicate body parts or shapes within the body of an organism. In nature and biology, symmetry is always approximate. For example, plant leaves – while considered symmetrical – rarely match up exactly when folded in half. Symmetry creates a class of patterns in nature, where the near-repetition of the pattern element is by reflection or rotation. The body plans of most multicellular organisms exhibit some form of symmetry, whether radial, bilateral, biradial, or spherical. A small minority, notably among the sponges, exhibit asymmetry. Positional terms have long been used in anatomy to describe the spatial symmetry of the impressive diversity of organismal forms of both plants, animals, and other organisms. Axes and planes are applicable to many bilateral organisms as well as other symmetries.



DOI: 10.1098/rsfs.2015.0032

Figure Intro-4. Schematic of A) spherical B), radial, C) biradial and D) bilateral symmetries.

A. Axes

An **anatomical axis** is a hypothetical axis used to transect an anatomical entity in a straight line through space. The primary or main axis is considered the **anterior-posterior (AP) axis**, which extends longitudinally from head to tail. The **dorsal-ventral (DV) axis** is recognized in that ventral typically faces toward, and dorsal away, from a substrate (meaning towards the ground for land-dwelling organisms or towards the ocean or river/lake bottom for marine or aquatic organisms), whereas the **left-right (LR) axis** is defined in relation to a plane running along the anterior-posterior midline.

- AP axis (Synonyms: anterior-posterior axis, longitudinal axis, cephalocaudal axis; craniocaudal axis; rostrocaudal axis; rostral/caudal) - an axis that extends through an organism from head end to opposite end of body or tail.
- LR axis (Synonyms: left to right axis; dextro-sinister axis; R-L axis; L-R axis; LR axis; right to left axis; right-left axis; RL axis) - an axis that bisects an organism from left to right sides of body, through a sagittal plane.
- DV axis (Synonyms: dorso-ventral axis; dorsoventral axis; D-V axis.) - an axis that is approximately perpendicular to the anterior-posterior axis and that extends through the horizontal plane of the body.
- AA axis (Synonyms: Adaxial-abaxial axis) - an anatomical axis that extends from the side of the anatomical entity that is closer to an axis (adaxial) to the side that is further from the same axis (abaxial).
- Proximal-distal axis (Synonyms: proximal/distal; proximodistal) - an axis that extends from the point of attachment of a structure (proximal) to the point furthest away from the plane of attachment (distal).
- Apical-Basal axis (Synonyms: apical/basal) - an axis that extends through an organism or organism part from the part of the organism or organism part attached to a substrate (basal) to the furthest from the attachment (apical). Note that the apical-basal axis is often used for organismal parts where there is attachment via a basal lamina or other structure. OR an axis of a plant structure that is determined by the direction of apical growth, either by an apical meristem or an apical cell. Apical is toward the direction of apical growth: toward the tip of a growing shoot axis, root, thallus, or non-vascular leaf. Basal is away from the direction of apical growth: toward the root-shoot junction in the case of the primary root or stem, toward the primary root or stem for higher order roots or branches, toward the point of attachment for non-vascular leaves, and toward the original point of growth (as determined in the embryo) for thalli.
- Oral-aboral axis - An axis that extends from the oral opening to the furthest point in an organism that is directly opposite.

B. Planes & Sections

An **anatomical plane** is a flat 2D plane intersecting an anatomical continuant (a thing that retains its identity even though its states and relations may change), dividing it into two adjacent portions. An anatomical plane is also called a section, anatomical cross-section, plane, or anatomical section.

- Transverse (Synonyms: axial plane; axial section; cross section) - anatomical plane that divides body into anterior and posterior parts.
- Frontal (Synonyms: horizontal anatomical plane; longitudinal section; horizontal section; coronal plane) - anatomical plane that divides bilateral body into dorsal and ventral parts.
- Sagittal (Synonyms: left/right plane; sagittal section; longitudinal section; median plane) - anatomical plane that divides a bilateral body into left and right parts, not necessarily of even size.

11. On Figures Figure Intro-5 (human and fish only) and Figure Intro-6 (giraffe and human only) draw, color, and label the three planes: sagittal (yellow); frontal (blue); transverse (red).

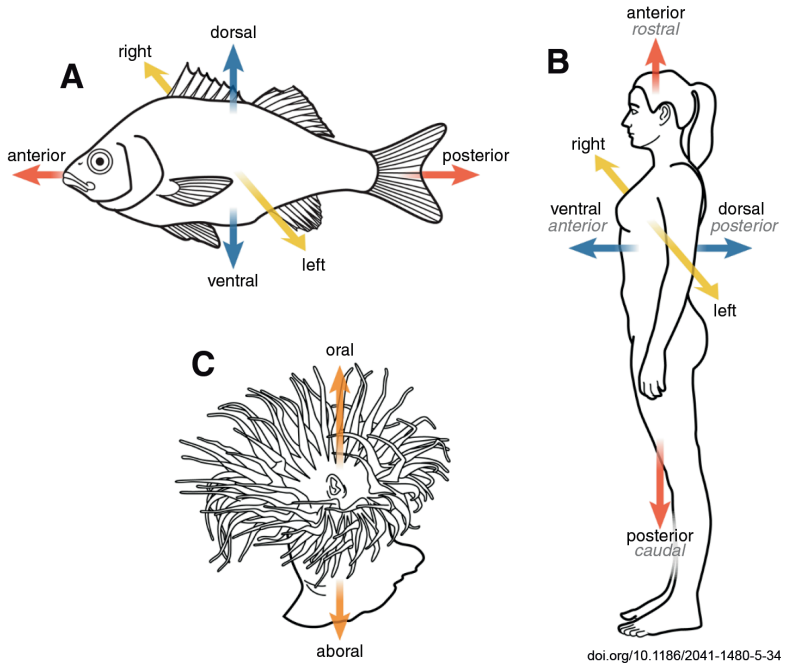


Figure Intro-5. Comparison of primary organismal axes designated in a diversity of species and their representation in Biological Spatial Ontology (BSPO). **A)** In fishes and **B)** in humans, 'anterior-posterior axis' (narrow synonym 'rostral-caudal axis' in humans) is shown in red, 'dorsal-ventral axis' (narrow synonym 'anterior-posterior axis' in humans) shown in blue, and 'left-right axis' shown in yellow. A cnidarian (sea anemone) (C) has an 'oral-aboral axis', shown in orange.

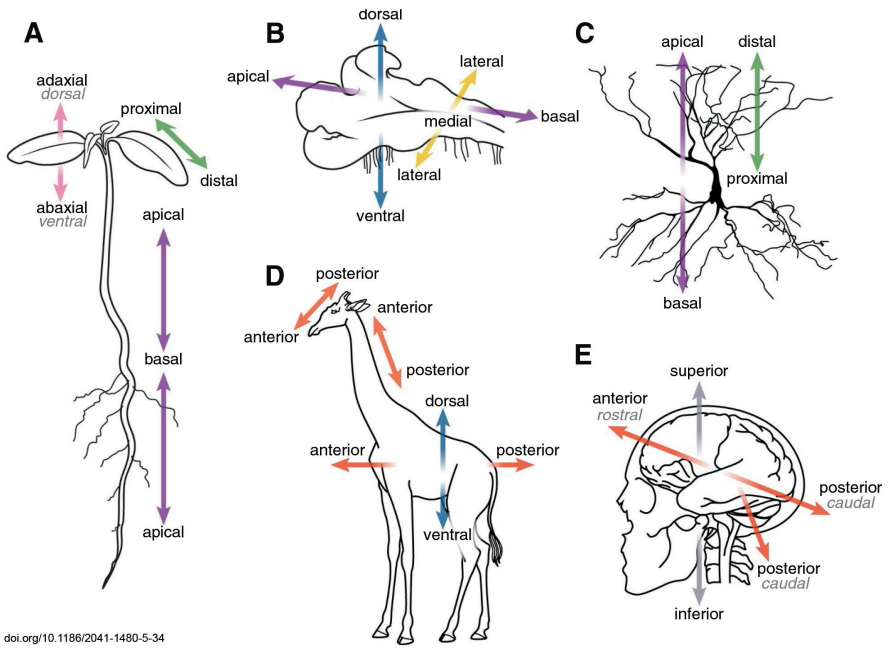
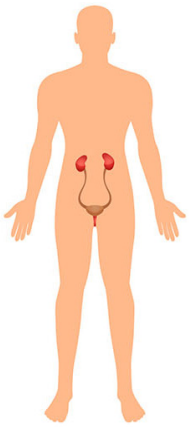


Figure Intro-6. Axes applied to organism parts. **A)** In vascular and **B)** non-vascular plants, the 'apical-basal axis relative to direction of growth'(purple) runs in the direction of apical growth, in both shoots and roots. **A)** For lateral organs such as branches or leaves, the primary axis is the 'proximal-distal axis' (green) and the 'adaxial-abaxial axis' (pink). **B)** In plants or organisms with a thalloid growth form, the 'apical-basal axis relative to direction of growth' often runs parallel to the substrate, resulting in a 'dorsal-ventral axis' that runs perpendicular to the substrate and a 'medial-lateral axis' that is perpendicular to the 'apical-basal axis'. **C)** Hippocampal pyramidal neuron, showing the application of the BSPO classes 'apical-basal axis relative to substrate' and 'proximal-distal axis' to the whole cell or portions thereof. **D)** AP axes for the head, neck and trunk of the giraffe. Note that these axis definitions delineate a "bent" version of the primary AP axis. **E)** AP axis of the human brain (double-headed red arrow) relative to the AP axis of the body (single red arrow). Note the use of "superior" and "inferior" to refer to structures relative to the substrate. https://doi.org/10.1186/2041-1480-5-34.

Unit I. Urinary System



Urinary System

Objectives

- Learn about the urinary system.
- Study the potential of hydrogen (pH) and electrical conductivity in urine using Vernier computer interfaces (Go!Link) and software (Logger Pro).
- Study macromolecules by determining the concentration of two types of macromolecules in urine using a spectrophotometer.
- Become familiar with the scientific method and experimental design.
- Demonstrate the movement of water and ions using two NaCl solutions, water, and change in potato mass.
- Learn the dependent nature of **hypotonic (hypo- beneath or below), hypertonic (hyper- over, above, beyond), and isotonic (iso- as equal, uniform) solutions.**
- Calculate the rate of movement using Vernier electrical conductivity sensors and software.
- Learn about and calculate descriptive statistics; mean and standard deviation.
- Create tables and figures with corresponding captions using Microsoft® 365 Excel and Word.

Terms & Definitions

Aliquot - a portion of a larger whole, especially a sample taken for chemical analysis or other treatment.

Atom - the smallest unit of ordinary matter that forms a chemical element. Every atom is composed of a nucleus and one or more electrons bound to the nucleus. The nucleus is made of one or more protons and a number of neutrons:

A. Proton - a positively charged subatomic particle. The number of protons in an atom is called the atomic number which defines each type of element.
B. Electron - a negatively charged subatomic particle with the least amount of mass.
C. Neutron - an uncharged subatomic particle with the greatest amount of mass.

Active transport - the transport of a solute across a membrane against its gradient (from a region of low concentration to a region of higher concentration). Requires an input of energy. Primary active transport that uses adenosine triphosphate (ATP), and secondary active transport that uses an electrochemical gradient.

Antidiuretic - Affects water retention in kidneys; controls blood pressure

Buffer - a compound that acts to minimize pH fluctuations in the fluids of living organism . Buffer systems can raise of lower pH as needed.

Compound - a substance consisting of two or more elements.

Concentration - the amount of solute dissolved in a unit volume of solution.

Chemical - a unique form of matter with constant chemical composition and characteristic properties. Chemical substances may take the form of a single element or chemical compounds.

Chemical bonds - hold molecules together and create temporary connections that are essential to life:

A. Covalent bond - the sharing of electrons between atoms. This type of bonding occurs between two atoms of the same element or of elements close to each other in the periodic table. This bonding occurs primarily between nonmetals; however, it can also be observed between nonmetals and metals.
B. Glycosidic bond - a bond formed between two sugar molecules.
C. Hydrogen bonds - a weak chemical attraction between a partially positive hydrogen atom of a polar molecule and a partially negative atom of another polar molecule.
D. Ionic bonding - is the complete transfer of valence electron(s) between atoms. It is a type of chemical bond that generates two oppositely charged ions. In ionic bonds, the metal loses electrons to become a positively charged cation, whereas the nonmetal accepts those electrons to become a negatively charged anion. Ionic bonds require an electron donor, often a metal, and an electron acceptor, a nonmetal.
E. Peptide -a covalent bond joining the α-amino group of one amino acid to the carboxyl group of another with the loss of a water molecule.
F. Phosphodiester - two of the hydroxyl groups in phosphoric acid react with hydroxyl groups on other molecules to form two ester bonds.

Chemical reactions - the formation and breaking of chemical bonds, resulting in a change in the composition of substances:

A. Anabolic - a metabolic pathway that involves the synthesis of larger molecules from smaller precursor molecules. Such reactions usually require an input of energy
B. Catabolic - a metabolic pathway in which a molecule is broken down into smaller components, usually releasing energy (exergonic).
C. Condensation - a type of chemical reaction in which two molecules are combined to form a single molecule, usually with the loss of a small molecule such as water.
D. Dehydration - a type of condensation reaction in which a molecule of water is lost.
E. Endergonic - chemical reactions that require an addition of free energy and do no proceed spontaneously.
F. Exergonic - refers to chemical reactions that release free energy and occur spontaneously.
G. Hydrolysis - any chemical reaction in which a molecule of water breaks one or more chemical bonds.

Dialysis - the separation of particles in a liquid on the basis of differences in their ability to pass through a membrane.

Electronegative - the tendency to attract electrons to form a chemical bond.

Equilibrium (chemistry) - occurs when the rate of the forward reaction is balanced by the rate of the reverse reaction; in ecology, the situation in which the population size stays the same.

Equilibrium potential - the membrane potential at which the flow of an ion is at equilibrium, with no net movement in either direction.

Electrolytes (ionic) - substances in water that dissociate into cations and anions. The resulting solution can then conduct an electrical current:

A. Acid - releases hydrogen ions (h+; protons) in solution; give up protons during chemical reactions.
B. Base - lowers the h+ concentration; accepts protons during chemical reactions.
C. Hydrogen ion concentration - the concentration of hydrogen ions (protons) in a solution expressed usually in moles per liter or in pH units.
D. pH - the mathematical expression of a solution's hydrogen ion (H+) concentration, defined as the negative logarithm to the base 10 of the H+ concentration.
E. pH scale - a logarithmic scale that indicates the concentration of hydrogen ions. The scale goes form 0-14, with zero representing an extremely high concentration of free H+ ions and 14 representing the lowest concentration.
F. Salt - a chemical compound consisting of an ionic assembly of positively charged cations and negatively charged anions, which results in a compound with no net electric charge. A common example is table salt, with positively charged sodium ions and negatively charged chloride ions.

Homeostasis - the tendency toward a relatively stable equilibrium between interdependent elements, especially as maintained by physiological processes.

Interstitial cells - any cell that lies in the spaces between the functional cells of a tissue.

Interstitial fluid - a fluid found in the spaces around cells. It comes from substances that leak out of blood capillaries (the smallest type of blood vessel). It helps bring oxygen and nutrients to cells and to remove waste products from them. As new interstitial fluid is made, it replaces older fluid, which drains towards lymph vessels. When it enters the lymph vessels, it is called lymph. Also called tissue fluid.

Ions - atoms or molecules that gain or lose one or more electrons and acquires a net electric charge:

A. Anions - an ion that has a net negative charge.
B. Cations - ions that have a positive net charge.

Macromolecule - molecules bonded together to form a polymer:

A. Carbohydrate - organic molecules often with the general formula c(H_2O); a carbon-containing compound that includes starches, sugars, and cellulose.
B. Lipid - a molecule composed predominately of hydrogen and carbon atoms; nonpolar and insoluble in water.
C. Nucleic acids - an organic molecule composed of nucleotides. The two types of nucleic acids are deoxyribonucleic acid (DNA) and ribonucleic acid (RNA)
D. Protein - a functional unit composed of one or more polypeptides. Each polypeptide is composed of a linear sequence of amino acids.

Membrane potential - the difference between the electric charges outside and inside a cells; also called potential difference.

Membrane transport - the movement of ions or molecules across a cell membrane.

Metabolism - the sum total of all chemical reactions that occur within an organism:

A. Anabolism - a metabolic pathway that results in the synthesis of cellular molecules and macromolecules; requires an input of energy.
B. Catabolism - a metabolic pathway in which a molecule is broken down into smaller components, usually releasing energy (exergonic).Membrane transport

Monomer - an organic molecule that can be used to form larger molecules (polymers) consisting of many repeating units of the monomer.

Nucleotides - organic molecules having three components: one or more phosphate groups, a five-carbon sugar (either deoxyribose or ribose), and a singe or double ring of carbon and nitrogen atoms known as a base.

A. Pyrimidine - single ring structured base; cytosine, thymine, and uracil (RNA only).
B. Purines - two-ring structured base; adenine and guanine.

Passive transport - a type of membrane transport that does not require energy to move substances across cell membranes:

A. Diffusion - in a solution, the process that occurs when a solute moves from a region of high concentration to a region of lower concentration.
B. Facilitated diffusion - the process of spontaneous passive transport (as opposed to active transport) of molecules or ions across a biological membrane via specific transmembrane integral proteins.
C. Filtration - movement of water and solute molecules across the cell membrane due to hydrostatic pressure generated by the cardiovascular system.
D. Osmosis - the movement of water across membranes to balance solute concentrations. Water diffuses from a solution that is hypotonic (lower solute concentration) into a solution that is hypertonic (higher solute concentration).

Peptide - short chains of amino acids linked by covalent peptide bonds.

Pressure - force per unit area:

A. Hydrostatic pressure - the physical force exerted by a fluid on a structure (HB). Blood pressure is the force exerted per unit area by the blood as it presses against the internal surface of the vessel wall. Interstitial fluid hydrostatic pressure is the force of interstitial fluid on the external surface of the blood vessel.
B. Pressure gradient - a physical quantity that describes in which direction and at what rate the pressure increases the most rapidly around a particular location.
C. Osmotic pressure - the minimum pressure which needs to be applied to a solution to prevent the inward flow of its pure solvent across a semipermeable membrane.

pH scale - a logarithmic scale used to specify the acidity or basicity of aqueous solutions; inversely indicates the activity of hydrogen cations (H^+) in the solution. Acidic solutions (higher concentrations of hydrogen cations) are measured to have lower pH values than basic or alkaline solutions. The scale goes from 0-14, with zero representing an extremely high concentration of free H^+ ions and 14 representing the lowest concentration.

Phospholipid - a class of lipids that are similar in structure to triglycerides, but the third hydroxyl group of glycerol is linked to a phosphate group instead of a fatty acid; a key component of biological membranes.

Phospholipid bilayer - the basic framework of the cellular membrane, consisting of two layers of lipids.

Polymer - a large molecule formed by linking many smaller molecules called monomers.

Secretion - 1) the export of a substance from a cell; 2) the process in which some solutes are actively transported into the tubules of the excretory organ; this supplements the amount of solute that would normally be removed by filtration alone.

Selectively Permeable - the property of membranes that allows the passage of certain ions or molecules but not others.

Solute - a substance dissolved in a liquid.

Solution - a liquid that contains one or more dissolved solutes.

Stock solutions - concentrated solutions of known, accurate concentrations that will be diluted for future laboratory use.

Spectrum - used to classify something, or suggest that it can be classified, in terms of its position on a scale between two extreme or opposite points:
A. Absorption spectrum - a diagram that depicts the wavelengths of electromagnetic radiation that are absorbed by a pigment.
B. Action spectrum - the rate of photosynthesis plotted as a function of different wavelengths of lights.
C. Electromagnetic spectrum - all possible wavelengths of electromagnetic radiation, from relatively short wavelengths to much longer wavelengths.

Standard curve (calibration curve) - a method to determine the concentration of a substance in an unknown sample by comparing the unknown to a set of standard samples of known concentration.

Tonicity - a measure of the effective osmotic pressure gradient; the water potential of two solutions separated by a partially-permeable cell membrane:
A. Hyper- a greater concentration of non-permeating solutes than another solution.
B. Hypo- a lower concentration of solutes than another solution.
C. Iso- the concentration is the same as that of another solution.

Wavelength - the distance from the peak of one wave to the next.

Solute - a substance dissolved in a liquid.

Solution - a liquid that contains one or more dissolved solutes.

Spectrum - used to classify something, or suggest that it can be classified, in terms of its position on a scale between two extreme or opposite points:
A. Absorption spectrum - a diagram that depicts the wavelengths of electromagnetic radiation that are absorbed by a pigment.
B. Action spectrum - the rate of photosynthesis plotted as a function of different wavelengths of lights.
C. Electromagnetic spectrum - all possible wavelengths of electromagnetic radiation, from relatively short wavelengths to much longer wavelengths.

Standard curve (calibration curve) - a method to determine the concentration of a substance in an unknown sample by comparing the unknown to a set of standard samples of known concentration.

Stock solutions - concentrated solutions of known, accurate concentrations that will be diluted for future laboratory use.

Tonicity - a measure of the effective osmotic pressure gradient; the water potential of two solutions separated by a partially-permeable cell membrane:
A. Hyper- a greater concentration of non-permeating solutes than another solution.
B. Hypo- a lower concentration of solutes than another solution.
C. Iso- the concentration is the same as that of another solution.

Wavelength - the distance from the peak of one wave to the next.

Urinary system - the structures that collectively act to filer blood or hemolymph and excrete wastes, while recapturing useful compounds. Relating to or denoting the system of organs, structures, and ducts by which urine is produced and discharged, in mammals comprising the kidneys, ureters, bladder, and urethra; maintain fluid and electrolyte balance, purify blood and excrete liquid waste (urine):

Arteries - blood vessels that deliver oxygen-rich blood from the heart to the tissues of the body:
A. Interlobar/Cortical radial - vessels of the renal circulation which supply the renal lobes. The interlobar arteries branch from the lobar arteries which branch from the segmental arteries, from the renal artery.
B. Arcuate - are vessels of the renal circulation. They are located at the border of the renal cortex and renal medulla.
C. Interlobular - renal blood vessels given off at right angles from the side of the arcuate arteries looking toward the cortical substance.

Arterioles - a small branch of an artery leading into capillaries:
A. Afferent - group of blood vessels that supply the nephrons in many excretory systems.
B. Efferent - supply the blood for the extensive network of capillaries that surround the cortical and medullary tubular system of the kidneys, known as the peritubular capillary network.

Calyx - a cuplike structure of the kidney through which urine passes:
A. Minor - surround the apex of the renal pyramids.
B. Major - urine passes before continuing through the renal pelvis into the ureter.

Capsular Space - slit like space between the visceral and parietal layers of the capsule of the renal corpuscle

Collecting Duct - the final component of the kidney to influence the body's electrolyte and fluid balance.

Filtrate - to liquid which has passed through the process of filtration

Glomerular:
A. Capsule - A double-walled, cup-shaped structure around the glomerulus of each nephron of the vertebrate kidney. It serves as a filter to remove organic wastes, excess inorganic salts, and water.
B. Filtration - the process by which the kidneys filter the blood, removing excess wastes and fluids.
C. Capillaries - a network of small blood vessels (capillaries) known as a tuft, located at the beginning of a nephron in the kidney.

Glomerulus - a network of small blood vessels (capillaries) known as a tuft, located at the beginning of a nephron in the kidney.

Loop of Henle - is the portion of a nephron that leads from the proximal convoluted tubule to the distal convoluted tubule. Creates a concentration gradient in the medulla of the kidney:
A. Descending - low permeability to ions and urea while being highly permeable to water. The loop has a sharp bend in the renal medulla going from descending to ascending thin limb.
B. Ascending - impermeable to water, but it is permeable to ions.

Kidney - of organs located in the right and left side of the abdomen. The kidneys remove waste products from the blood and produce urine. As blood flows through the kidneys, the kidneys filter waste products, chemicals, and unneeded water from the blood.

Nephron - is the minute or microscopic structural and functional unit of the kidney.

Peritubular capillary - tiny blood vessels, supplied by the efferent arteriole, that travel alongside nephrons allowing reabsorption and secretion between blood and the inner lumen of the nephron.

Renal:
A. Artery - paired arteries that supply the kidneys with blood.
B.Capsule - a tough fibrous layer surrounding the kidney and covered in a layer of perirenal fat known as the adipose capsule of kidney.
C. Corpuscle - the blood-filtering component of the nephron of the kidney.
D Cortex - the outer portion of the kidney between the renal capsule and the renal medulla.

E. Medulla - the innermost part of the kidney.
F. Pelvis - is the funnel-like dilated part of the ureter in the kidney.
G. Tubule - a long pipe like structure containing the tubular fluid filtered through the glomerulus
H. Vein - veins that drain the kidney. They connect the kidney to the inferior vena cava. They carry the blood filtered by the kidney.

Tubules:
A. Proximal - the segment of the nephron in kidneys which begins from the renal pole of the Bowman's capsule to the beginning of loop of Henle.
B. Distal Convoluted - a portion of kidney nephron between the loop of Henle and the collecting tubule.

Tubular:
A. Reabsorption - a passive process whereby drugs are reabsorbed into the systemic circulation from the lumen of the distal tubules
B. Secretion - the transfer of materials from peritubular capillaries to the renal tubular lumen and occurs mainly by active transport and passive diffusion.

Ureter - the duct by which urine passes from the kidney to the bladder or cloaca.

Urine - filtrate after it has passed through the nephron and undergone filtration, reabsorption, and secretion.

Urinary Bladder - a muscular sac in the pelvis, just above and behind the pubic bone

Vasa Recta - the capillary networks that supply blood to the kidney medulla.

Veins - blood vessels that carry blood towards the heart. Most veins carry deoxygenated blood from the tissues back to the heart; exceptions are the pulmonary and umbilical veins, both of which carry oxygenated blood to the heart. In contrast to veins, arteries carry blood away from the heart.
A. Interlobar - drain the renal lobes.
B. Arcuate - located at the border of the renal cortex and renal medulla.
C. Interlobular - run alongside the interlobular arteries and collect venous blood from the capillary plexus of the cortex.

Zona - zones of the adrenal cortex:
A. Fasciculata - the middle and also the widest zone of the adrenal cortex, sitting directly beneath the zona glomerulosa. Constituent cells are organized into bundles.
B. Glomerulosa - the most superficial layer of the adrenal cortex, lying directly beneath the renal capsule. Its cells are ovoid and arranged in clusters or arches.
C. Reticularis - the innermost layer of the adrenal cortex, lying deep to the zona fasciculata and superficial to the adrenal medulla. The cells are arranged cords that project in different directions giving a net-like appearance.

Background

There are four homeostatic functions of the human urinary/renal system; 1) regulate blood volume and blood pressure by adjusting volume of water lost in urine and releasing hormones that control red blood cell production and blood pressure, 2) regulate plasma concentrations of sodium, potassium, and chloride ions by controlling quantities lost in urine and calcium ion concentration through synthesis of the hormonally active metabolite of vitamin D, 3) help stabilize blood pH by controlling loss of hydrogen ions and bicarbonate ions in urine, and 4) conserve valuable nutrients while excreting organic waste products. Human urine is a liquid that is secreted by the urinary system. The kidneys filter blood to remove waste and extra fluid which is collected within the bladder as urine and excreted through the urethra. Urine is composed of 91–96% water and the remainder can be broadly characterized into inorganic salts, urea, organic compounds, organic ammonium salts, and small amount of epithelial cells from the bladder and external urethra. **Epithelial cells in the urine can increase from a urinary tract infection or some other cause of inflammation. Diseases can also cause urine to contain white blood cells (infection), red blood cells (kidney disease, a blood disorder or another underlying medical condition, such as bladder cancer), bacteria or yeasts (infection), tube-shaped proteins called casts (kidney disorders), and crystals (sign of kidney stones).**

A. Urinalysis

Urinalysis evaluates samples of urine to detect and assess a wide range of disorders, such as urinary tract infections, kidney disease, and diabetes. It involves examining the appearance, concentration and content of urine. Abnormal urinalysis results may point to a disease or illness. Most routine clinical tests are completed by using a commercial dipstick. A dipstick has absorbent paper impregnated with specific chemicals and several different chemical tests can be performed with one dipstick. Dipsticks can screen for the presence of excessive glucose or protein in urine. Other tests that can be performed using a dipstick include detecting the presence of bilirubin, blood, leukocytes, ketones, nitrite, urobilinogen, and pH. Urine can also be used for pregnancy testing and drug screenings. For example, pregnancy testing measures a hormone called human chorionic gonadotropin (HCG). Drug screenings detect specific drugs or their metabolic products, depending on the purpose of the testing. Quantitative tests for pH, conductivity, and macromolecules in urine are not routinely done. During the Unit I - Urinalysis & Dialysis we will study a variety of urine variables and measure them quantitatively.

pH & Conductivity

The pH level of urine indicates the amount of acid in urine. Abnormal pH levels may indicate a kidney or urinary tract disorder and possibly the formation of stones. Electrical conductivity (EC) can show how concentrated the urine is and can indicate dehydration. Also, red blood cells, pus cells, calcium oxalate monohydrate, calcium oxalate dihydrate, uric acid, sodium, and phosphates that can be found in urine and are correlated with high EC.

- pH - 4.6 to pH 8.0
- Conductivity (EC) - 2.1- 8.1 dS/m (decisiemens per meter)

Macromolecules

The major classes of macromolecules can be detected in urine. Analysis of urine for the major classes of macromolecules is clinically important in assisting diagnosis metabolism disorders and understanding the pathologic significance of the disorders.

Carbohydrate - There are eight carbohydrates that are often tested for: maltose, lactose, D-mannose, D-glucose, D-ribose, D-xylose, L-arabinose and D-galactose. Detection of glucose in a urine test usually calls for follow-up testing for diabetes. **Glucose - not usually found in urine. If it is, further testing is needed. Normal glucose range in urine: 0 - 0.15 mg/ml**

Protein - Low levels of protein in urine are normal but large increases of protein may indicate a kidney problem such as diabetic kidney damage. More than 150 mg/24 hours is considered excessive and can be caused by a variety of conditions, from benign to serious. Transient increases can occur with a fever, exposure to cold, emotional stress or severe exercise. However, persistent increases are most commonly associated with kidney diseases such as polycystic kidney disease, and nephrotic syndrome. **Protein - 0 to 0.2 mg/ml in a random urine sample. The normal value is less than 0.8 mg/ml in a 24-hour urine collection.**

Nucleic Acids - Urine is not considered an ideal source of nucleic acids because there are few nucleated cells found in urine but those that are found are typically white blood cells and epithelial cells. The need for the use of urine as an identification tool may arise from a crime scene, or in a toxicology laboratory. At a crime scene, urine may be used to identify the perpetrator of a crime, or to place a victim at a particular site. In a laboratory, DNA analysis may be needed to positively identify an individual as the submitter of a particular urine sample.

Lipid - Human urine usually contains only very small amounts of lipids. However, under certain nephrotic syndromes the urinary excretion of cholesterol, cholesterol esters, triglycerides, free fatty acids and phospholipids is considerably increased. Many of the lipids found in urine come from the phospholipids of cell membranes.

B. Filtration

In order to survive, all organisms need to move molecules in and out of their cells. Molecules such as gases (e.g., O_2, CO_2), water, food, and wastes pass across the cell membrane. There are two ways that the molecules move through the membrane: **passive transport** and **active transport.** While active transport requires that the cell uses chemical energy to move substances through the cell membrane, passive transport does not require such energy expenditures. Passive transport occurs spontaneously, using heat energy from the cell's environment. **Diffusion is the movement of molecules by passive transport from a region in which they are highly concentrated to a region in which they are less concentrated.** Diffusion continues until the molecules are randomly distributed throughout the system. **Osmosis, the movement of water across a membrane, is a special case of diffusion. Water always flows down a pressure gradient or up a solute concentration gradient**. Water molecules are small and can easily pass through the membrane. Other molecules, such as proteins, DNA, RNA, and sugars are too large to diffuse through the cell membrane. The membrane is said to be semipermeable, since it allows some molecules to diffuse through but not others.

Kidneys filter blood in a three-step process to produce urine. Nephrons, the functional unit of the kidney, are composed of a Glomerular capsule (Bowman's capsule, renal corpuscle) and a long convoluted renal tubule (proximal convoluted tube + loop of Henle + distal convoluted tubule). Nephrons are involved in the formation of filtrate by 1) renal ultrafiltration of the blood plasma, 2) selective reabsorption of most of the filtered water and other small molecules (amino acids, glucose, and sodium ions), and the 3) secretion of some excretory products (Potassium ions K+, Hydrogen ions H+, Ammonium ions NH4+, Creatinine, Urea, some hormones, some

drugs). Renin (blood pressure regulation), erythropoietin (stimulate production of erythrocytes), and calcitriol (activated vitamin D promoting intestinal absorption of calcium and the renal reabsorption of phosphate) are hormones produced by the nephrons.

Glomerular filtration filters out almost all solutes, except for proteins, due to high blood pressure and specialized membranes in the **afferent arteriole**. A process called **tubular reabsorption** allows almost all nutrients to be reabsorbed in the **proximal convoluted tubule** (PCT). Reabsorption of water and some key electrolytes are regulated and can be influenced by hormones. Sodium (Na^+) is the most abundant ion and most of it is reabsorbed by active transport and then transported to the **peritubular capillaries**. Because Na^+ is actively transported out of the tubule, water follows it to even out the osmotic pressure. Water is also independently reabsorbed into the peritubular capillaries due to the presence of aquaporins, or water channels, in the PCT. This occurs due to the low blood pressure and high osmotic pressure in the peritubular capillaries. However, every solute has a transport maximum and the excess is not reabsorbed. In the loop of Henle, the filtrate continues to exchange solutes and water with the renal medulla and the **peritubular capillary network**. Water is also reabsorbed during this step. Then, additional solutes and wastes are secreted into the kidney tubules during **tubular secretion**.

Name:_____ Team #:_____ Section #:_____

1. Figure UI-1, label male and female, color the structures accordingly.

1. Ribs (yellow)
2. Kidney (light purple)
3. Ureter (light blue)
4. Bladder (pink)
5. Urethra (dark purple)
6. Rectum (brown)
7. Anus (light brown)

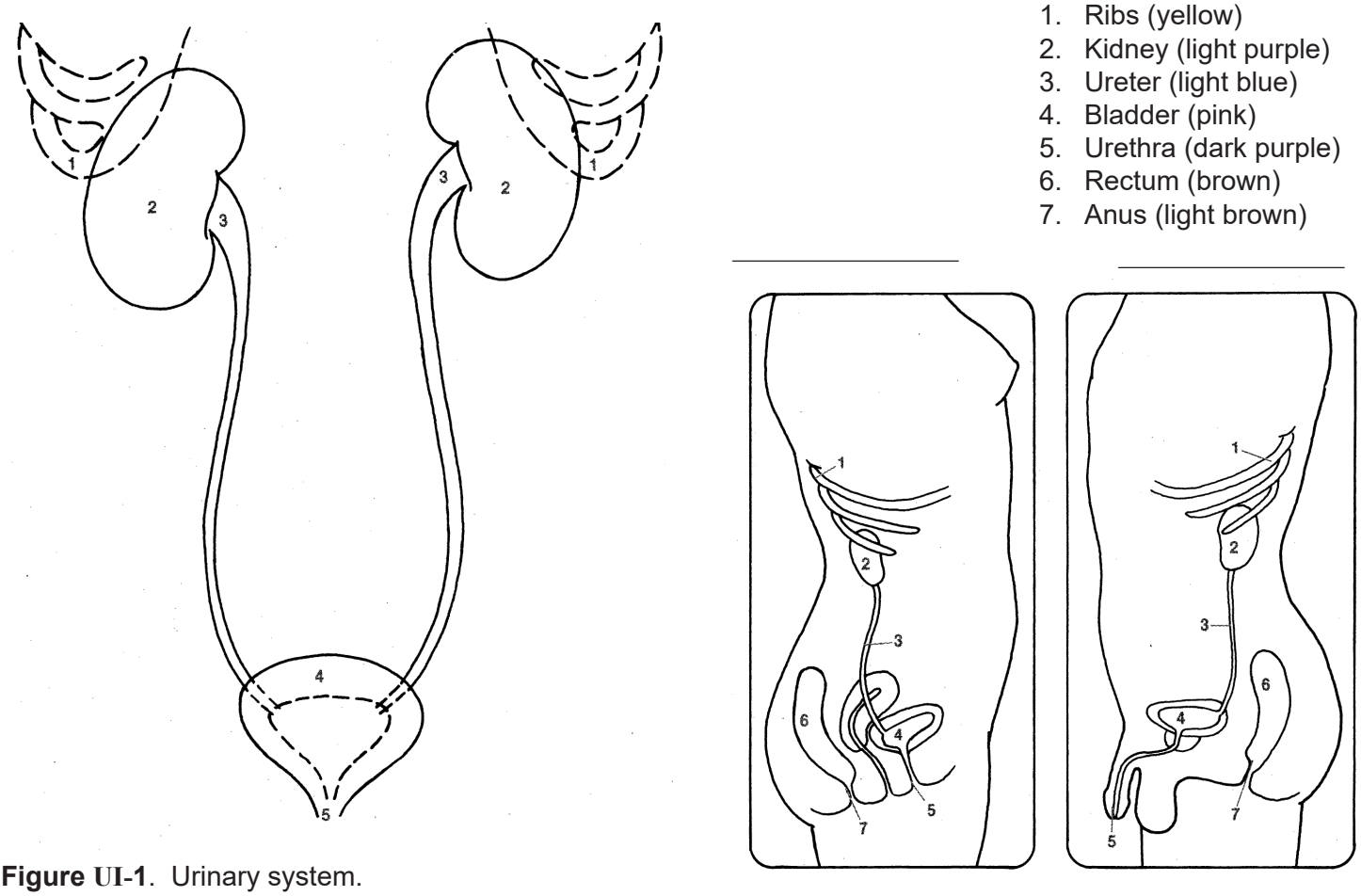
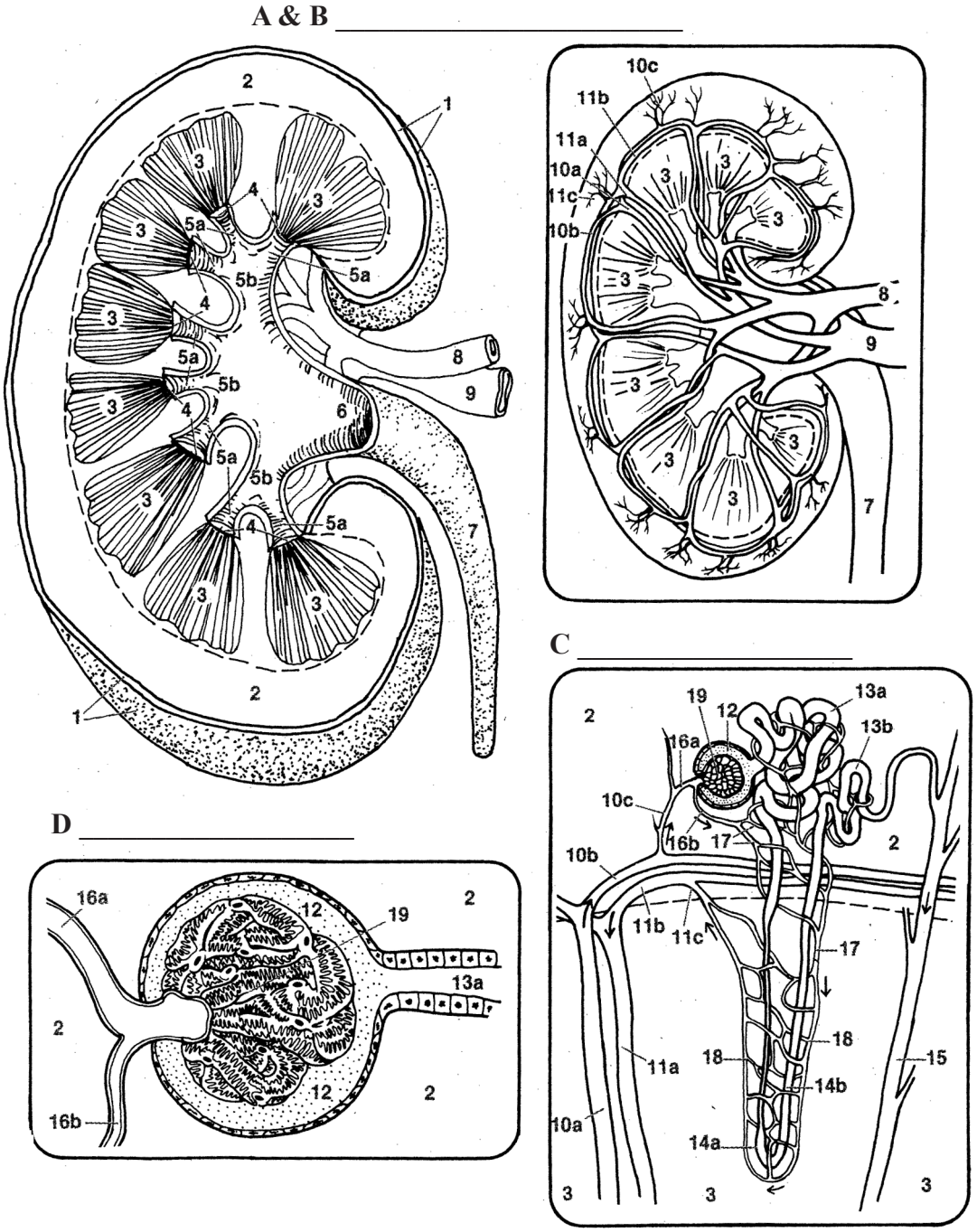


Figure UI-1. Urinary system.

2. In Figure UI-2, label structures A-D, color the structures accordingly, and compare the structures to the model available in the lab.

☐ Observe the models in the teaching laboratory once you arrive.

3. On Figure UI-1, draw and label the bladder trigone?
4. On Figure UI-1, draw and label the adrenal glands. To which system does this organ belong? _____



1. Renal capsule (light purple)
2. Cortex (pink)
3. Medulla (orange)
4. Papilla (yellow)
5. Calyx (green)
   a. Minor
   b. Major
6. Renal pelvis (light green)
7. Ureter (light blue)
8. Renal artery (red)
9. Renal vein (dark blue)
10. Arteries (red)
    a. Interlobar
    b. Arcuate
    c. Interlobular
11. Veins (blue)
    a. Interlobar
    b. Arcuate
    c. Interlobular
12. Glomerular Bowman's capsule (gray)
13. Tubules (light brown)
    a. Proximal
    b. Distal convoluted
14. Loop of Henle (brown)
    a. Descending
    b. Ascending
15. Collecting duct (light orange)
16. Arterioles (red)
    a. Afferent
    b. Efferent
17. Peritubular capillary network (red)
18. Vasa recta (blue)
19. Glomerulus/Glomerular capillaries (purple)

Figure UI-2. Kidney cross section with increasing levels of magnification; A & B) kidney, C) nephron, and D) glomerulus. Note that the Vasa recta are the tiny capillaries that surround Henle's loop and provide nutrients and oxygen to the renal medulla while peritubular capillaries are the capillaries that surround the proximal and distal tubules and provide nutrients and oxygen to the renal cortex.

Part 1. pH & Conductivity

An electrolyte is any solute that produces ions in solution such as sodium, potassium, chloride calcium, and hydrogen ions controlled by the urinary system. The resulting solution can then conduct an electrical current. Most biological organisms require electrolytes to maintain the pumps and channels across their plasma membranes. If the balance of electrolytes between the inside and outside of a membrane is disrupted due to dehydration or salt reduction, cardiac and neurological complications can occur in most multicellular organisms.

The electrolyte that we most commonly recognize is salt but most acids and bases are also electrolytes. All of these solutes produce ions in solution:

NaCl (table salt) when dissolved in water produces Na^+ and Cl^- ions.

HCl (gastric acid) when dissolved in water produces H^+ and Cl^- ions.

The potential of a solution to pass an electric current is called electrical conductivity (EC) and it is usually measured in microSiemens per centimeter (µS/cm). This is often expressed simply as an 'EC Unit'. Strong acids, such as sulfuric acid or hydrochloric acid, and strong bases, such as sodium hydroxide or potassium hydroxide, are strong electrolytes because when they dissolve in water, almost every molecule dissociates to produce ions. On the other hand, weak electrolytes, such as weak acids and weak bases, produce relatively few ions when dissolved in water. Citric acid and acetic acid (in vinegar) are weak acids. Baking soda and ammonia are weak bases. When weak electrolytes dissolve in water, the solution is a poor conductor. As the concentration of ions in a solution increases, so does the EC reading. In this lab we **will use deciSiemens per meter (dS/m)** to read conductivity.

☐ Write you Team number on the top of the first page next to your name.

☐ Connect the Go!Links to the computer USB hub and the pH and conductivity sensors to the Go!Links (UI-1). The sensors can fit in the clamp together.

☐ Open the Urinalysis.cmbl file found in the Documents > 2227L folders on the lab computers. The .cmbl file will be opened by the Logger Pro software.

☐ You may need to set the conductivity sensor range to 20,000 µS using the switch on the sensor.

☐ A window will open. Click on the 'Use Sensor Settings' button.

Put on goggles and gloves

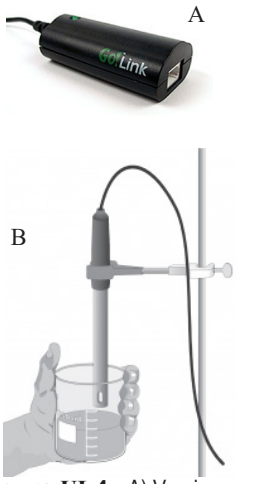


Figure UI-4. A) Vernier computer interface; Go!Link. B) Conductivity sensor attached to ring stand. You will attach both sensors to the ring stand using one clamp.

IMPORTANT: Before, between, and after each use - rinse the tips of the pH and conductivity sensors thoroughly using the wash bottle and plastic beaker, then dry with a KimWipe.
Do not let the pH electrode dry out. Keep the tip covered in the buffer of the storage vial between tests and after use.

A. Controls

☐ Obtain specimen vials for the controls:
- positive - 0.85 NaCl mg/ml (37 mEq/L)
- negative - distilled (deionized) water

☐ Test the controls:
- Uncap a control sample and set it on the ring stand base. Lower the sensors into the sample.
- Briefly swirl the solution and then let the sensors sit in the solution for 3 minutes before taking a recording.
- Click the Collect button. The pH and EC will be tested in a 20 second run by the Logger Pro software.
- Once collection is complete, click the ½ button.

Table UI-1. pH and EC of control and urine samples tested.

	pH	EC
Negative control		
Positive Control		
Urine Sample 1		
Urine Sample 2		
Urine Sample 3		

☐ Record the mean pH and EC of the 20 second run in the appropriate column of Table UI-1.
- Recap the sample, wash and dry the sensors.
- Repeat for the other control.

B. Urine Samples

☐ Obtain a specimen vial for one of the three different urine samples.

☐ Repeat the steps used to test the pH and EC of the controls; then repeat for the other two samples.

☐ Record the mean pH and EC of the 20 second run in the appropriate column of Table UI-1.

☐ Recap the sample, wash and dry the sensors.

Specimen:_____

Total Magnification: _____

Specimen:_____

Total Magnification: _____

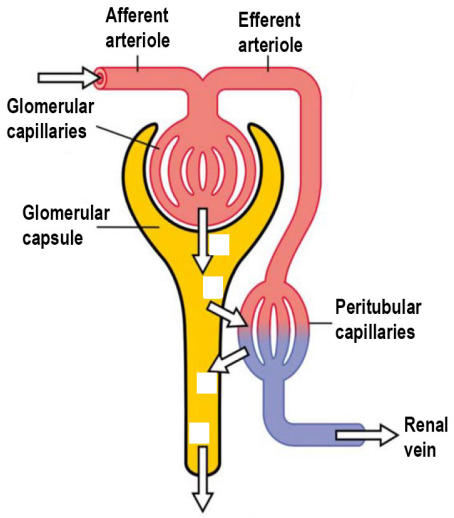


Figure UI-3. Diagram showing the basic physiologic mechanisms of the kidney and the three steps involved in urine formation. Urinary Excretion = Filtration - Reabsorption + Secretion.

Species:_____

Kingdom: _____

Domain: _____

C. Interpretation

5. Figure UI-3, label the arrows; 1) filtration, 2) reabsorption, 3) secretion, and 4) excretion.
6. View and sketch the urine and kidney slides using the **10X** objective lens. Label the following:
   A. Urine - epithelial cells and casts
   B. Kidney - renal capsule, cortex, glomerular capsule and arterioles
   C. Specimen.
   D. Total magnification

☐ Open the 3_Urinalysis Part 1 workbook file found in the Documents > 2227L folder on the lab computers. Add your group's data to the Part 1 spreadsheet table. The graph will automatically populate.

7. Open a Word document and at the top right of the document type:
   - Biol 2227L
   - Unit I: Urinary System (pH & Conductivity)
   - Your section #
   - Your team #
   - Names of everyone on your team
8. Insert the table and figure into the document.
9. Create a table caption ABOVE the table.
10. Create a figure caption BELOW the figure.
11. Type the answers to the following questions into the document:
    A. Why were controls used?
    B. How did the control measurements compare to the sample measurements?
    C. Which sample(s) had the greatest pH and conductivity?
    D. Which sample(s) were out of range for a normal (healthy) urine sample for each type of measurement?
    E. What type of disorders do you think could be causing the abnormal measurements?
    F. What components of urine result in pH and conductivity; Why?
    G. What type of investigator error could have caused the controls to show a response similar to the urine samples?

☐ **Send the document to your lab instructor using your ISU Google Account Gmail.**

Name:_____ Team #:_____ Section #:_____

Part 2. Macromolecules

1. Before attending Lab 3, use the suggested reading on Canvas to complete Table UI-2 by filling in the characteristics of each of the four classes of biologically important large molecules (proteins, nucleic acids, carbohydrates, and lipids).

Table UI-2. The characteristics of A) PROTEINS, B) CARBOHYDRATE, C) NUCLEIC ACID, and D) LIPIDS.

A) PROTEIN

Sketch the general structure of a **protein monomer** and name all characteristic chemical groups:
Sketch the general structure of a **protein polyme**r and name all characteristic chemical group:
Name of bond between monomers:

B) CARBOHYDRATE

Sketch the general structure of a **carbohydrate monomer** and name all characteristic chemical groups:
Sketch the general structure of a **carbohydrate polymer** and name all characteristic chemical group:
Name of bond between monomers:

C) NUCLEIC ACID

Sketch the general structure of a **nucleic acid monomer** and name all characteristic chemical groups:
Sketch the general structure of a **nucleic acid polyme**r and name all characteristic chemical group:
Name of bond between monomers:

D) LIPID

Sketch the general structure of a **triglyceride molecule**, name the characteristic chemical groups that form the molecule, and label the chemical linkages between the groups:
Sketch the general structure of a **phospholipid molecule**, name the characteristic chemical groups that form the molecule, and show how phospholipids align to create cell membranes.
Name the characteristic that distinguishes all **lipids** from other large molecules:

☐ Go to Biolab > Training > Technology > Spectrophotometers (https://www.isu.edu/biology/biolab/instructor-training/technology/spectrophotometers/#d.en.90794) and study the content at that web page.

A standard curve is a quantitative research technique where multiple samples with known concentrations are measured and graphed. Concentrations can be determined for other samples by following the line from the y-axis measurement of the graph to where it intersects the standard curve. The corresponding value on the x-axis is the concentration of substance in the unknown samples. Today we will use the spectrophotometers and a standard curve to determine the concentration of two types of macromolecules found in urine; protein and carbohydrate (glucose).

☐ Write you Team number on the top of the first page next to your name.

☐ Remove the plastic protective cover of the Genesys 20 Spectrophotometer and turn on the spectrophotometer using the power switch located on the back of the machine, lower left side. Once it has warmed up (it may take a few minutes), it should automatically be set to absorbance (A) mode. If it is not, press the A/T/C button to select absorbance (A).

A: Protein

The Bradford assay is mediated by Coomassie Brilliant Blue dye and generates a protein-dependent color change response that will vary with regard to protein type and concentration. The assay is accurate but non-linear and is used in many research and clinical applications. Binding of the dye to protein stabilizes the blue anionic dye form, detected at 595 nm.

☐ Using a permanent marker, label six cuvette tubes **near the top** of the tube with the sample information: B, N, P, 4, 5, and 6.

Put on goggles and gloves

☐ Aliquot 3 ml of the Blank solution into the B cuvette tube. Stretch Parafilm over the top of the tube. You will use the Blank (B) cuvette in Parts 2A and 2B.

☐ Prepare samples (swirl solutions before aliquoting):
- Aliquot 1 ml of each sample into its corresponding cuvette tube (N, P and 4, 5, and 6).
- Aliquot 2 ml of Bradford reagent into each tube N, P and 4, 5, and 6.
- Cover the tubes by stretching Parafilm over the top of each tube and then mix by inverting the tube three times.
- Let the tubes sit for ten minutes.
- Mix by inverting each tube three times.

☐ Set the wavelength to 595 nm by pressing either the buttons.

☐ Insert the B cuvette tube into the cell holder of the sample chamber. The tube should be sitting on the bottom of the cell.

Important: Do not cross-contaminate; use a different transfer pipette for each sample.

Table UI-3. Urine sample with Bradford reagent absorbance at 595 nm and determined protein concentration.

	Absorbance	Absorbance Adjusted	Protein Concentration (mg/ml)
Negative **-** Control (N)			
Positive **+** Control (P)			
Urine Sample **4**			
Urine Sample **5**			
Urine Sample **6**			

☐ Close the sample chamber.

☐ Press the 0 ABS 100% T button to set the blank to 0 concentration.

☐ Measure the Negative - (N) control sample by inserting the cuvette tube into the cell holder and closing the sample chamber.

☐ **Record the absorbance in Table UI-3.**

☐ Repeat for the Positive + (P) albumin control and the urine samples 4, 5, and 6.

☐ **Reserve the B cuvette tube for Part 2B.**

☐ Cleaning the cuvette tubes:
- Empty tubes N, P, 4, 5, and 6 into the correct waste container.
- Scrub the tubes with water and dish detergent.
- Rub off the marker label.
- Invert the tubes on the tube rack.

Calculations

☐ Open the 4_Urinalysis Part 2 spreadsheet found in the Documents > 2227L folder on the lab computers.

☐ Using the absorbance you recorded in Table UI-3, determine the **adjusted absorbance** by typing your data into the 4_Urinalysis Part 2 spreadsheet table protein column 2.
- It subtracts the absorbance of the negative control from positive control and the sample (4, 5, and 6).
- The adjusted absorbance is calculated by the spreadsheet for all samples in Table UI-3.

☐ Determine the **protein concentration** of the urine samples:
- Using the standard curve (Fig. UI-6A) of albumin dilutions, find the adjusted absorbance of each sample on the y-axis and determine the concentration from the x-axis.
- Use a ruler to draw a line from the adjusted absorbance (from y-axis) to the standard curve down to the protein concentration (x-axis).

☐ **Record the protein mg/ml for all controls and samples in Table UI-3.**

☐ Record the concentration in protein column 4 of the 4_Urinalysis Part 2 spreadsheet. The graph will automatically populate.

B: Carbohydrate - Glucose

Glucose is oxidized by Copper (Cu^{2+}) and copper is reduced by glucose. Gluconic acid and a reddish precipitate of copper oxide are formed when glucose is oxidized by the copper ions in the Benedict's reagent. Color change is measured spectroscopically. The amount of the red precipitate is proportional to the amount of glucose in the sample. Glucose concentration in a sample can be measured by measuring the absorption at 520 nm.

☐ Using a permanent marker, label five cuvette tubes **near the top** of the tube with the sample information: N, P, 7, 8, and 9.

☐ Prepare samples (swirl solutions before aliquoting):
- Aliquot 0.5 ml of each sample into its corresponding cuvette tube (N, P and 7, 8, and 9).
- Aliquot 2 ml of Benedict reagent into each tube N, P and 7, 8, and 9 BUT NOT the B cuvette.

☐ Water bath 70°C:
- Place the cuvette tubes in the bath for 20 minutes.
- Remove the tubes and let them cool for 5 minutes.

☐ Cover the cuvette tubes:
- Stretch Parafilm over the top of each tube.
- Mix by inverting or flicking the bottom of the tube three times.

☐ Set the wavelength to 520 nm by pressing either the  buttons.

Table UI-4. Urine sample with Benedict reagent absorbance at 520 nm and determined glucose concentration.

	Absorbance	Absorbance Adjusted	Glucose Concentration (mg/ml)
Negative **-** Control (N)			
Positive **+** Control (P)			
Urine Sample **7**			
Urine Sample **8**			
Urine Sample **9**			

☐ Insert the B cuvette tube into the cell holder of the sample chamber. The tube should be sitting on the bottom of the cell.

☐ Close the sample chamber.

☐ Press the 0 ABS 100% T button to set the blank to 0 concentration.

☐ Measure the Negative - (N) control sample by inserting the cuvette tube into the cell holder and closing the sample chamber.

☐ **Record the absorbance in Table UI-3.**

☐ Repeat for the Positive + (P) glucose control and the urine samples 7, 8, and 9.

☐ Cleaning the cuvette tubes:
- Empty tubes B, N, P, 7, 8, and 9 into the correct waste container.
- Scrub the tubes with water and dish detergent.
- Rub off the marker label.
- Invert the tubes on the tube rack.

Calculations

☐ Open the 4_Urinalysis Part 2 spreadsheet found in the Documents > 2227L folderson the lab computer.

☐ Using the absorbance you recorded in Table UI-4, determine the **adjusted absorbance** by typing your data into the 4_Urinalysis Part 2 spreadsheet table glucose column 2.
- It subtracts the absorbance of the negative control from positive control and the sample (4, 5, and 6).
- The adjusted absorbance is calculated by the spreadsheet for all samples in Table UI-4.

☐ Determine the **glucose concentration** of the urine samples:
- Using the standard curve (Fig. UI-6B) of glucose dilutions, find the adjusted absorbance of each sample on the y-axis and determine the concentration from the x-axis.
- Use a ruler to draw a line from the adjusted absorbance (from y-axis) to the standard curve down to the glucose concentration (x-axis).

☐ **Record the glucose mg/ml for all controls and samples in Table UI-4.**

☐ Record the concentration in glucose column 4 of the 4_Urinalysis Part 2 spreadsheet. The graph will automatically populate.

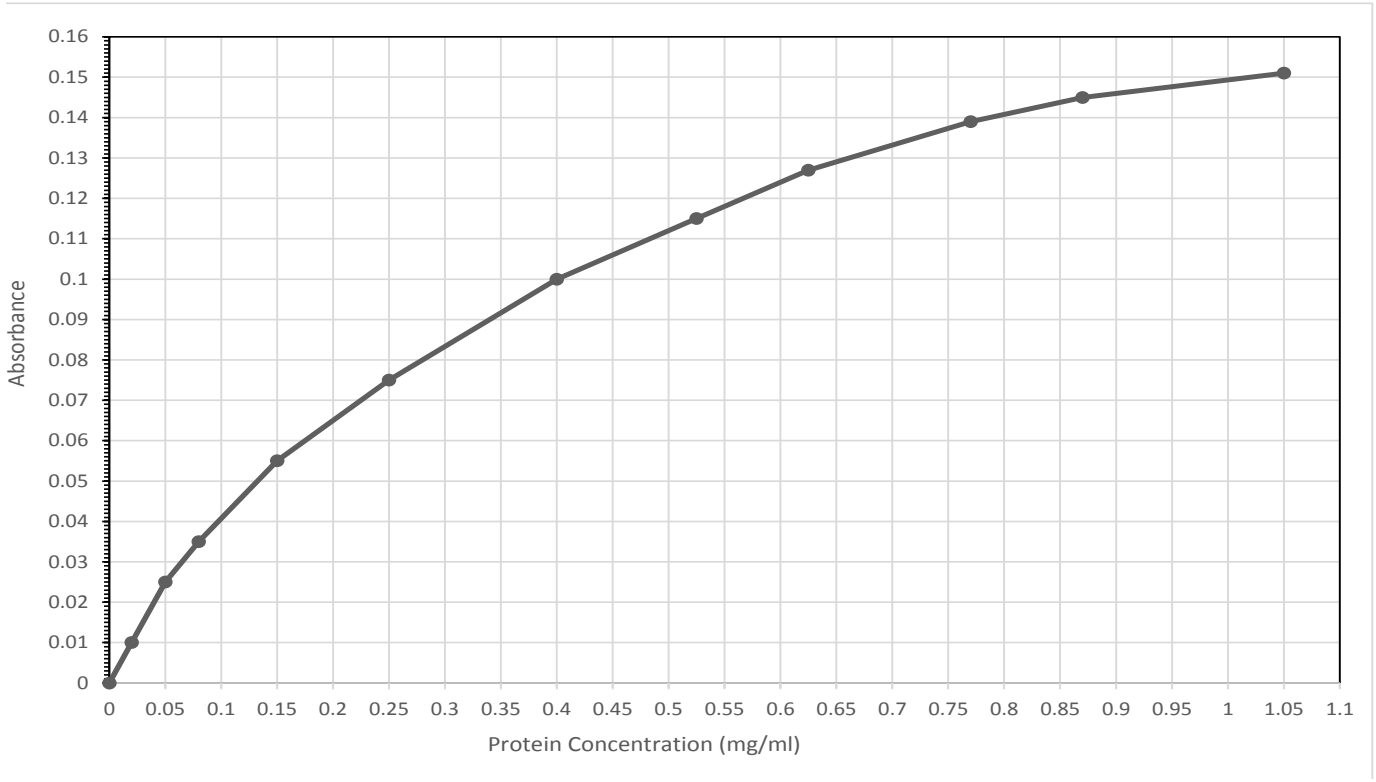
C: Interpretation

☐ Download and open the Urinalysis workbook found on Canvas. Add your group's data spreadsheet table. The graph will automatically populate.

2. Open a Word document and at the top right of the document type:
   - Biol 2227
   - Unit I: Urinary System (Macromolecules)
   - Your section #
   - Your team #
   - Names of everyone on your team
3. Insert the table and graph into the document.
4. Create a table caption ABOVE the table.
5. Create a figure caption BELOW the figure.
6. Type the answers to the following questions into the document:
   A. What are macromolecules?
   B. Which sample(s) had the greatest concentration of protein and glucose?
   C. Which sample(s) were out of range for a normal (healthy) urine sample for each type of macromolecule?
   D. What type of disorders do you think could be causing the abnormal measurements?
   E. Explain how the concentrations were determined using the spectrophotometer and standard curves?

☐ **Send the document to your lab instructor using your ISU Google Account Gmail.**

A



B

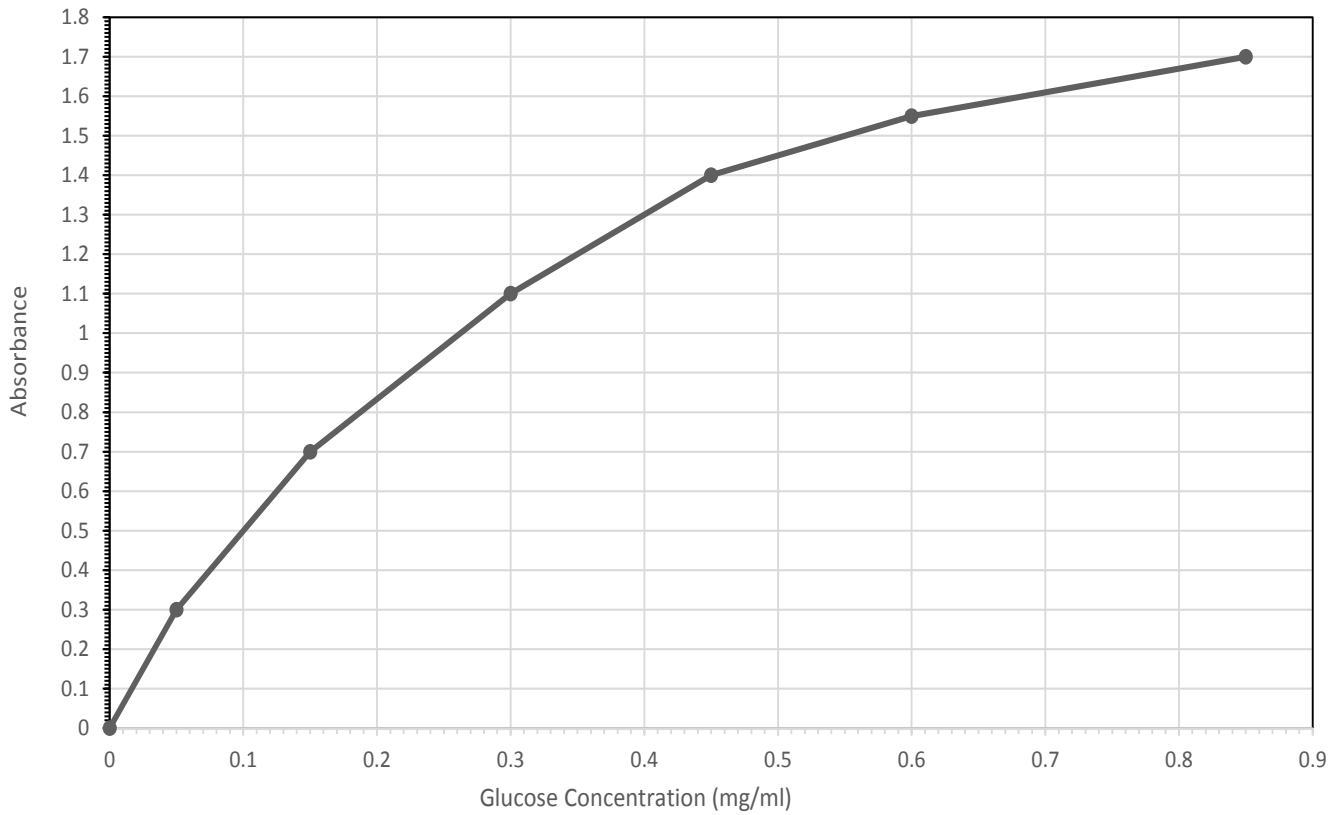


Figure UI-6. Standard curve created from; **A)** albumin standard showing protein concentration (mg/ml) and absorbance at wavelength 595 nm but adjusted for the absorbance of the Bradford Reagent, and **B)** glucose standard showing glucose concentration (mg/ml) and absorbance at wavelength 520 nm but adjusted for the absorbance of the Benedict Reagent. Concentrations were determined for controls, samples 4-6 (protein), and 7-9 (glucose).

Name:_____ Team #:_____ Section #:_____

Part 3. Osmosis & Diffusion

Water that contains ions is able to conduct electricity. The more ions in solution, the easier it is for an electric current to flow. The potential of a solution to pass an electric current is called electrical conductivity (EC) and it is usually measured in microSiemens per centimetre (µS/cm) or deciSiemens per metre (dS/m). This is often expressed simply as an 'EC Unit'. As ion concentration of a solution increases, so to does the EC reading. In this lab will determine the change in solution conductivity and potato mass as well as the rate of osmosis (dS/m/minute) to study diffusion/osmosis in a potato tuber and to learn the dependent nature of **hypotonic (hypo- beneath or below), hypertonic (hyper- over, above, beyond), and isotonic (iso- as equal, uniform) solutions**.

The part of the potato plant (*Solanum tuberosum,* Domain Eukarya, Kingdom Plantae) we eat is called the tuber, which is actually an enlarged underground stem. Potato tubers are mostly made of parenchyma. Parenchyma is a plant ground tissue that forms, among other things, the cortex and pith of stems, the cortex of roots, the mesophyll of leaves, the pulp of fruits, and the endosperm of seeds. Parenchyma cells are living cells and may remain meristematic at maturity—meaning that they are capable of mitosis if stimulated. Each tuber consists of individual parenchyma cells with cellulose **cell walls** cemented together with pectins and weak cell membranes. The cell membrane is a thin bilayer of phospholipids with protein molecules that separates the interior of the cell from its environment. Cell membranes are selectively permeable, controlling what moves into and out of the cell. Inside each cell is a **nucleus** and **cytoplasm** where respiration and starch synthesis occurs. **Starch grains** can be observed in the cytoplasm of most tuber cells.

A. Experimental Design

☐ Write you Team number on the top of the first page next to your name

☐ Observe the Pre-Lab video to find the question, hypothesis, and prediction.

☐ Open Word document, answer question 1 and 2, and then save the document.

1. At the top right of the document type:
   - Biol 2227L
   - Unit I- Urinary System (Osmosis & Diffusion)
   - Your section #
   - Your team #
   - Names of everyone on your team
2. Type the answers to the following questions into the Word document.
   A. Background: What is the species name of your model organism? To which domain and kingdom does it belong? Plants store energy as starch, what type of macromolecule is starch? Plant cell walls are made of what type of organic compound? What type of macromolecule is the organic compound?
   B. Question?
   C. Determine your variables: Which is your response (dependent) variable? Which is your predictor (independent) variable? Are the variables categorical and/or quantitative?
   D. Describe the control and experimental groups for your experiment:
   E. Describe the type of relationship between the variables (positive, negative, or neutral).
   F. Develop a hypothesis.
   G. What is your prediction as to the outcome of your experiment.

B. Data Collection

☐ Connect the Go!Link to the computer USB hub and the conductivity sensor to the Go!Link.

☐ Open the **Osmosis.cmbl** file found in the Documents > 2227L folder on the lab computers. The .cmbl file will be opened by the Logger Pro software.

- You may need to set the conductivity sensor range to 20,000 µS using the switch on the sensor.
- A window will open. Click on the 'Use Sensor Settings' button.

Important: Rinse and dry the beaker and sensor before and after use.

☐ Pour 100 mL of dechlorinated water into a 250-mL glass beaker.

☐ Lower the conductivity sensor into the solution until the top of the oblong opening containing the metal rod is covered.

☐ Potato (initial):
- The instructor will have six fresh half slices on a paper towel for you.
- Blot the potato slices dry using the paper towel.
- On the scale, push the blue lid back and place the washed and dry weigh boat on the scale.
- Zero the scale.
- Place all the potato slices in the weigh boat.
- Weigh all the slices together in grams (g).

☐ **Record the initial weight in Table UI-5.**

☐ The slices need to be vertically positioned around the sensor tip that is submerged in the solution. Let the sensor/potatoes sit in the solution for 30 seconds.

☐ Run:
- Set a timer for 18 minutes.
- Click the 'Collect' button.
- Swirl the solution every two minutes of the run. Keep the sensor in the solution but gently slide the beaker around on the ring stand or tray.

Table UI-5 The initial and final potato mass (g) and percent change (%Δ) when raw potato is placed in three solutions (water, 0.9% NaCl, and 10% NaCl) for 18 minutes. %Δ is calculated by subtracting the initial mass FROM the final mass, dividing by the initial mass, and then multiplying by 100.

Solution	Potato Mass (g)		%Δ Mass
	Initial	Final	
Dechlorinated water			
0.9% NaCl			
10% NaCl			

☐ **Record the initial and final dS/m at time 18 minutes in Table UII-6.**

☐ Move your data to a stored run. To do this, choose Store Latest Run from the Experiment menu. DO NOT close Logger Pro.

☐ Potato (final):
- Pour out the water from the beaker and dump the potato slices onto a paper towel.
- Blot the slices dry.
- On the scale, push the blue lid back and place the washed and dry weigh boat on the scale.
- Zero the scale.
- Place all the potato slices in the weigh boat.
- Weigh all the slices together in grams (g).

☐ **Record the final mass of the potato slices in Table UI-5.**

☐ Repeat the above steps using the 0.9% NaCl solution and fresh potato slices and then again with the 10% NaCl solution and another set of fresh potato slices.

☐ DO NOT close Logger Pro.

☐ Rate of osmosis (dS/m/minute):
- Go back to the open Logger Pro with all your stored runs.
- Select a run by clicking on the corresponding graph. Roll the mouse over the graph and place the cursor at the lower left portion of the graph.
- Left click the mouse and, while keeping the left button depressed, drag the shaded area up and right over the first 10 minutes of the run.
- Release the left mouse button.
- Click on the Linear Fit  button.

☐ **Record the value of the slope, m, in Table UI-6**

☐ Percent change (%Δ): %Δ=(f- i)/i X 100:
- Potato mass - subtract the initial mass FROM the final mass, divide by the initial mass, and then multiply by 100. **Record the value in Table UI-5.**
- Electrical conductivity (dS/m) - subtract the initial dS/m FROM the final dS/m, divide by the initial dS/m, and then multiply by 100**. Record the value in Table UI-6.**

☐ **Record in rate of osmosis, %Δ potato mass, and %Δ conductivity <u>in the Osmosis workbook on the instructor computer.</u>**

Table UI-6. The initial and final electrical conductivity (dS/m), percent change (%Δ) of electrical conductivity, and the rate of osmosis (dS/m/min) when raw potato is placed in three solutions (water, 0.9% NaCl, and 10% NaCl) for 18 minutes. %Δ is calculated by subtracting the initial dS/m FROM the final dS/m, dividing by the initial dS/m, and then multiplying by 100.

Solution	dS/m			Rate (dS/m/minute)
	Initial	Final	%Δ	
Dechlorinated water				
0.9% NaCl				
10% NaCl				

C. Interpretation

3. In Figure UI-4, label and color the listed subcellular structures:

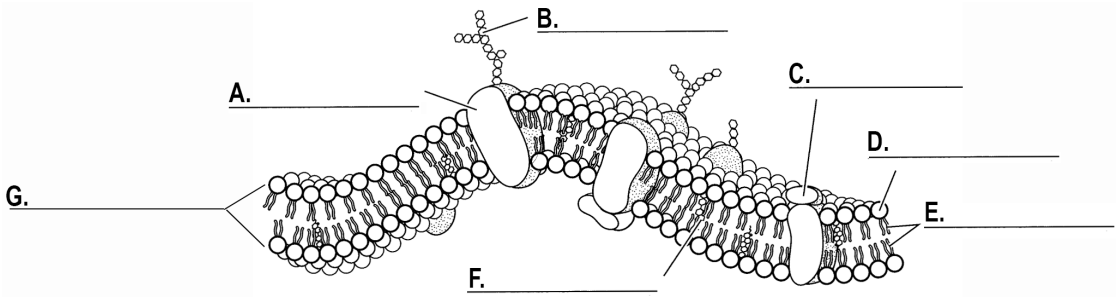
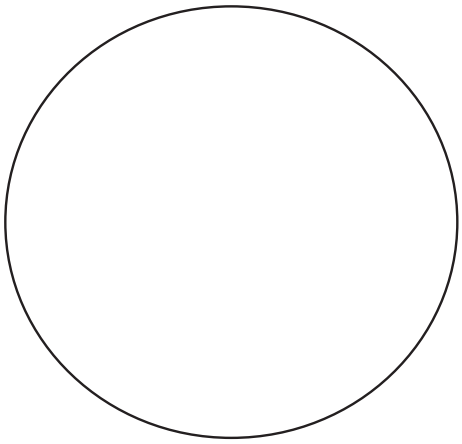


Figure UI-4. Plasma membrane diagram, subcellular structures; A. Integral protein (green), B. Carbohydrate chain (purple), C. Peripheral (dark blue), D. Phosphate molecule (brown), E. Lipid Layer, F. Cholesterol molecule (yellow), and G phospholipid bilayer.

4. View and sketch the potato tuber slide using the 40X objective lens. Label the following:
   A. Cell wall and starch grains.
   B. Specimen, Species, Domain, and Kingdom names.
   C. Total magnification.



☐ Your instructor will send your lab section's completed workbook to each student's ISU Google Account Gmail.

☐ Open the Word document from Section A.

5. Insert the table and graph into the document.
6. Create a table caption ABOVE the table.
7. Create a figure caption BELOW the figure.
8. Type the answers to the following questions into the Word document you created and saved in section A (Questions 1 & 2):
   H. Look at the 2-D column chart that you created showing mean %Δ potato mass:
   - Which potato/solution had the greatest positive change in mass? What caused the change?
   - Which potato/solution had the greatest negative change in mass? What caused the change?
   - Which solution showed the least change in mass? Why?

Specimen:_____

Species::_____

Domain:_____

Kingdom: _____

Total Magnification _____

I. Look at the 2-D column chart that you created showing mean %Δ solution conductivity:
- Which potato/solution had the greatest positive change in conductivity? What caused the change?
- Which potato/solution had the greatest negative change in conductivity? What caused the change?
- Which solution showed the least change in conductivity? Why?

J. We calculated rate of osmosis (dS/m/minute) by taking the slope of the lines in the graph generated by LoggerPro:
- What does rate of osmosis mean if slope is rate of change, what was changing?
- Which solution had a fairly flat slope. Was equilibrium achieved? What does that mean?
- Did one of the solutions have a negative slope? What does that mean?

K. After looking at the slide of a potato tuber section; describe how water and ions were moving with respect to the potato tuber cells and each solution.

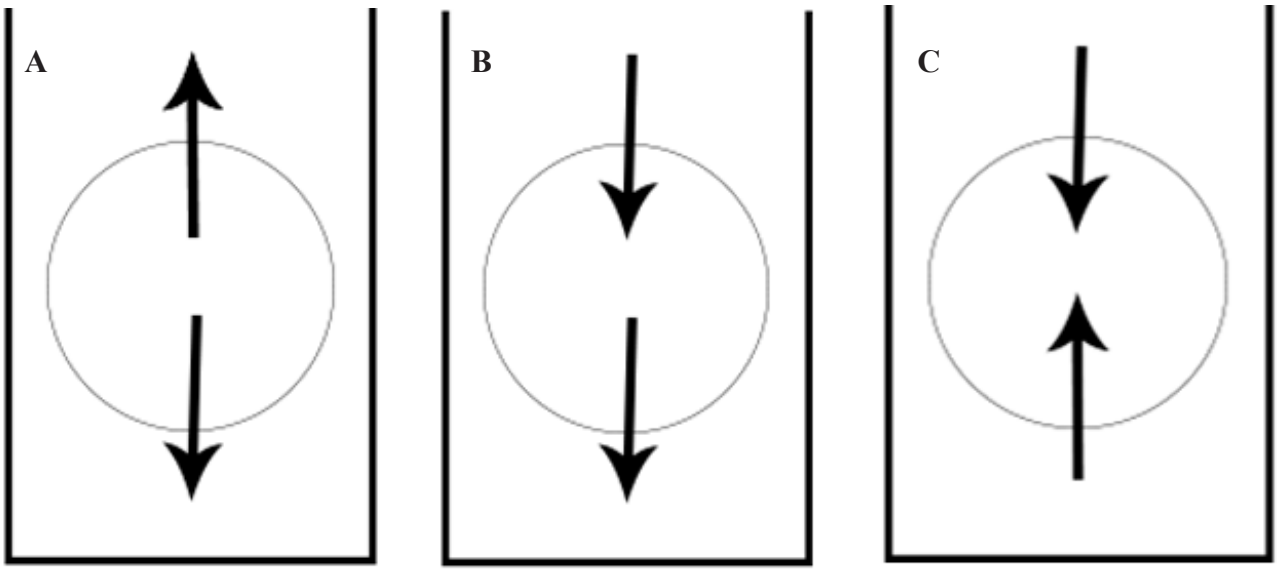
L. Did you reject or support your hypothesis**?** WHY?

M. Compare the raw data to the means and standard deviations that were calculated. Which data points could have caused the standard deviation to widen. What type of investigator error could have caused the standard deviation that you see?

N. Using your understanding of osmosis, diffusion, dialysis, and compare and contrast glomerular filtration with the movement of water and ions into and out of the potato cells.

☐ **Send the document to your lab instructor using your ISU Google Account Gmail.**

9. Fill in Figure UI-5 (represents a beaker with solution and the circle is potato) using the class %Δ potato mass data to:
A. Determine which diagram (A-C) represents the movement of water in each of your potato/solution experiments and fill in the figure and figure caption.
B. Determine the tonicity of the potato and solution relative to each other in each diagram.



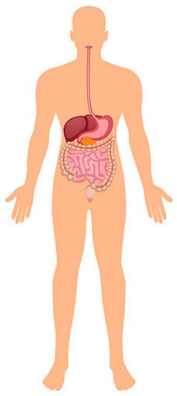
Solution type_____ Solution type_____ Solution type_____

Potato tonicity:_____ Potato tonicity:_____ Potato tonicity:_____

Solution tonicity:_____ Solution tonicity:_____ Solution tonicity:_____

Figure UII-5. Tonicity and water movement in a beaker containing a solution and potato slices. Three solutions types: ___) water, ___)0.9% NaCl, and ___)10% NaCl. Arrows depict the direction of water movement into and out of potato cells.

Unit II - Digestive System: Energy & Metabolism



Digestive System

Objectives

- Learn about the digestive system.
- Continue to practice the scientific method through experimental design, hypothesis construction, and descriptive statistics (mean and standard deviation).
- Learn about enzymes and describe their activity in cells.
- Observe and measure changes in gas pressure and CO_2 concentration during metabolism of sugars by yeast.
- Determine the rate of respiration and fermentation by yeast using different sugars.
- Create tables and figures with corresponding APA captions using Microsoft® Office Excel and Word.

Terms & Definitions

Activation energy - an initial input of energy is a chemical reaction that allows the molecules to get close enough to cause a rearrangement of bonds

Aerobic processes - refers to a process that occurs in the presence of oxygen; a form of metabolism that does requires oxygen.

Anaerobic processes - refers to a process that occurs in the absence of oxygen; a form of metabolism that does not require oxygen.

Adenosine triphosphate (ATP) - a common energy source for all cells; it converts either to adenosine diphosphate (ADP) or to adenosine monophosphate (AMP).

Catalyst - a substance that lowers the activation energy of a chemical reaction, thereby speeding up the reaction:

A. Active site - the location in an enzyme where a chemical reaction takes place
B. Amylase - a digestive enzyme in saliva and the pancreas involved in the digestion of starch.
C. Coenzyme - substances such as vitamins that help enzymes catalyze chemical reactions.
D. Enzymes - macromolecular biological catalysts that are usually proteins that accelerate chemical reactions. Enzymes are biological catalysts and catalyze the rate of a chemical reaction.
E. Induced fit - a change in shape of the active site of an enzyme so that it binds tightly to a substrate.
F. Isomerase - a general class of enzymes that convert a molecule from one isomer to another such as fructose to glucose.
G. Lactase - a family of enzymes involved in the hydrolysis of the disaccharide lactose into constituent galactose and glucose monomers. It is located in the brush border of the small intestine of humans and other mammals.
H. Maltase - an enzyme that catalyses the hydrolysis of the disaccharide maltose to glucose monomers.
I. Sucrase/invertase - an enzyme that catalyzes the hydrolysis (breakdown) of sucrose (table sugar) into fructose and glucose monomers.
J. Substrate - the substance upon which an enzyme reacts.
K. Specificity - phenomenon of enzyme shape determining the reaction the enzyme catalyzes.

Cellular respiration - a process by which living cells obtain energy from organic molecules and release waste products.

Cellulose - a structural polysaccharide found in cell walls and composed of glucose molecules.

Chitin - a tough, nitrogen-containing polysaccharide that forms the external skeleton of many insects and the cell walls of fungi.

Citric acid cycle (KREBS) - a cycle that results in the breakdown of carbohydrate to co2; also known as the krebs cycle.

Digestion - the process of breaking down food by mechanical and enzymatic action in the gastrointestinal tract into substances that can be used by the body.

Digestive system – the system that take in food and liquids, breaks them down into substances that the body can use for energy, growth, and tissue repair, and remove waste products. The system is made up of the gastrointestinal tract (GI tract), liver, pancreas, and gallbladder:

1. Gallbladder – a small, pear-shaped organ on the right side of the abdomen, just beneath the liver storing bile produced by the liver.
   A. Cystic Duct – a tube that carries bile from the gall bladder.
2. GI tract – a series of hollow organs joined in a long, twisting tube from the mouth to the anus.
   A. Pharynx – a portion of the vertebrate alimentary canal; also known as the throat.
   B. Esophagus - the part of the gastrointestinal tract that connects the mouth to the stomach; the gullet. In humans and other vertebrates it is a muscular tube lined with mucous membrane.
   C. Intestine:
      i. Large (cecum, colon, rectum and anus) – portion of the GI tract most responsible for absorption of water from the indigestible residue of food.
      ii. Small – most of the end absorption of nutrients and minerals from food takes place.
         a. Duodenum - the first part of the small intestine immediately beyond the stomach, leading to the jejunum.
         b. Jejunum – the part of the small intestine between the duodenum and ileum.
         c. Ileum – the third portion of the small intestine, between the jejunum and the cecum.
   D. Stomach – a muscular, hollow organ in the gastrointestinal tract of humans and many other animals, including several invertebrates.
      i. Chief Cells – a type of gastric gland cell that releases pepsinogen and gastric lipase.
      ii. Circular fold – large valvular flaps projecting into the lumen of the small intestine.
      iii. Fundus – the upper part of the stomach, which forms a bulge higher than the opening of the esophagus (farthest from the pylorus).
      iv. Gastric Gland – any of the branched tubules in the inner lining of the stomach that secrete gastric juice and protective mucus
      v. Gastric Pits - indentations in the stomach which denote entrances to the tubular shaped gastric glands.
      vi. Pylorus – connects the stomach to the duodenum.

vi. Pylorus Sphincter – a band of muscle that marks the junction between the stomach and the duodenum.
E. Rectum – the final straight portion of the large intestine in humans and some other mammals, and the gut in others.
F. Anus – the final portion of the alimentary canal through which solid wasters are expelled.
3. Liver – accessory digestive organ used to produce bile.
4. Pancreas – an organ of the digestive system and endocrine system of vertebrates; it is a mixed or heterocrine gland, i.e. it has both an endocrine and a digestive exocrine function
A. Pancreatic Islets – the regions of the pancreas that contain its endocrine cells.
5. Villi – fingerlike or threadlike projections from the surface of certain membranous structures, typically serving to increase surface area and facilitate the passage of fluid or nutrients.
A. Microvilli – microscopic cellular membrane protrusions of simple cuboidal and simple columnar epithelium that increase the surface area for diffusion and minimize any increase in volume, and are involved in a wide variety of functions, including absorption, secretion, and cellular adhesion.
B. Brush-border membrane – the microvilli of the enterocytes that line the intestinal villi

Electron Transport Chain - a group of protein complexes and small organic molecules within the inner membranes of mitochondria and chloroplasts and the plasma membrane of prokaryotes. the components accept and donate electrons to each other in a linear manner and produce a H+ electrochemical gradient.

Energy - the capacity of a body to do work.

Enteroendocrine cells - specialized cells found within the gastrointestinal tract, stomach and pancreas. They produce and release hormones in response to a number of stimuli.

Enterocytes - a cell of the intestinal lining.

Epiglottis - a flap of cartilage at the root of the tongue, which is depressed during swallowing to cover the opening of the windpipe.

Disaccharide - a carbohydrate composed of two monosaccharides:
A. Lactose - β-D galactose + β-D glucose “milk sugar” (lactase)
B. Maltose - α-D-glucose + α-D-glucose “malt sugar. (maltase)
C. Sucrose - glucose + fructose; “table sugar” (sucrase/invertase)

Fermentation - the breakdown of organic molecules to produce energy without any net oxidation.

Fermentation (Lactic Acid) - a metabolic process by which glucose or other six-carbon sugars (also, disaccharides of six-carbon sugars, e.g. sucrose or lactose) are converted into cellular energy and the metabolite lactate, which is lactic acid in solution.

Flavin adenine dinucleotide (FAD) - a redox-active coenzyme associated with various proteins, which is involved with several important enzymatic reactions in metabolism. Fad can exist in four different redox states, which are the flavin-n(5)-oxide, quinone, semiquinone, and hydroquinone. Fad is converted between these states by accepting or donating electrons. FAD, in its fully oxidized form, or quinone form, accepts two electrons and two protons to become FADH2 (hydroquinone form). The semiquinone (FADH·) can be formed by either reduction of fad or oxidation of FADH2 by accepting or donating one electron and one proton, respectively.

Falciform ligament - is the thin, sickle-shaped, fibrous structure that connects the anterior part of the liver to the ventral wall of the abdomen.

Fungi - a eukaryotic kingdom of the domain eukarya that is composed of heterotrophic unicellular, multicellular, or syncytial spore-producing organisms, including molds, yeast, mushrooms, and toadstools.

Glands - a group of cells in an animal’s body that synthesizes substances for release into the bloodstream (endocrine) or into cavities inside the body or its outer surface (exocrine):
A. Endocrine gland - a gland that performs communication within the body using hormones; relating to or denoting glands which secrete hormones or other products directly into the blood.
B. Exocrine gland (holocrine, merocrine, or apocrine) - A gland that makes substances such as sweat, tears, saliva, milk, and digestive juices, and releases them through a duct or opening to a body surface.
C. Intestinal Glands - imaginations of the intestinal mucosa that secrete intestinal juice. (AKA crypts).
D. Mammary gland - an exocrine gland in humans and other mammals that produces milk to feed young offspring.
E. Salivary Glands - exocrine glands that produce saliva through a system of ducts. Humans have three paired major salivary glands (parotid, submandibular, and sublingual), as well as hundreds of minor salivary glands.
F. Sweat Gland - small tubular structures of the skin that produce sweat. Sweat glands are a type of exocrine gland, which are glands that produce and secrete substances onto an epithelial surface by way of a duct.

Glycogen - a polysaccharide found in animals cells (animals starch) and fungus.

Glycolysis - a metabolic pathway that breaks down glucose to pyruvate.

Glycoprotein - compound containing carbohydrate (or glycan) covalently linked to protein.

Glucagon - a hormone formed in the pancreas which promotes the breakdown of glycogen to glucose in the liver.

Goblet Cells - a column-shaped cell found in the respiratory and intestinal tracts, which secretes the main component of mucus.

Lamina Propria - a type of connective tissue found under the thin layer of tissues covering a mucous membrane.

Mitochondrial Matrix - the space within the inner membrane; contains the mitochondria’s DNA, ribosomes, soluble enzymes, small organic molecules, nucleotide cofactors, and inorganic ions. The enzymes in the matrix facilitate reactions responsible for the production of ATP, such as the citric acid cycle, oxidative phosphorylation, oxidation of pyruvate, and the beta oxidation of fatty acids.

Monosaccharide - a simple sugar:
A. Fructose - hexose monosaccharide “fruit sugar” (isomerase to convert to glucose)
B. Galactose - hexose monosaccharide (five different types of enzymes)
C. Glucose - hexose monosaccharide is found in all living cells and is often referred to as “blood sugar”Mucosa

Mesenteric Capillaries - take blood from the aorta and distribute it to a large portion of the gastrointestinal tract. Mucosa - a membrane that lines various cavities in the body and covers the surface of internal organs. It consists of one or more layers of epithelial cells overlying a layer of loose connective tissue.

Mucous Membranes - is a membrane that lines various cavities in the body and covers the surface of internal organs

Muscularis Mucosa - is a thin layer (lamina) of muscle of the gastrointestinal tract, located outside the lamina propria, and separating it from the submucosa.

Nutrients - substances that provide nourishment.

Nutrient cycling - the process by which decomposers break down dead organisms or waste products, release the chemical elements locked in the biological material, and return them to the environment.

Nicotinamide adenine dinucleotide phosphate (NADP+_) - a cofactor used in anabolic reactions, such as the calvin cycle and lipid and nucleic acid syntheses, which require NADPH as a reducing agent. It is used by all forms of cellular life. NADPH is the reduced form of NADP+. NADP+ differs from NAD+ in the presence of an additional phosphate group on the 2' position of the ribose ring that carries the adenine moiety.

Oxidation - a process that involves the removal of electrons; occurs during the breakdown of small organic molecules.

Oxidative phosphorylation - a process during which NADH and FADH2 are oxidized to make more ATP via the phosphorylation of ADP.

Parietal Cells - are epithelial cells in the stomach that secrete hydrochloric acid (HCl) and intrinsic factor.

Peristalsis - the rhythmic, spontaneous waves of muscle contractions that propel food through the digestive system

Pyruvate - the simplest of the alpha-keto acids, with a carboxylic acid and a ketone functional group; an intermediate in several metabolic pathways throughout the cell.

Ribose - the five-carbon sugar in RNA.

Rugae - a series of ridges produced by folding of the wall of an organ. Most commonly rugae refers to the gastric rugae of the internal surface of the stomach.

Simple Columnar Epithelium - a single layer of columnar epithelial cells which are tall and slender with oval-shaped nuclei located in the basal region, attached to the basement membrane. In humans, simple columnar epithelium lines most organs of the digestive tract including the stomach, and intestines. Simple columnar epithelium also lines the uterus.

Serosa - a smooth tissue membrane of mesothelium lining the contents and inside wall of body cavities, which secrete serous fluid to allow lubricated sliding movements between opposing surfaces.

Starch - a polysaccharide composed of repeating glucose units that is produces by the cells of plants and some algal protists.

Stratified Squamous Epithelium - squamous (flattened) epithelial cells arranged in layers upon a basal membrane.

Submucosa - a thin layer of tissue in various organs of the gastrointestinal, respiratory, and genitourinary tracts.

Spectrum - used to classify something, or suggest that it can be classified, in terms of its position on a scale between two extreme or opposite points:
A. Absorption spectrum - a diagram that depicts the wavelengths of electromagnetic radiation that are absorbed by a pigment.
B. Action spectrum - the rate of photosynthesis plotted as a function of different wavelengths of lights.
C. Electromagnetic spectrum - all possible wavelengths of electromagnetic radiation, from relatively short wavelengths to much longer wavelengths.

Teeth - a hard, calcified structure found in the jaws (or mouths) of many vertebrates and used to break down food.

Yeast - a fungus that can occur as a single cell and that reproduces by budding.

Tongue - a muscular organ in the mouth of a typical vertebrate. It manipulates food for mastication and swallowing as part of the digestive process, and is the primary organ of taste.

Trachea - a cartilaginous tube that connects the larynx to the bronchi of the lungs, allowing the passage of air, and so is present in almost all air-breathing animals with lungs.

Background

Digestive System

The digestive system is made up of the gastrointestinal tract (9 meters long) and the liver, pancreas, and gallbladder. The wall of the gastrointestinal tract has four layers; 1) Mucosa, 2) Submucosa, 3) Muscular layer, and 4) serosa. The mucosa is the innermost layer of the wall. It lines the lumen of the gastrointestinal tract. The mucosa consists of simple columnar epithelial tissue, an underlying loose connective tissue layer called lamina propria, and a thin layer of smooth muscle called the muscularis mucosa. In the mouth and anus, where thickness for protection against abrasion is needed, the epithelium is stratified squamous tissue. The stomach and intestines have a thin simple columnar epithelial tissue layer for secretion and absorption. This tissue in the intestines is made up of enterocytes (absorptive cells) and are coated with digestive enzymes. Villi on the apical surface of the small intestine increase its surface area. This facilitates transport of numerous small molecules into the enterocyte from the intestinal lumen. These include broken down proteins, fats, and sugars, as well as water, electrolytes, vitamins, and bile salts. Enterocytes also have an endocrine role, secreting hormones such as leptin. The submucosa is a thick layer of loose connective tissue that surrounds the mucosa. This layer also contains blood vessels, lymphatic vessels, nerves, and embedded glands. The smooth muscle responsible for movements of the digestive tract is arranged in two layers, an inner circular layer and an outer longitudinal layer with the myenteric plexus is between. Above the diaphragm, the outermost layer of the digestive tract is a connective tissue called adventitia. Below the diaphragm, it is called serosa.

Food (macromolecules, minerals, vitamins, and fluids) enter the body through the digestive system. Macromolecules (lipids, proteins, complex carbohydrates, nucleic acids) are mechanically and enzymatically broken down into small and absorbable units principally in the small intestine and then cross the mucosa and enter the lymph or the blood. Digestion allows molecules to traverse the intestinal epithelium and enter the bloodstream for use in the body and is a form of catabolism. Mechanical digestion involves physically breaking down food substances into smaller particles to more efficiently undergo chemical digestion. The role of chemical digestion is to further degrade the molecular structure of the ingested compounds by digestive enzymes into a form that is absorbable into the bloodstream. Digestion of the major macromolecule is an orderly process involving the action of a large number

of enzymes. Enzymes from the salivary and the lingual glands digest carbohydrates and fats, enzymes from the stomach digest proteins, and enzymes from the exocrine glands of the pancreas digest carbohydrates, proteins, lipids, RNA, and DNA. Other enzymes that help in the digestive process are found in the luminal membranes and the cytoplasm of the cells that lines the small intestine. The action of the enzymes is promoted by the hydrochloric acid (HCl), which is secreted by the stomach, and bile from the liver.

Energy intake depends on the amount of nutrients absorbed by the brush-border membrane (BBM) of small intestinal epithelial cells. Complex carbohydrates reaching the small intestine must first be hydrolyzed to monosaccharides such as glucose or galactose in order to be absorbed across the BBM. The BBM not only absorbs the monosaccharides but is also secretes the enzymes lactase, sucrase, and maltase that breakdown complex carbohydrates into monosaccharides. Microvilli are the microscopic cellular membrane protrusions of an enterocyte that increases the surface area (but not volume) for this required absorption and secretion. All the microvilli of the enterocytes that line the intestinal villi create the BBM. The absorption of glucose at the interface of the BBM and the intestinal lumen uses sodium-dependent glucose cotransporters (plasma membrane proteins) that facilitate the transport of glucose across the plasma membrane, a process known as facilitated diffusion. Once the monosaccharides enter the enterocytes they are exported from the cells into the mesenteric capillaries and then transported; 1) to the liver where they are processed and stored as glycogen or 2) through the rest of the bloodstream where they are used for cellular respiration.

Name:_____ Group #:_____ Section #:_____

Part 1. Enzymes

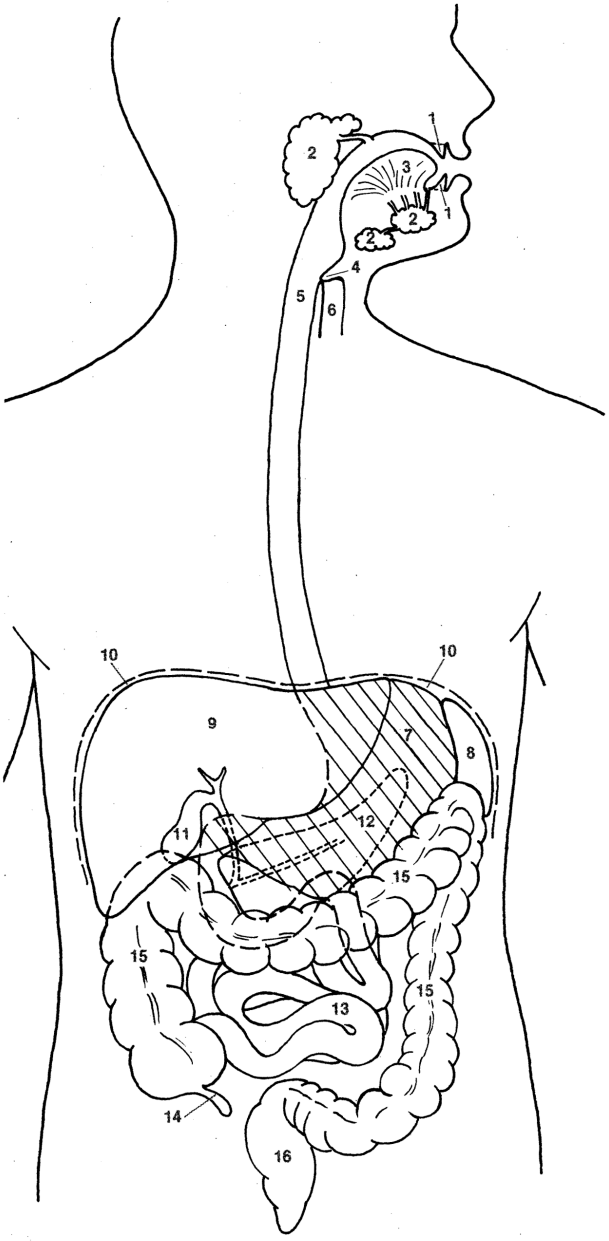
Living organisms perform a multitude of chemical reactions very rapidly because of the participation of enzymes. Enzymes are biological catalysts, compounds that speed up chemical reactions without being used up or altered in the reaction. The material with which the catalyst reacts, the substrate, is modified during the reaction to form a new product. Because the enzyme itself emerges from the reaction unchanged, a small amount of enzyme can alter a relatively large amount of substrate. The active site of an enzyme will bind with the substrate, forming an enzyme-substrate complex. It is here that the reaction takes place, and when it is complete, the complex dissociates into the enzyme and a product or products. Enzymes are, in part or in whole, proteins that are highly specific in function. Because enzymes lower the energy of activation needed for reactions to take place, they accelerate the rates of reactions. They do not, however, determine the direction in which a reaction will go or its final equilibrium. Enzyme activity is affected by many factors. Varying environmental conditions such as temperature and pH may alter the three-dimensional shape of an enzyme, thereby affecting its rate of activity. Similarly, the amount of enzyme relative to the amount of substrate can have an affect on the rate of enzyme activity.

Sugars are a vital source of food for all living organisms but much be broken down in order to be used. Glycoside hydrolase is a class of enzyme involved in the hydrolysis of the glycosidic linkage/bond of a disaccharide. A hydrolysis reaction is a chemical reaction that utilizes water to break apart molecules. This class of enzyme includes sucrase, maltase, and lactase. The disaccharides sucrose, maltose, and lactose have glycosidic bonds that under go a hydrolysis reaction with the aid of their respective hydrolase enzyme and water.

Saccharomyces cerevisiae (Domain Eukarya, Kingdom Fungi) is the species name of the yeast used as a leavening agent in baking bread where it converts the fermentable sugars present in the dough into carbon dioxide and ethanol. Brewer's yeast is another strain of *S. cerevisiae* commonly used in alcoholic fermentation (beer and wine). It is a single-cell microorganism found on and around the human body and was domesticated by humans long ago. Yeast cells are unable utilize all of the sugars equally well but they do synthesize a range of enzymes with some more effective than others. Yeast metabolizes sugars aerobically (in the presence of oxygen) the process is known as respiration; when yeast metabolizes sugars anaerobically (in the absence of oxygen) the process is known as fermentation. *Saccharomyces cerevisiae* is glucophilic, meaning glucose will be used at a faster rate than fructose. For example, sucrose is a disaccharide and yeast cells use the **enzyme sucrase/invertase** to break it into glucose and fructose. Finally, it uses the glucose in respiration or fermentation. Depending on the strain of *S. cerevisiae* or the conditions of yeast incubation, the yeast may not produce enough maltase enzyme to split maltose in the time frame we are running the experiments. We generally see (but not always), (**1 being the sugar that yeast utilize at the greatest rate**):

1. Sucrose - disaccharide = glucose + fructose; "table sugar." (sucrase/invertase)
2. Glucose - hexose monosaccharide; found in all living cells; often referred to as "blood sugar."
3. Maltose - disaccharide = α-D-glucose + α-D-glucose; "malt sugar. (maltase)
4. Fructose - hexose monosaccharide; "fruit sugar."
5. Galactose - hexose monosaccharide; (five different types of enzymes)
6. Lactose - disaccharide = β-D galactose + β-D glucose; (lactase)

1. Figures UII-1 color the structures accordingly.

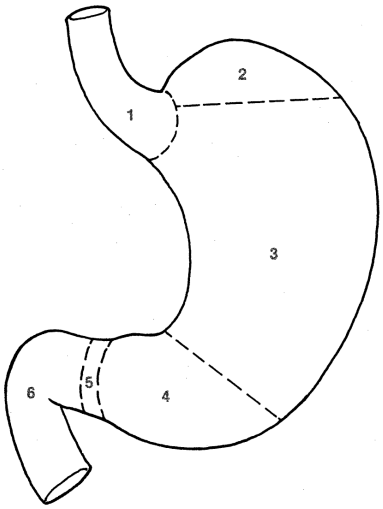


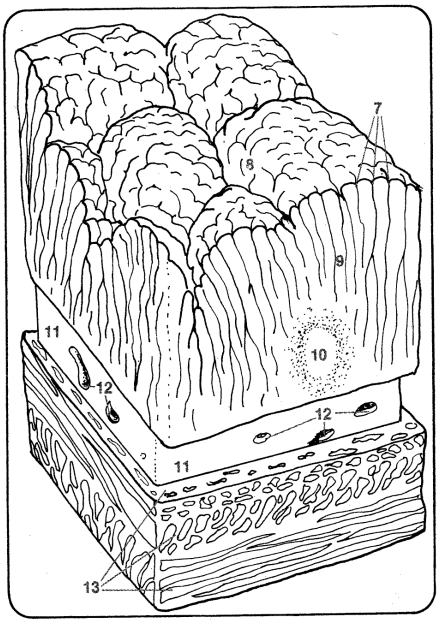
1. Teeth (yellow)
2. Salivary glands (turquoise)
3. Tongue (pink)
4. Epiglottis
5. Esophagus (yellow-green)
6. Trachea (blue)
7. Stomach (green)
8. Spleen (purple)
9. Liver (light brown)
10. Diaphragm (red)
11. Gallbladder (gray)
12. Pancreas (light green)
13. Small intestine (light blue)
14. Appendix (orange)
15. Large intestine (dark blue)
16. Rectum (brown)

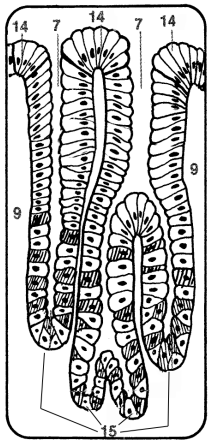
Figure UII-1. Digestive system.

2. Figure UII-2,
   a. label diagrams A-D
   b. color the structures accordingly
   c. label and color the muscularis mucosa (orange) and serosa (gray) on diagram A

A _____

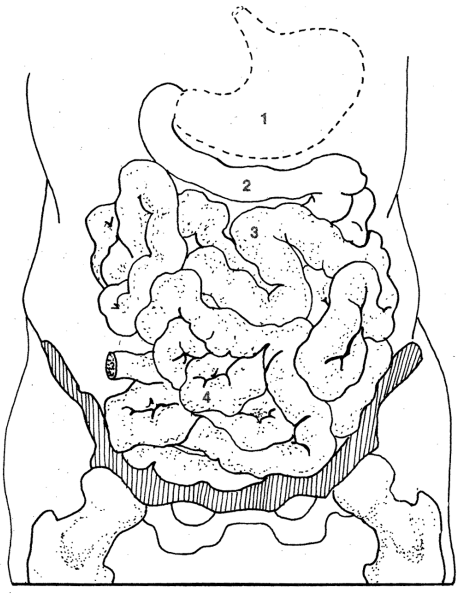


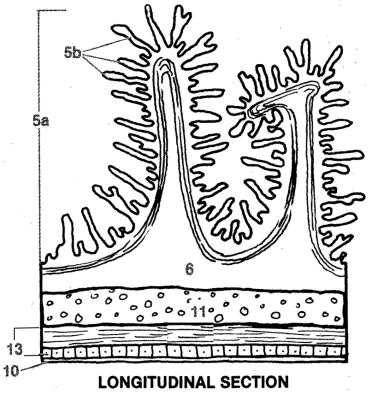




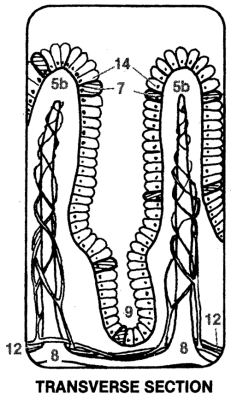
1. Esophagus (yellow-green)
2. Fundus (light green)
3. Body (green)
4. Pylorus (light blue)
5. Pylorus sphincter (blue)
6. Duodenum (dark blue)
7. Gastric pits leading to the gastric glands (yellow)
8. Rugae (green)
9. Lamina propria (purple)
10. Lymph nodule (brown)
11. Submucosa (pink)
12. Blood vessels (red)
13. Smooth muscle; inner circular layer and outer longitudinal layer with myenteric plexus between (light purple)
14. Surface mucous cells (light brown)
15. Gastric Gland composed of:
    a. Mucous cells
    b. Parietal cells
    c. Chief cells
    d. Enteroendocrine cells

B _____

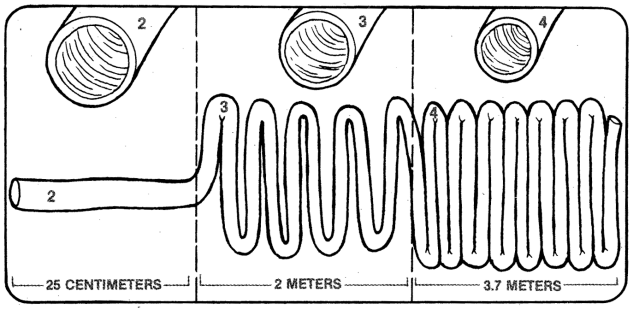


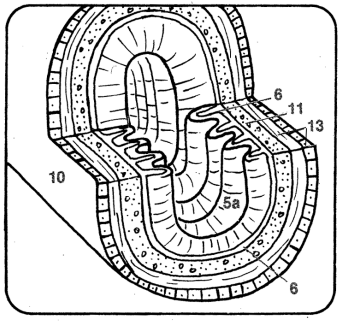


LONGITUDINAL SECTION



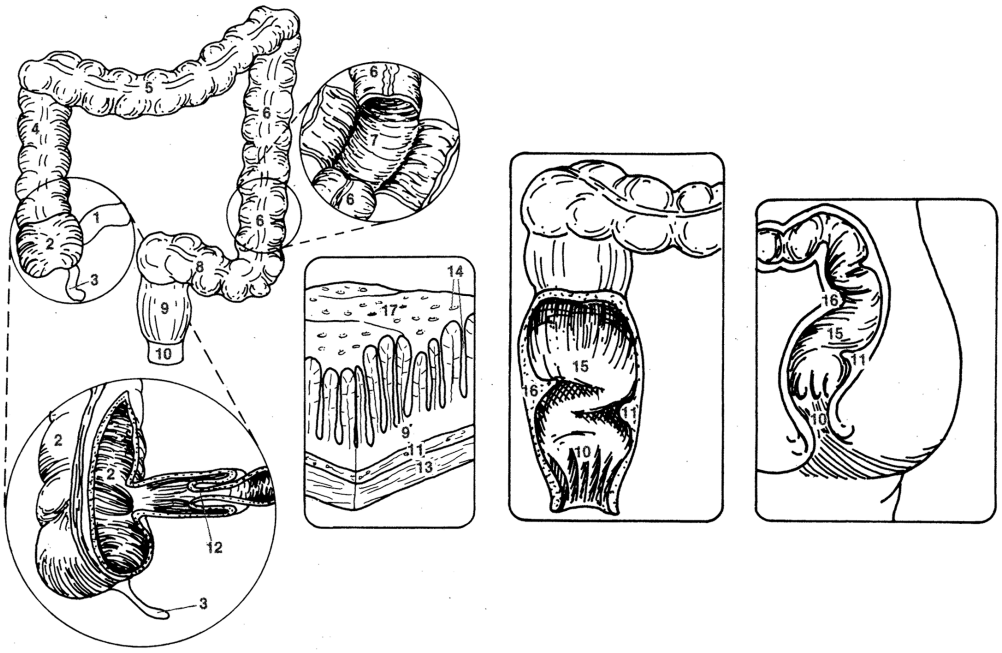
TRANSVERSE SECTION





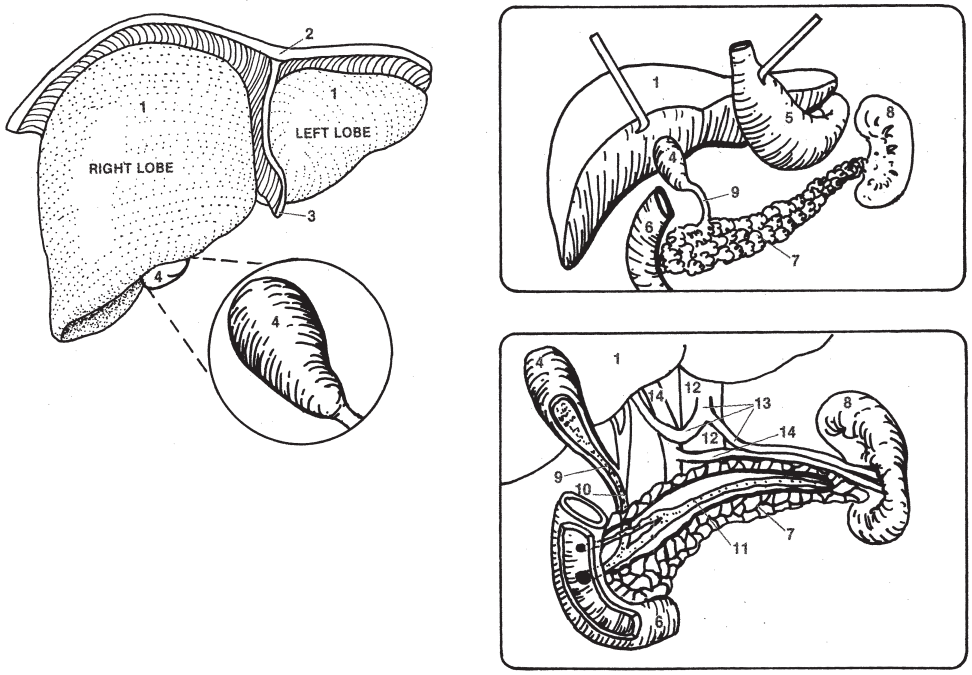
1. Stomach (green)
2. Duodenum (dark blue)
3. Jejunum (light blue)
4. ileum (blue)
5. a. Circular fold with b. villi (light green)
6. Muscularis mucosa (orange)
7. Goblet cells
8. Lymphatic vessel (brown)
9. Intestinal glands (yellow)
10. Serosa (gray)
11. Submucosa (pink)
12. Blood vessels (red)
13. Smooth muscle; inner circular layer and outer longitudinal layer with myenteric plexus between (light purple)
14. Enterocytes (light brown)

C _____



1. ileum (blue)
2. Cecum (red)
3. Appendix (orange)
4. Ascending colon (yellow-green)
5. Transverse colon (light green)
6. Descending colon (green)
7. Interior of descending colon (light blue)
8. Sigmoid colon (turquoise)
9. Lamina propria (purple)
10. Anal canal and anus (light brown)
11. Submucosa (pink)
12. ileocecal valve (dark blue)
13. Smooth muscle; inner circular layer and outer longitudinal layer with myenteric plexus between (light purple)
14. Intestinal glands (yellow)
15. Rectum (brown)
16. Rectal transverse folds
17. Stratified squamous epithelial tissue (light brown)

D _____



1. Liver (brown)
2. Diaphragm (light purple)
3. Falciform ligament
4. Gallbladder (purple)
5. Stomach (green)
6. Duodenum (dark blue)
7. Pancreas (light green)
8. Spleen (blue)
9. Cystic duct (light orange)
10. Common bile duct (yellow)
11. Pancreatic duct (yellow-green)
12. Aorta (red)
13. Celiac, splenic, and hepatic arteries (pink)
14. splenic and hepatic portal veins (light blue)
15. Appendix (orange)

Figure UII-2. Regions of the digestive system with increasing levels of magnification; A) stomach, B) small intestine, C) large intestine, and D) accessory organs.

Part 2. Respiration

The following equation shows that in the presence of oxygen, glucose is converted by yeast through the process of cellular respiration to water, carbon dioxide and energy:

$$C_6H_{12}O_6 + 6\ O_2 \rightarrow 6\ H_2O + 6\ CO_2 + \text{energy}$$

During this process, oxygen gas (O_2) is consumed at the same rate that carbon dioxide (CO_2) is produced. The rate of cellular respiration in yeast can be determined by measuring either the amount of O_2 consumed or the amount of CO_2 produced in a specified period of time.

A. Experimental Design

☐ Write you Team number on the top of the first page next to your name.

☐ Observe the Pre-Lab video to find the question, hypothesis, and prediction.

☐ Open Word document, answer question 1 and 2, and then save the document.

3. At the top right of the document type:
   - Biol 2227L
   - Unit II - Digestive System (Respiration)
   - Your section #
   - Your team #
   - Names of everyone on your team
4. Type the answers to the following questions into the Word document.

A. Background: What is the species name of your model organism? To which domain and kingdom does it belong? Yeast cell walls are made of what type of organic compound? What type of macromolecule is the organic compound?
B. Question?
C. Determine your variables: Which is your response (dependent) variable? Which is your predictor (independent) variable? Are the variables categorical and/or quantitative?
D. Describe the control and experimental groups for your experiment:
E. Describe the type of relationship between the variables (positive, negative, or neutral).
F. Develop a hypothesis.
G. What is your prediction as to the outcome of your experiment.

B. Data Collection

You will work in a team of two or three students, depending on the number of people in your lab section. Part of your team should prepare your computer for collecting data while others on your team prepare the glassware and reagents for conducting your experiment. Everyone should read the entire procedure before beginning.

☐ Connect the Go!Link to the computer USB hub and the CO_2 sensor (Figure UII-1) to the Go!Link.

☐ Open the **Respiration.cmbl** file found in the Documents > 2227L folders on the lab computers. The .cmbl file will be opened by the Logger Pro software.

☐ Remove the CO_2 sensor from the reaction chamber and calibrate it following your instructor's direction; it should fluctuate between 300 and 400 ppm. Make sure the switch on the sensor is set to LOW 0 - 10,000ppm.

Figure UII-3. CO_2 sensor. Keep the sensor upright at all times.

☐ Label four separate tubes with the control solution names and the test solution names, then fill each tube with 4 mL of the corresponding solution:
- Control solutions - Water (tube 1) & Sucrose (tube 2)
- Test solutions - Lactose (tube 3) & Lactose with enzyme lactase (tube 4)

☐ Water bath (38-42°C):
- Place the large beaker at the base of the ring stand.
- Fill ½ up with water from the electric hot water bath.
- Attach the 250-mL reaction chamber to the ring-stand clamp.
- Lower the chamber into the water with the bottom half submerged in the beaker water.
- Continuously monitor and maintain the bath temperature at 38-40°C; using the Styrofoam cups and basters, move the water between the beaker and the electric hot water bath. Cooler water from the beaker can be added back to the electric hot water bath.

Incubation= I Before each run you need to maintain a 38-42°C water bath to incubate your tubes for 10 minutes.

Run= R During each run, as you collect data, you need to maintain a 38-42°C water bath to incubate your tubes.

NA= no incubation or run at this time.

	Time in minutes																																
Solution	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35
Water	I	I	I	I	I	I	I	I	I	I	R	R	R	R	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Sucrose	NA	NA	NA	NA	NA	NA	NA	I	I	I	I	I	I	I	I	I	I	R	R	R	R	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Lactose	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	I	I	I	I	I	I	I	I	I	I	R	R	R	R	NA	NA	NA	NA	NA	NA	NA
Lactose w/ lactase	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	I	I	I	I	I	I	I	I	I	I	R	R	R	R

Figure UII-4. Respiration incubation and run schedule.

Important: Start the incubation for the second tube AFTER the first seven (7) minutes of the first tube's pre-incubation. See Figure UII-4.

Table UII-1. Rate of respiration (ppm/min) by yeast, as determined from the slope of the best-fit linear regression line of the plot of CO_2 gas versus time.

Solution (tube)	°C	Respiration rate
Water (1)		
Sucrose (2)		
Lactose (3)		
Lactose w/ enzyme (4)		

☐ Pre-incubation (See Figure UII-4):
- Gently swirl the yeast suspension flask to resuspend the cells.
- Transfer 4 mL of the yeast suspension into the tube and gently mix.
- Transfer the solution from the tube into the reaction chamber and incubate for 10 minutes.
- Continuously monitor and maintain the bath temperature at 38-42°C.

☐ Run (See Figure UII-4):
- At the 10 minute pre-incubation time, place the CO_2 gas sensor shaft into the opening of the reaction chamber.
- Begin measuring CO_2 concentration for four (4) minutes by clicking the collect button.
- Continuously monitor and maintain the bath temperature at 38-42°C.
- DO NOT click on the Stop button. The software will stop collection on its own.
- **Store Latest Run from the Experiment menu. DO NOT close Logger Pro.**

☐ Repeat the pre-incubations and runs for the other three tubes.

☐ Disconnect the CO_2 sensor from the Go!Link and set it upright in a dry beaker.

☐ Rinse and shake dry the respiration chamber.

☐ Click on the Linear Fit button. Note: you can use the buttons to view the slope region of interest.

☐ **Record the value of the slope, m, for each of the equations that describe the straight-line relationships, y = mx + b, in Table UII-1 AND to the Respiration workbook on the instructor computer.**

☐ Cleaning the tubes:
- Scrub the tubes with water and dish detergent.
- Rub off the marker label.
- Invert the tubes on the wooden tube rack.

C. Interpretation

☐ Your instructor will send your lab section's completed workbook to each student's ISU Google Account Gmail.

☐ Open the Word document from Section A.

5. Insert the table and graph into the document.
6. Create a table caption ABOVE the table.
7. Create a figure caption BELOW the figure.
8. Type the answers to the following questions into the document:
   H. Which solution was the positive control and which was the negative control?
   I. Describe the patterns shown by your graphs and discuss what the patterns are indicating.
   J. Do yeast utilize all lactose and sucrose for respiration equally? Why does this difference between the sugars exist?
   K. Did you reject or support your hypothesis? Explain in DETAIL why it was supported or rejected?
   L. Using the following terms (active site, substrate, activation energy, enzyme, disaccharide, monosaccharide) describe how lactose is modified by yeast with and without lactase.
   M. Humans have lactose intolerance. Do you think the intolerance is based on a similar mechanism? Why?
   N. Compare the raw data to the means and standard deviations that were calculated. Which data points could have caused the standard deviation to widen. What type of investigator error could have caused the standard deviation that you see?

☐ **Send the document to your lab instructor using your ISU Google Account Gmail.**

Name:_____ Group #:_____ Section #:_____

Part 3. Fermentation

During the process of fermentation, which occurs when oxygen (O_2) is not available, yeast converts glucose to ethanol, carbon dioxide (CO_2) and energy:

$$C_6H_{12}O_6 \rightarrow 2\ CH_3CH_2OH + 2\ CO_2 + \text{energy}$$

CO_2 is still produced, but ethanol is produced instead of water. Ethanol is an undesirable waste product from the perspective of yeast because it can eventually reach toxic concentrations. By measuring the change in pressure in a vessel in which it is possible to determine whether yeast is using a particular sugar as a source of food.

A. Experimental Design

☐ Observe the Pre-Lab video to find the question, hypothesis, and prediction.
☐ Open Word document, answer question 1 and 2, and then save the document.

1. At the top right of the document type:
   - Biol 2227L
   - Unit II - Digestive System (Fermentation)
   - Your section #
   - Your team #
   - Names of everyone on your team
2. Type the answers to the following questions into the Word document.
   A. Background: What is the species name of your model organism? To which domain and kingdom does it belong? Yeast cell walls are made of what type of organic compound? What type of macromolecule is the organic compound?
   B. Question: What is one question about fermentation and sugar type?
   C. Determine your variables: Which is your response (dependent) variable? Which is your predictor (independent) variable? Are the variables categorical and/or quantitative?
   D. Describe the control and experimental groups for your experiment.
   E. Describe the type of relationship between the variables (positive, negative, or neutral).
   F. Develop a hypothesis.
   G. What is your prediction as to the outcome of your experiment.

B. Data Collection

☐ Connect the Go!Link to the computer USB hub and the gas pressure sensor (Figure UII-5A) to the Go!Link.

☐ Open the **Fermentation.cmbl** file found in the Documents > 2227L folder on the lab computer. The .cmbl file will be opened by the Logger Pro software.

☐ Label four separate tubes with the control solution names and the test solution names, then fill each tube with 4 mL of the corresponding solution:
   - Control solutions - Water (tube 1) & Sucrose (tube 2)
   - Test solutions - Maltose (tube 3) & Galactose (tube 4)
     OR Glucose (tube 3) & Fructose (tube 4)

☐ Continuously monitor and maintain the water bath temperature between 38-42°C:
   - Place the large beaker at the base of the ring stand.
   - Fill ½ up with water from the electric hot water bath.
   - Using the Styrofoam cups and basters, move the water between the beaker and the electric hot water bath. Cooler water from the beaker can be added back to the electric hot water bath. (Figure UII-5B).

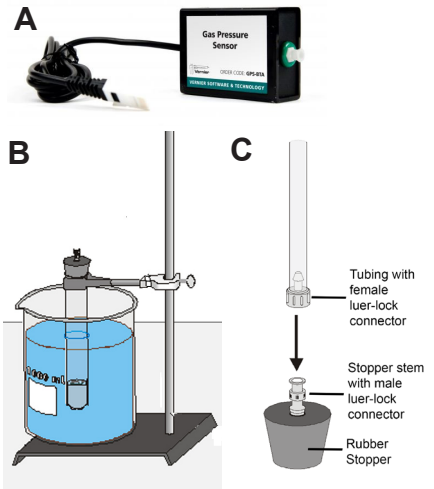


Figure UII-5. A) Gas pressure sensor. **B)** Reaction tube suspended in a water bath using a ring stand and clamp. Do not connect to the pressure sensor until the end of incubation, once you begin to collect data, and **C)** Connector.

Incubation= I Before each run you need to maintain a 38-42° C water bath to incubate your tubes for 10 minutes.
Run= R During each run, as you collect data, you need to maintain a 38-42° C water bath to incubate your tubes.
NA= no incubation or run at this time.

Time in minutes

Solution	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39
Control Solutions																																								
Water (tube 1)	I	I	I	I	I	I	I	I	I	I	R	R	R	R	R	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Sucrose (tube 2)	NA	NA	NA	NA	NA	NA	NA	NA	I	I	I	I	I	I	I	I	I	R	R	R	R	R	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Test solutions																																							
_____ (tube 3)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	I	I	I	I	I	I	I	I	I	R	R	R	R	R	NA	NA	NA	NA	NA	NA	NA	NA	NA
_____ (tube 4)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	I	I	I	I	I	I	I	I	I	I	R	R	R	R	R

Figure UII-6. Fermentation incubation and run schedule.

Important: Start the incubation for the second tube AFTER the first eight (8) minutes of the first tube's pre-incubation. See Figure UII-6.

Table UII-2. Rate of fermentation (kPA/min) by yeast, as determined from the slope of the best-fit linear regression line of the plot of gas pressure versus time.

Solution (tube)	°C	Fermentation rate
Controls		
Water (1)		
Sucrose (2)		
Assigned test solutions		
_____(3)		
_____(4)		

☐ Pre-incubation:
- Gently swirl the yeast suspension flask to resuspend the cells.
- Transfer 4 mL of the yeast suspension into the tube and gently mix.
- Attach the tube to the ring-stand clamp.
- Lower the tube into the water with the bottom half submerged.
- Add a drop of vegetable oil to the surface of the yeast-sugar mixture to create an anaerobic seal. Do not drip oil on the inside wall of the test tube, as it will keep a seal from forming.
- Insert the black rubber stopper into the test tube (Figure UII-6A). DO NOT connect the pressure sensor to the stopper. Gently twist the stopper into the tube to create an airtight seal (but do not twist so hard that you break the glass tube!).
- Incubate the test tube for 10 minutes.
- Continuously monitor and maintain the bath temperature at 38-40°C.

☐ Run:
- After 10 minutes of pre-incubation, connect the free end of the plastic tubing to the connector in the rubber stopper (Figure UII-5C).
- Begin measuring gas pressure for five (5) minutes by clicking the collect button.
- Continuously monitor and maintain the bath temperature at 38-40°C.
- DO NOT click on the Stop button. The software will stop collection on its own.
- **Store Latest Run from the Experiment menu. DO NOT close Logger Pro.**

☐ Repeat the pre-incubations and runs for the other three tubes.

☐ Click on the Linear Fit button. Note: you can use the buttons to view the slope region of interest.

☐ **Record the value of the slope, m, for each of the equations that describe the straight-line relationships, y = mx + b, in Table UII-1 AND to the Fermentation workbook on the instructor computer.**

☐ Cleaning the tubes:
- Scrub the tubes with water and dish detergent.
- Rub off the marker label.
- Invert the tubes on the wooden tube rack.

C. Interpretation

☐ Your instructor will send your lab section's completed workbook to each student's ISU Google Account Gmail.

☐ Open the Word document from Section A.

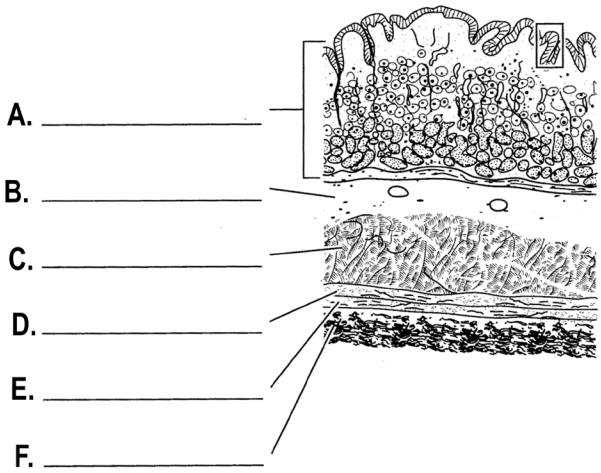
3. Insert the table and graph into the document.
4. Create a table caption ABOVE the table.
5. Create a figure caption BELOW the figure.
6. Type the answers to the following questions into the document:
   H. Describe the patterns shown by your graphs and discuss what the patterns are indicating.
   I. Do yeast equally utilize all sugars for fermentation?
   J. Why does this difference between sugars with respect to fermentation?
   K. Did you reject or support your hypothesis? Explain in DETAIL why it was supported or rejected?
   L. Using the following terms (active site, substrate, activation energy, enzyme, disaccharide, monosaccharide) describe how sucrose is modified by yeast. Is this similar to human use of sucrose? Why?
   M. Describe the anatomical (hint: enterocytes, BBM,etc.) and physiological pathway that leads to the production of the glucose needed for cellular respiration through the consumption of complex carbohydrates by animals.
   N. What happens during animal cell lactic acid fermentation.

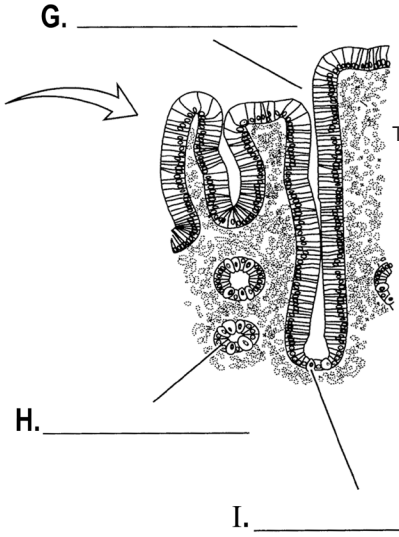
☐ **Send the document to your lab instructor using your ISU Google Account Gmail.**

7. Match diagrams in Figure UII-7 to the slides you observed, color, label accordingly, and include total magnification.

Specimen: _____

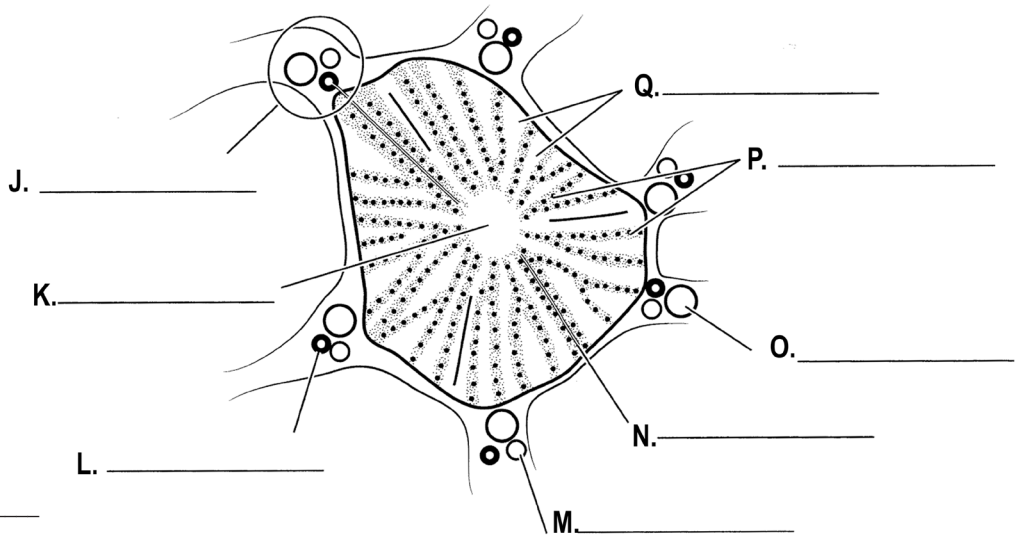
Total Magnification:_____





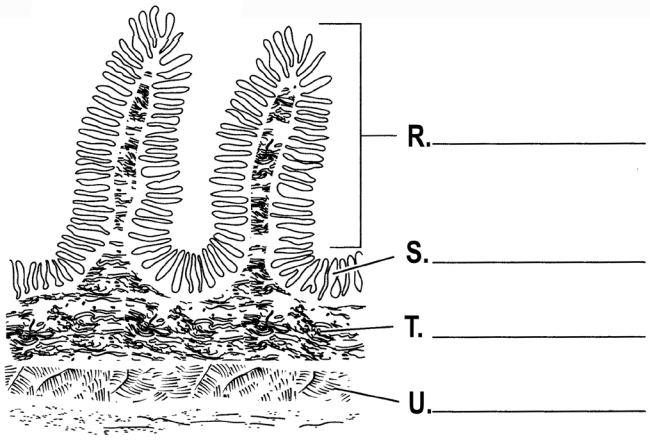
Total Magnification:_____

Specimen: _____ Total Magnification:_____



Specimen: _____

Total Magnification:_____

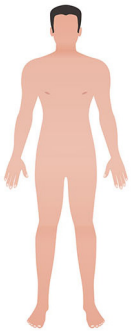


Total Magnification:_____

V. _____ W._____

Figure UII-7. Digestive system cross-sections with the following structures; A. Mucosa (red), B. Submucosa, Muscle layers (C. Oblique (pale blue), D. Circular (yellow), E. Longtudinal), F. Serosa, G. Gastric pit (light purple), H. Chief cell, I. Parietal cell, J. Portal dria, K. Central vein (dark purple), L. Bile duct, M. Hepatic artery branch, N. Bile canaliculus, O. Portal vein branch, P. Hepatocytes, Q. Sinusoids, R. Circular fold, S. Villi, T. Submucosa, U. Muscularis, V. Jejunum, and W. Ileum.

Unit III. Integumentary System



Integumentary System

Objectives

- Name, identify, and define the various structures of the integumentary system.
- Compare the rate of recovery from cold in two different skin regions.
- Correlate rate of recovery with vascularity.
- Create tables and figures with corresponding APA captions using Microsoft® Office Excel and Word.

Terms & Definitions

Adipose - a loose connective tissue composed mostly of fat cells (adipocytes).

Cell junctions (Intercellular bridges) - a cellular structures consisting of protein complexes that provide adhesion between neighboring cells or between a cell and the extracellular matrix in animals

Areolar Connective Tissue - a type of connective tissue that holds organs in place and attaches epithelial tissue to other underlying tissues.

Capillaries - any of the fine branching blood vessels that form a network between the arterioles and venules.

Circulatory - circulate blood in order to transport nutrients, waste, hormones, O2, CO2, and aid in maintaining pH and temperature

Collagen - the main structural protein in the extracellular matrix found in the body's various connective tissues.

Elastin - a protein that exists as fibers in the extracellular spaces of many connective tissues.

Evaporation - the process of turning from liquid into vapor.

Evaporative Cooling - the conversion of liquid water into vapor using the thermal energy in the air, resulting in a lower air temperature.

Fibroblast - the most common type of cell found in connective tissueExtracellular fluid (ECF) - the fluid outside of cells.

Glands - a group of cells in an animal's body that synthesizes substances for release into the bloodstream (endocrine) or into cavities inside the body or its outer surface (exocrine).

A. Endocrine gland - a gland that performs communication within the body using hormones; relating to or denoting glands which secrete hormones or other products directly into the blood.
B. Exocrine gland (holocrine, merocrine, or apocrine) - A gland that makes substances such as sweat, tears, saliva, milk, and digestive juices, and releases them through a duct or opening to a body surface.
C. Intestinal Glands - imaginations of the intestinal mucosa that secrete intestinal juice. (AKA crypts).
D. Mammary gland - an exocrine gland in humans and other mammals that produces milk to feed young offspring.
E. Salivary Glands - exocrine glands that produce saliva through a system of ducts. Humans have three paired major salivary glands (parotid, submandibular, and sublingual), as well as hundreds of minor salivary glands.
F. Sweat Gland - small tubular structures of the skin that produce sweat. Sweat glands are a type of exocrine gland, which are glands that produce and secrete substances onto an epithelial surface by way of a duct.

Homeostasis - the tendency toward a relatively stable equilibrium between interdependent elements, especially as maintained by physiological processes.

Thermoregulation - ability of an organism to keep its body temperature within certain boundaries, even when the surrounding temperature is very different.

A. Endothermy - use internal metabolism to regulate body temperature.
B. Ectothermy - use an external source of temperature to regulate body temperature.
C. Hyperthermia - the condition of having a body temperature greatly above normal.
D. Hypothermia - the condition of having an abnormally low body temperature, typically one that is dangerously low.

Integumentary system - the set of organs forming the outermost layer of an animal's body. It comprises the skin and its appendages, which act as a physical barrier between the external environment and the internal environment:

A. Bulb - the base of the hair follicle.
B. Dermal Papillae - fingerlike projections arranged into double rows, increasing the surface area between the epidermis and dermis, thereby strengthening the juncture with the epidermis and increasing the amount exchange of oxygen, nutrients, and waste.
C. Dermis - the thick layer of living tissue below the epidermis that contains blood capillaries, nerve endings, sweat glands, hair follicles, and other structures.
D. Epidermis - outermost layer that provides a barrier to infection from environmental pathogens and regulates the amount of water released from the body into the atmosphere.
E. Follicle - a tunnel-shaped structure in the epidermis (outer layer) of the skin.
F. Hair Root - the part of a hair that is embedded in the hair follicle.
G. Hair Shaft - the part of your hair that can be seen above your scalp.
H. Hypodermis - layer of cells lying immediately below the dermis. This layer connects the dermis layer to your muscles and bones, insulates, helps regulate body temperature, and produces fat cells to store energy.
I. Meissner Corpuscle - a type of nerve ending in the skin that is responsible for sensitivity to light touch. In particular, they have their highest sensitivity (lowest threshold) when sensing vibrations between 10 and 50 hertz. They are rapidly adaptive receptors. They are most concentrated in thick hairless skin, especially at the finger pads.

J. Melanocyte - are melanin-producing neural crest-derived cells located in the bottom layer (the stratum basale) of the skin's epidermis.
L. Sebaceous Gland - a microscopic exocrine gland in the skin that opens into a hair follicle to secrete an oily or waxy matter, called sebum, which lubricates the hair and skin of mammals.
M. Stratum Basale - the deepest layer of the five layers of the epidermis, the external covering of skin in mammals.
N. Stratum Corneum - the outermost layer of the epidermis.
O. Stratum Granulosum - a thin layer of cells in the epidermis lying above the stratum spinosum and below the stratum corneum (stratum lucidum on the soles and palms)
P. Stratum Lucidum - a thin, clear layer of dead skin cells in the epidermis named for its translucent appearance under a microscope.
Q. Stratum Spinosum - a layer of the epidermis found between the stratum granulosum and stratum basale.
R. Pacinian Corpuscle - is one of the four major types of mechanoreceptors (specialized nerve ending with adventitious tissue for mechanical sensation) found in mammalian skin.

Keratin - a fibrous protein forming the main structural constituent of hair, feathers, hoofs, claws, horns, etc.

Matrix - is the material (or tissue) in between a eukaryotic organism's cells.

Simple Columnar Epithelium - single layer of columnar epithelial cells which are tall and slender with oval-shaped nuclei located in the basal region, attached to the basement membrane. In humans, simple columnar epithelium lines most organs of the digestive tract including the stomach, and intestines. Simple columnar epithelium also lines the uterus.

Stratified Squamous Epithelium - squamous (flattened) epithelial cells arranged in layers upon a basal membrane.

Perspiration/Sweat - is the production of fluids secreted by the sweat glands in the skin of mammals.

Temperature - physical quantity that expresses hot and cold. It is the manifestation of thermal energy, present in all matter, which is the source of the occurrence of heat, a flow of energy, when a body is in contact with another that is colder or hotter.

Tissues - the association of many cells of the same type:
A. Connective - the tissue that supports, protects, and gives structure to other tissues and organs in the body; develops from the mesoderm.
B. Epithelial - line the outer surfaces of organs and blood vessels throughout the body, as well as the inner surfaces of cavities in many internal organs; develops from the ectoderm or endoderm.
C. Muscle - a soft tissue that makes up the different types of muscle in animals, and gives the ability of muscle to contract; develops from the mesoderm.
D. Nervous - the main tissue component of the nervous system; develops from the ectoderm.

Vasoconstriction - the narrowing (constriction) of blood vessels by small muscles in their walls.

Vasodilation - the dilatation of blood vessels, which decreases blood pressure.

Background - Integumentary System

The consists of skin and its appendages (i.e. hair, nails, exocrine glands) and is the heaviest organ in the human and most animal bodies. It accounts for approximately 16% of human body weight. This system has four major functions; **1)** protect the body from various kinds of damage (water loss, ultraviolet light, mechanical, chemical, or thermal damage), **2)** provide sensory information using receptors for touch, pressure, pain, and temperature, **3)** regulate temperature using integument appendages, sweat glands (in certain animals like humans), and subcutaneous adipose tissue, and **4)** aid in metabolic functions such as triglyceride storage in the subcutaneous adipose tissue, waste excretion, and Vitamin D synthesis. In order to accomplish this task, the system is composed of several tissues. The **epidermis** is the outer keratinizing stratified squamous epithelium. The ectoderm germ layer gives rise to the epidermis during embryo development. It is made of four or five layers of epithelial cells, depending on its location in the body. It does not have any blood vessels within it (i.e., it is avascular). Reptile and bird scales develop from epidermal tissue. Hair and feather follicles, sebaceous glands, sweat glands, apocrine glands, and mammary glands are considered epidermal glands or epidermal appendages, because they develop as invaginations of the epidermis into the dermis. Hair, feathers, and scales are made of keratin that is produced by the epidermis. The **dermis** is made of two layers of connective tissue that compose an interconnected mesh of elastin and collagenous fibers, produced by fibroblasts. The dermis is derived from the mesoderm germ layer. The dermis contains blood and lymph vessels, nerves, and other structures, such as the epidermal follicles and glands. Dermal papillae are projections of dermis into the epidermis. Fish scales develop from dermal tissue and, in many fishes, age can be determined by counting scale growth rings. The **hypodermis** consists of well-vascularized, loose, areolar connective tissue, and adipose tissue, which functions as a mode of fat storage and provides insulation and cushioning for the integument. Hypodermis is also derived from the mesoderm germ layer.

Name:_____ Team #:_____ Section #:_____

1. Figure UIII-1, label and color the structures listed in the figure caption.

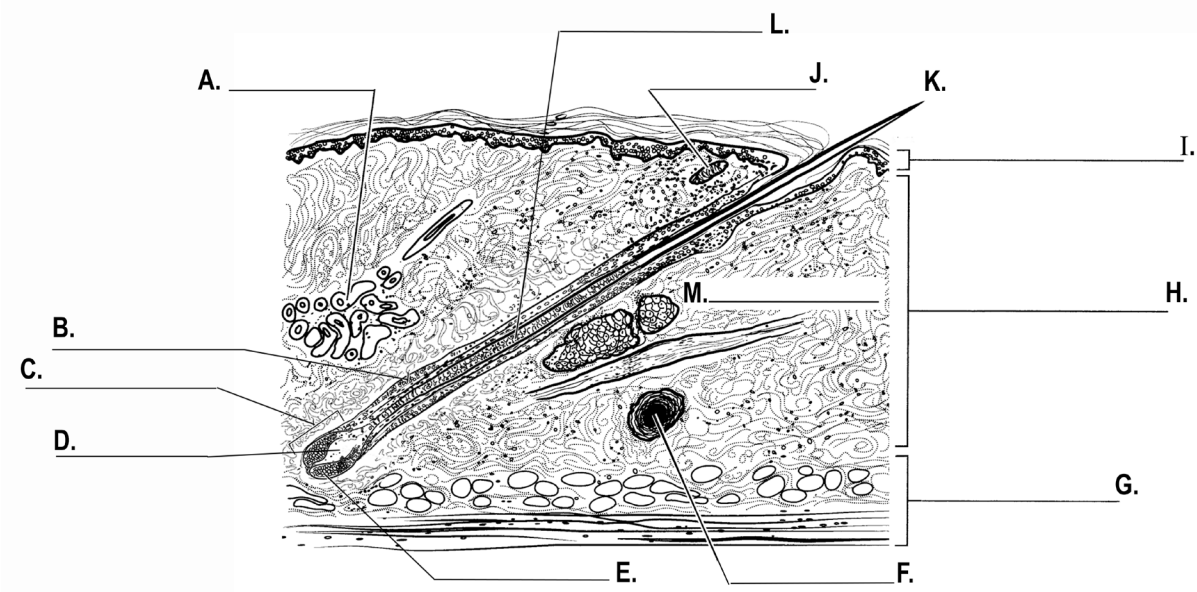


Figure UIII-1. Cross-section diagram of the integumentary system: A. Sweat gland (light-green), B. Follicle (red), C. Bulb, D. Dermal papillae (pink), E. Matrix, F. Pacinian corpuscle, G. Hypodermis, H. Dermis, I. Epidermis, J. Meissner corpuscle (purple), K. Hair shaft (yellow), L. Hair root (yellow), and M. Sebaceous gland (green).

2. Figure UIII-2, label and color the structures listed in the figure caption.

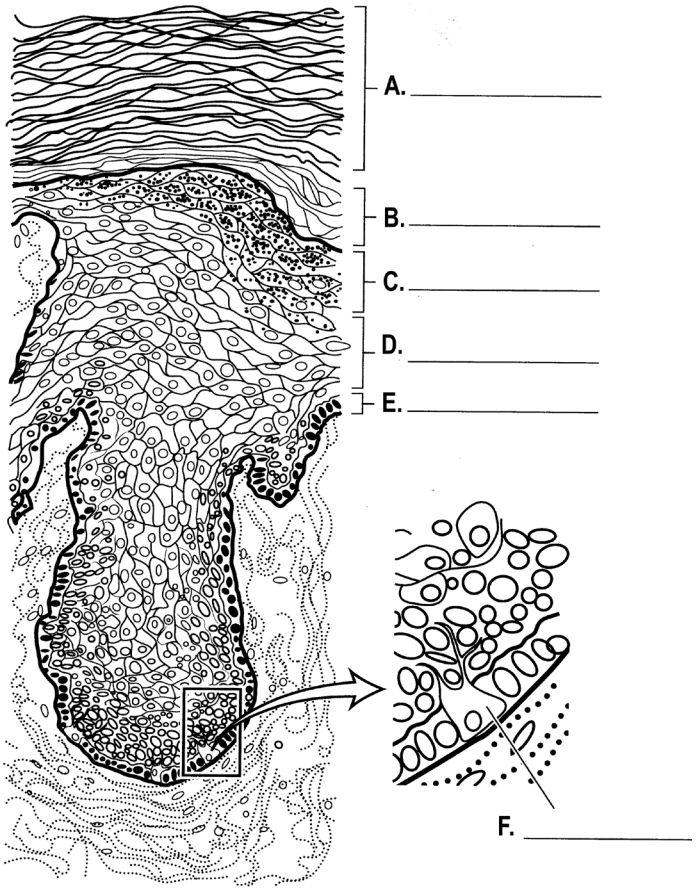


Figure UIII-2. Cross-section diagram of the epidermis of the integumentary system; A. Stratum corneum (light-brown), B. Stratum lucidum (orange), C. Stratum granulosum, D. Stratum spinosum (light-orange), E. Stratum basale, F. Melanocyte (dark brown).

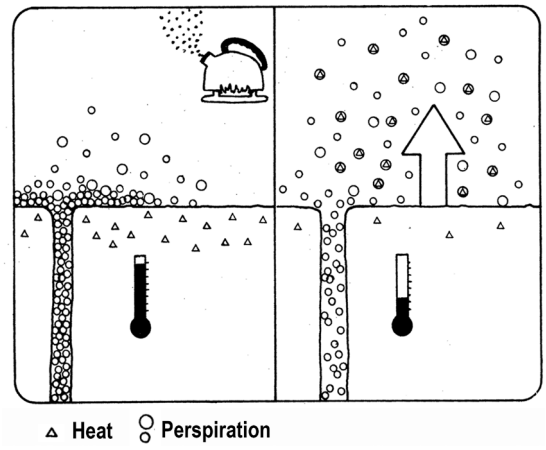


Figure UIII-3. Evaporative cooling of the skin.

Part 1. Skin Vascularity

Homeostasis refers to the body's ability to maintain internal conditions (e.g., temperature, pH, hydration) within the narrow limits that are optimal for the continuation of metabolic processes. When these optimal conditions are disturbed by a change in the environment, body systems work to return them to normal.

Many of the chemical reactions and cellular processes necessary to sustain human life occur most readily at a body temperature of approximately 37.0°C (98.6°F). Homeostatic mechanisms work to maintain this temperature, regardless of changes in the external environment. Changes in temperature are sensed by the skin, which is well-designed to counteract these changes. Beneath the protective epidermal layer of the skin lies the dermis (Figure UIII-1), which contains sweat and oil glands and a rich blood supply.

Your body makes use of the evaporative process when perspire. Sweat, which consists of 90 percent water, starts to evaporate (see Figure UIII-3). The necessary heat of evaporation is extracted from the sweat itself, which leads to a heat transfer from the liquid into the gaseous state. This results in a cooling effect (called evaporative cooling) that helps to maintain body temperature and cools the body down when it gets too hot. The degree of cooling is dependent on the evaporation rate and heat of evaporation. A similar process occurs with boiling water in a tea kettle.

The integumentary system influences body temperature by either allowing or diverting the blood's access to the surface of the skin. When an area is exposed to the cold, small capillaries in the dermis constrict, allowing less than normal blood flow to that area and protecting core body temperature. When cold is removed, the blood supply to the skin increases again as the arterioles dilate. Exposure to heat causes blood vessels to dilate, allowing heat to dissipate from the skin's surface, and resulting in a flushed or red appearance. Other examples of the regulation of body temperature through the action of the skin's blood supply include the dilatation seen with embarrassment and the constriction and resulting paleness that occurs with fear. Dilatation also occurs with ingestion of alcohol.

A. Experimental Design

Human skin temperature is maintained at approximately 33°C (91°F). This temperature can be referred to as the skin temperature "set point." Regions of skin with higher vascularity (containing more blood vessels) will return more quickly to the set point after a disturbance than regions with less vascularity. In this experiment, you will compare the rate of recovery from cold in two different skin regions and draw conclusions about the vascularity of these areas.

☐ Open Word document, answer question 1 and 2, and then save the document.

3. At the top right of the document type:
   - Biol 2227L
   - Unit III - Integumentary System (Vascularity)
   - Your section #
   - Your team #
   - Names of everyone on your team

4. Type the answers to the following questions into the Word document.
   A. Background: What is the species name of a human subject? To which domain and kingdom does a human belong? What is the integumentary system and what are the three tissue layers that make up the system? Which layer(s) are vascular?
   B. Question?
   C. Determine your variables: Which is your response (dependent) variable? Which is your predictor (independent) variable? Are the variables categorical and/or quantitative?
   D. Describe the control and experimental groups for your experiment.
   E. Describe the type of relationship between the variables (positive, negative, or neutral).
   F. Develop a hypothesis.
   G. What is your prediction as to the outcome of your experiment.

B. Data Collection

☐ Connect the Go!Link to the computer USB hub and the Surface Temperature sensor (Figure UIII-4) to the Go!Link.

☐ Open the **Skin.cmbl** file found in the Documents > 2227L folderson the lab computers. The .cmbl file will be opened by the Logger Pro software.

Upper Arm Skin

☐ Remove excess oil from the skin over the biceps with the rubbing alcohol and the cotton balls available.

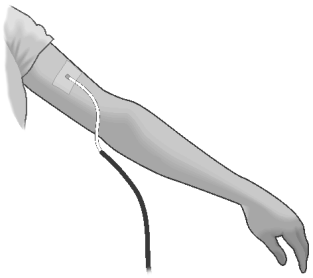


Figure UIII-4. Surface Temperature Sensor attached to the upper arm.

☐ **Baseline (A-C)**

A. Tape the thermistor end (the tip) of the Surface Temperature Sensor directly to the upper arm, over the area of the biceps (Figure UIII-4).

B. Click to begin data collection. Collect data for 50 seconds to obtain a baseline recording of the temperature.

C. After the run, select the run by clicking on the graph.
- Hold down the left mouse button. Drag the pointer across the entire graph.
- Release the left mouse button.
- Click on the Linear Fit button. Note: you can use the buttons to view the slope region of interest.

Table UIII-1. Rate of temperature recovery (°C/s) by skin, as determined from the slope of the best-fit linear regression line of the plot of surface temperture (°C) versus time (s).

	Rate	
Skin	Baseline	Cold Recovery
Upper arm		
Facial cheek		

☐ **Record the value of the slope, m, in Table UIII-1 AND to the Skin workbook on the instructor computer.**

☐ Remove the Surface Temperature Sensor from the arm.

☐ **Cold Recovery (A-E)**

A. Hold an ice cube in place for 30 seconds on the area of the upper arm to which the Surface Temperature Sensor was affixed.

B. Remove the ice and quickly blot the area dry with a towel. DO NOT RUB as friction can cause an increase in skin temperature.

C. Tape the Surface Temperature Sensor to the upper arm again, in the same area of the biceps where the ice was held.

D. Click to begin data collection. Data will be collected for 120 seconds.

E. After the run, select the run by clicking on the graph.
- Hold down the left mouse button. Drag the pointer across the entire graph.
- Release the left mouse button
- Click on the Linear Fit button. Note: you can use the buttons to view the slope region of interest.

☐ **Record the value of the slope, m, in Table UIII-1 AND to the Skin workbook on the instructor computer.**

Facial Skin

☐ Remove excess oil from the skin below the cheek bone, approximately 3 cm from the corner of the mouth (Figure UIII-5) with the rubbing alcohol and the cotton balls available.

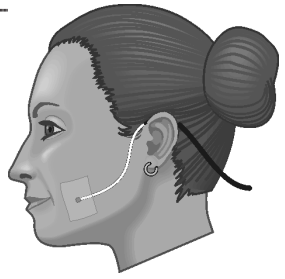


Figure UIII-5. Surface Temperature Sensor attached to the cheek.

☐ **Baseline (A-C)**

A. Tape the Surface Temperature Sensor to the same area, looping the sensor wire over the ear for stability (Figure UIII-5).

B. Click to begin data collection. Collect data for 50 seconds to obtain a baseline recording of the temperature.

C. After the run, select the run by clicking on the graph.
- Hold down the left mouse button. Drag the pointer across the entire graph.
- Release the left mouse button.
- Click on the Linear Fit button. Note: you can use the buttons to view the slope region of interest.

☐ **Record the value of the slope, m, in Table UIII-1 AND to the Skin workbook on the instructor computer.**

☐ Remove the Surface Temperature Sensor from the arm.

☐ **Cold Recovery (A-E)**

A. Hold an ice cube in place for 30 seconds on the area of the cheek to which the Surface Temperature Sensor was affixed.
B. Remove the ice and quickly blot the area dry with a towel. DO NOT RUB as friction can cause an increase in skin temperature.
C. Tape the Surface Temperature Sensor to the cheek again, in the same area where the ice was held.
D. Click to begin data collection. Data will be collected for 120 seconds.
E. After the run, select the run by clicking on the graph.
- Hold down the left mouse button. Drag the pointer across the entire graph.
- Release the left mouse button.
- Click on the Linear Fit button. Note: you can use the buttons to view the slope region of interest.

☐ **Record the value of the slope, m, in Table UIII-1 AND to the Skin workbook on the instructor computer.**

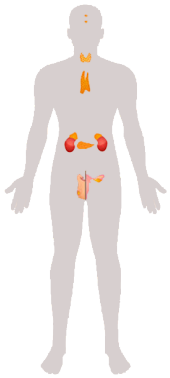
C. Interpretation

☐ Your instructor will send your lab section's completed workbook to each student's ISU Google Account Gmail.

5. Insert the table and graph into the document.
6. Create a table caption ABOVE the table.
7. Create a figure caption BELOW the figure.
8. Type the answers to the following questions into the document:
   H. Describe the patterns shown by your graphs and discuss what the patterns are indicating.
   I. Did you reject or support your hypothesis? Explain in DETAIL why it was supported or rejected?
   J. Is the baseline temperature recovered within the 2 minutes during which data is collected? Estimate how long it would take for full recovery to be achieved for each of the two runs. Relate this to everyday experiences where you have been exposed to cold.
   K. How does the anatomical location of the integument on the human body affect the recovery rate of surface temperature when the skin is exposed to the melting temperature of ice (0-10C)? Which area of skin tested (upper arm or face) had the most rapid recovery of temperature after cooling? Explain this result.
   L. A condition called hyperthermia (heat prostration) can result when the body's homeostatic mechanisms are no longer adequate to counter the effect of high external temperatures. On the basis of what you know about vasoconstriction and vasodilation as methods of temperature regulation in the body, describe the skin color of someone who is in the first stages of hyperthermia.
   M. Alcohol causes dilatation of dermal capillaries and a sensation of warmth. Would you recommend that someone who is stranded in the snow drink alcohol to keep warm? Why or why not?

☐ **Send the document to your lab instructor using your ISU Google Account Gmail.**

Unit IV - Endocrine System



Endocrine System

Objectives

- Learn about the endocrine system, hormones, and how they are connected to DNA, the cell cycle, protein synthesis, and meiosis.
- Gain a better understanding of the molecular structure of DNA and the process of DNA replication by constructing simple models.
- Transcribe and translate a gene to synthesize a protein.
- Use electrophoresis to observe variations in cleavage patterns produced by the action of restriction enzymes on samples of DNA.
- Name, identify, describe, and compare cell cycle events.
- Observe the cell cycle using prepared animal and plant tissue slides.

Terms & Definitions

Agarose gel - a jelly-like slab used to separate molecules on the basis of molecular weight.

Amino acid - monomer subunit of a protein. Contains an amino, a carboxyl, and a unique side group.

Bivalent/tetrad - the association of a pair of homologous chromosomes (4 sister chromatids) physically held together by at least one synaptic DNA crossover. This physical attachment allows for alignment and segregation of the homologous chromosomes in the meiosis I.

Blood - a fluid connective tissue in animals consisting of cells and (in mammals) cell fragments suspended in a solution of water containing dissolved nutrients, proteins, gases, and other molecules.

A. Basophils - a type of white blood cell. Basophils are the least common type of granulocyte, representing about 0.5% to 1% of circulating white blood cells
B. Clotting - coagulation, is an important process that prevents excessive bleeding when a blood vessel is injured. Platelets and proteins in plasma work together to stop the bleeding by forming a clot over the injury.
C. Erythrocyte (red blood cell, RBC) - the most common type of blood cell and the vertebrate's principal means of delivering oxygen (O2) to the body tissues through the circulatory system. RBCs take up oxygen in the lungs, or in fish the gills, and release it into tissues while squeezing through the body's capillaries.
D. Hemoglobin - an iron-containing protein that binds oxygen and is found within the cytosol of red blood cells.
E. Leukocyte (White blood cell, WBC) - A type of blood cell that is made in the bone marrow and found in the blood and lymph tissue and are part of the body's immune system (B & T Lymphocyte, Monocyte,Neutrophil,Eosinophil,Basophil)
F. Thrombocytes (Platelet) - cell fragments in the blood of mammals that play a crucial role in the formation of blood clots.
G. Plasma - fluid, composed of about 92% water, 7% vital proteins, clotting factors, and 1% mineral salts, sugars, fats, hormones and vitamins.

Blood group systems - blood type that is determined by the presence or absence of certain antigens on the cell membranes of erythrocytes.

Bone marrow - a semi-solid tissue found within the spongy or cancellous portions of bones. In birds and mammals, bone marrow is the primary site of new blood cell production or hematopoiesis. It is composed of hematopoietic cells, marrow adipose tissue, and supportive stromal cells.

A. Hematopoiesis - the production of blood cells and platelets, which occurs in the bone marrow.
B. Erythropoiesis - the production of red blood cells.

Blood Vessels - a vessel (arteries, veins, and capillaries) in the human or animal body in which blood circulates.

Cell cycle - the series of phases a eukaryotic cell progresses through from its origin until it divides by mitosis:

A. Interphase - it is the portion of the cell cycle during which the chromosomes are decondensed and found in the nucleus. G1 (first gap cycle), S (DNA synthesis phase), and G2 (second gap cycle) are stages of interphase.
B. Prophase - phase of mitosis during which the chromosomes condense and the nuclear membrane begins to vesiculate.
C. Prometaphase - phase of mitosis during which the mitotic spindle is completely formed.
D. Metaphase - the phase of mitosis during which the chromosomes are aligned along the metaphase plate -
E. Anaphase - the phase of mitosis during which the sister chromatids separate from each other and move to opacity poles; poles themselves also move farther apart.
F. Telophase - the phase of mitosis during which the chromosomes decondense and the nuclear membrane re-forms.
C. Cytokinesis - the division of the cytoplasm to produce two distinct daughter cells.

Cell cycle structures:

A. Centriole - a cylindrical organelle composed mainly of tubulin protein that helps anchor microtubules during cell division.
B. Centromere - a region (not a true structure) of a chromosome where sister chromatids are attached and to which microtubules bind.
C. Centrosome - a structure near the cell nucleus that forms the main microtubule organizing center during division. Each centrosome is composed of two centrioles at right angles to each other. Duplication occurs during the G1 phase and S Phase.
D. Kinetochore - a protein structure that can be found in the centromere region of a chromosome where the microtubules attach during cell division to pull sister chromatids apart.
e. Microtubule - protein structure that moves chromosomes around during mitosis and meiosis.

Cellular division - in eucaryotic cells, the process by which one cell divides into two cells:

A. Mitosis - the process in which nuclear division results in two nuclei, each of which receives the same complement of chromosomes.
B. Meiosis - the process by which haploid cells are produced from a cell that was originally diploid.

Chromosome - a discrete unit of genetic material composed of DNA and associated proteins. Eukaryotes have chromosomes in their cell nuclei and in their plastids and mitochondria:

A. Autosome - non-sex chromosome, of which there are 22 pairs in humans.

B. Chromatin - highly organized chromosome complex composed of DNA and histone proteins.
C. Histone - a group of proteins involved in the formation of nucleosomes that aid in the compaction of eukaryotic DNA.
D. Homologous (Homologs) - a pair of chromosomes consisting of one chromosome received from the father and one from the mother.
E. Nucleosome - the structural subunit of chromatin and is composed of eight histones wrapped with DNA.
F. Sex chromosome - a distinctive set of chromosomes that are different in males and females.
G. Sister chromatid - either of the two duplicated, identical copies of a chromosome formed after DNA synthesis. Acidophilic cells

Codon - a sequence of three nucleotides bases that specifies a particular amino acid or a stop codon; codons function during translation:
A. Anticodon - a three nucleotide sequence in tRNA that is complementary to a codon in mRNA.
B. Start - the three-base sequences (start usually aug) that specify the first animo acid in a polypeptide in translation
C. Stop - those that signal the end of translation (stop UAA, UAG, UGA)

Denature - the process where proteins unravel and change their native shape thus losing their biological activity.

Deoxyribonucleic acid (DNA) - the genetic material that serves as the code for building each unique organism.

Deoxyribonuclease (DNAse) - an enzyme that catalyzes the hydrolytic cleavage of phosphodiester linkages in the DNA backbone, thus degrading DNA

DNA fingerprinting - a technology that identifies particular individuals using properties of their DNA.

DNA replication - the copying of DNA strand.
A. Directionality - the end-to-end chemical orientation of a single strand of nucleic acid. In a single strand of DNA or RNA. The chemical convention of naming carbon atoms in the nucleotide pentose-sugar-ring means that there will be a 5′ end (typically contains a phosphate group attached to the 5′ carbon of the ribose ring) and a 3′ end that is unmodified from the ribose -OH substituent. In a DNA double helix, the strands run in opposite directions to permit base pairing between them, which is essential for replication or transcription of the encoded information.
B. Helicase - separates double-stranded DNA into single strands.
C. Lagging strand - a single DNA strand that is replicated in the 5′ – 3′ direction (opposite direction to the replication fork).
D. Leading strand - a single DNA strand that is replicated in the 3’ – 5’ direction (same direction as the replication fork). DNA is added to the leading strand continuously, one complementary base at a time.
E. Ligase - covalently attaches adjacent Okazaki fragments in the lagging strand.
F. Okazaki fragments - DNA added to the lagging strand in discontinuous sections.
G. DNA Polymerase - an enzyme involved in making new DNA molecules from the four nucleotide bases, using the existing DNA as a template. They can add nucleotides to the 3’-end of an existing nucleic acid, requiring a primer be bound to the template before DNA polymerase can begin a complementary strand.
H. DNA Primase - are enzymes that must be continuously active and catalyze the creation of small RNA molecules that are employed as DNA polymerase primers.
I. RNA primer - four to fifteen nucleotides long single-stranded nucleic acid used by all living organisms in the initiation of DNA synthesis.
J. Topoisomerase - enzymes that play essential roles in DNA replication, transcription, chromosome segregation, and recombination: type I, which makes single-stranded cuts in DNA, and type II enzymes, which cut and pass double-stranded DNA. They remove the tightened coils ahead of the replication fork.

Endocrine - communication within the body using hormones made by endocrine glands; relating to or denoting glands which secrete hormones or other products directly into the blood.
A. Follicular Cells - the major cell type in the thyroid gland, and are responsible for the production and secretion of the thyroid hormones thyroxine (T4) and triiodothyronine (T3).
B. Granulosa Cell - a somatic cell of the sex cord that is closely associated with the developing female gamete (called an oocyte or egg) in the ovary of mammals.
C. Parafollicular Cell - thyroid C cells, are neuroendocrine cells in the thyroid that secrete calcitonin.
E. Parathyroid - small glands of the endocrine system that control the amount of calcium in our blood and bones.
F. Pineal - a small, highly vascularized neuroendocrine gland that receives and conveys information about the current light-dark cycle from the environment and, consequently produces and secretes melatonin cyclically at night (dark period).
G. Pituitary (anterior & posterior) - - aka “master control gland” is a small gland connected directly to the hypothalamus and provides a key link between the brain and the endocrine system; the front part of the pituitary gland known as the anterior pituitary. This part of the gland makes these hormones that control other endocrine glands; the posterior pituitary is an extension of brain tissue from the hypothalamus. The posterior pituitary is where hormones made by the hypothalamus (vasopressin and oxytocin) are stored and released into the bloodstream.
H. Principal Chief Cells - one of the two cell types of the parathyroid glands, along with oxyphil cells. The chief cells are much more prevalent in the parathyroid gland than the oxyphil cells. Secretes parathyroid hormone (PTH).
I. Thyroid - an endocrine gland in vertebrates. In humans it is in the neck and consists of two connected lobes.

Essential Amino Acid - any of the amino acids that humans cannot synthesize and thus must be obtained from the diet. Gametogenesis - the process in which cells undergo meiosis to form gametes.

Gel Electrophoresis - a technique used to separate macromolecules by using an electric field that causes them to pass trough a gel matrix.

Genome - the complete genetic composition of a cell or a species.

Hormone - any member of a class of signaling molecules in multicellular organisms, that are transported to distant organs to regulate physiology and behavior
A. Calcitonin - a hormone secreted by the thyroid that has the effect of lowering blood calcium.
B. Epinephrine - aka adrenaline, a hormone secreted by the medulla of the adrenal glands. In medicine epinephrine is used chiefly as a stimulant in cardiac arrest, as a vasoconstrictor in shock, and as a bronchodilator and antispasmodic in bronchial asthma. Epinephrine is found in small amounts in the body and is essential for maintaining cardiovascular homeostasis because of its ability to divert blood to tissues under stress.
C. Estrogen - a group of hormones that play an important role in the normal sexual and reproductive development in women.
D. Glucocorticoids (Cortisol) - a type of corticosteroid hormone that is very effective at reducing inflammation and suppressing the immune system.
E. Gonadotropin Releasing (GNRH) - a hormone made by a part of the brain called the hypothalamus. Gonadotropin-releasing hormone causes the pituitary gland in the brain to make and secrete the hormones luteinizing hormone (LH) and follicle-stimulating hormone (FSH). In men, these hormones cause the testicles to make testosterone. In women, they cause the ovaries to make estrogen and progesterone. Also called GnRH, LH-RH, LHRH, and luteinizing hormone-releasing hormone.
F. Growth Hormone (GH) - Affects growth and development; stimulates protein production; affects fat distribution
H. Insulin - a hormone made by the pancreas that helps glucose in your blood enter cells in your muscle, fat, and liver, where it’s used for energy.
I. Norepinephrine - aka noradrenaline (NA) or noradrenalin, is an organic chemical that functions in the brain and body as both a hormone and neurotransmitter that is used to mobilize the brain and body for action. Norepinephrine release is lowest during sleep, rises during wakefulness, and reaches much higher levels during situations of stress or danger

J. Mineralocorticoids - a class of steroid hormones that regulate salt and water balances.
K. Oxytocin - a peptide hormone and neuropeptide/transmitter normally produced in the hypothalamus and released by the posterior pituitary that is involved in childbirth and breast-feeding.
L. Progesterone - a steroid hormone belonging to a class of hormones called progestogens. It is secreted by the corpus luteum, a secondary endocrine gland that the female body produces after ovulation during the second half of the menstrual cycle.
M. Prolactin (Prl) - a hormone made by the pituitary gland, a small gland at the base of the brain. Prolactin causes the breasts to grow and make milk during pregnancy and after birth. Prolactin levels are normally high for pregnant women and new mothers. Levels are normally low for nonpregnant women and for men
N. Steroid - a biologically active organic compound with four rings arranged in a specific molecular configuration. Steroids have two principal biological functions: as important components of cell membranes which alter membrane fluidity; and as signaling molecules. Hundreds of steroids are found in plants, animals and fungi.
O. Thymosin - a type of polypeptide hormone produced by thymic epithelial cells, can effectively increase T-cell numbers; support T-cell differentiation and maturation; and reduce cell apoptosis.
P. Thyroid Stimulating (TSH) - Stimulates the production and secretion of thyroid hormones
Q. Thyroxine (T4) - the main secretory product of the thyroid follicle cells and is converted to the active hormone, triiodothyronine (T3).
R. Triiodothyronine (T3) - a thyroid hormone that affects almost every physiological process in the body, including growth and development, metabolism, body temperature, and heart rate.

Hormone Response Element - a short sequence of DNA within the promoter of a gene, that is able to bind to a specific hormone receptor complex and therefore regulate transcription.

Ligand - a ligand is an ion or molecule (functional group) that binds to a central atom to form a coordination complex.

Locus - the physical location of a gene on a chromosome.

Nucelotide - organic molecules composed of a nitrogenous base (nucleobase), a pentose sugar (ribose or deoxyribose) and a phosphate containing one to three phosphates. They serve as monomeric units of the nucleic acid polymers – deoxyribonucleic acid (DNA) and ribonucleic acid (RNA).
A. Nucleotide bases (nucleobases, nitrogenous bases) - nitrogen-containing biological compounds; adenine (A), cytosine (C), guanine (G), thymine (T), and uracil (U)
B. Purine bases - guanine (G) and adenine (A) have a fused-ring skeletal structure derived of purine; characterized by their single amino group (−NH2), at the C6 carbon in adenine and C2 in guanine.
C. Pyrimidine bases - cytosine (C), thymine (T), and uracil (U) have a simple-ring structure derived of pyrimidine
D. Nucleosides - glycosylamines that can be thought of as nucleotides without a phosphate group.
D. Base pair - the structure in which two bases in opposite strands of DNA hydrogen-bond with each other.
F. Complementary - bases pair with each other by hydrogen bonding across the DNA helix; adenine with thymine and cytosine to guanine.

Nuclear Receptors - a double-membrane structure that encloses the cell's nucleus.

Ploidy - the number of sets of chromosomes in a cell, or in the cells of an organism.
A. Diploid - carrying two complete sets of chromosome; denotes by 2n.
B. Haploid - carrying one set of chromosomes; designated as 1n.

Pole - opposite ends of a sphere, such as a cell or of a planet.

Polymerase Chain Reaction - a technique used for replication DNA that can produce millions of copies of a DNA sequence in just a few hours from a small initial amount of DNA; primers are used that flank the region of DNA to be amplified.

Primers - short segments of RNA, typically 10-12 nucleotides in length, that are needed to begin DNA replication

Progenitor cell - a cell that can differentiate into specialized cell types but cannot maintain an undifferentiated status as does a stem cell.

Promoter - the site in DNA where transcription begins.

Protease - an enzyme that cuts proteins into smaller polypeptides.

Protein synthesis - the process whereby biological cells generate new proteins.

Proteolysis - a processing event within a cell in which enzymes called proteases cut proteins into smaller polypeptides.

Restriction enzyme - a protein isolated from bacteria that cleaves DNA sequences at sequence-specific sites along the phosphate backbone producing DNA fragments with a known sequence at each end.

Secondary Messenger - intracellular signaling molecules released by the cell in response to exposure to extracellular signaling molecules—the first messengers.

Somatic cell - the type of cell the constitutes all cells of an animal or plant body except those that give rise to gametes.

Stem cell - a cell that can go through mitotic cell division numerous times without differentiating into a specific cell type but they can also differentiate into specialized cell types.

Ribonucleic acid (RNA) - one of two classes of nucleic acids; the other is deoxyribonucleic acid (DNA). RNA consists of a single strand of nucleotides.
A. Messenger RNA (mRNA) - RNA that contains the information to specify a polypeptide with a particular amino acid sequence.
B. RNA Primers - short segments of RNA, typically 10 - 12 nucleotides in length, that are needed to begin DNA replication
C. Ribosomal RNA (rRNA) - an RNA that forms part of ribosomes, which provide the site where translation occurs.
D. Transfer RNA (tRNA) - is used to translate mRNA into polypeptides and carries an amino acid to the protein synthesizing ribosome.

TAQ Polymerase - is a thermostable DNA polymerase that is frequently used in the polymerase chain reaction (PCR).

Telomerase - an enzyme that helps prevent the degradation of the tips of chromosomes, active during development and sometimes reactivated in cancer cells.

Transcription - the use of a gene sequence to make a copy of RNA.

Transcription Factors - a protein that controls the rate of transcription of genetic information from DNA to messenger RNA, by binding to a specific DNA sequence. The function of TFs is to regulate genes in order to make sure that they are expressed in the right cell at the right time and in the right amount throughout the life of the cell and the organism.

Translation - the process of synthesizing a specific polypeptide on a ribosome.

Background - Endocrine System

The endocrine system is a system of ductless glands that secretes hormones directly into the circulatory system regulating all biological processes in the body from conception through adulthood and into old age, including the development of the brain and nervous system, the growth and function of the reproductive system, as well as the metabolism and blood sugar levels. The glands are typically well vascularized and the cells comprising the tissue are usually rich in intracellular vacuoles or granules that store hormones prior to release. Endocrine signaling is typically slow to initiate but is prolonged in response; this provides a counterpoint to the more rapid and short-lived nervous system signals. Hormones are chemical messengers that are released into the blood stream to act on an organ in another part of the body. Although hormones reach all parts of the body, only target cells with compatible receptors are equipped to respond. Over 50 hormones have been identified in humans and other vertebrates. Hormones control or regulate many biological processes and are often produced in exceptionally low amounts within the body. Much like a lock and key, many hormones act by binding to receptors that are produced within cells. When a hormone binds to a receptor, the receptor carries out the hormone's instructions, either by altering the cell's existing proteins or turning on genes that will build a new protein. The hormone-receptor complex switches on or off specific biological processes in cells, tissues, and organs (Table UIV-1).

Hormones can alter cell activity by binding with a receptor. Receptors can either directly influence gene expression and thus cell activity, or induce a secondary signaling cascade that will in turn influence cell activity. Receptors that can directly influence gene expression are termed **nuclear receptors**. Located within the cytosol or nucleus, **nuclear receptors are the target of steroid and thyroid hormones** that are able to pass through the cell membrane. **Nuclear receptors can bind directly to DNA to regulate specific gene expressions and are, therefore, classified as transcription factors.** Nuclear receptors can be classified into two broad classes according to their mechanism of action and their sub-cellular distribution in the absence of ligand. Type I nuclear receptors (Figure UIV-2) are located in the cytosol. Upon binding to a hormone the receptor and hormone translocate into the nucleus, and bind to specific sequences of DNA known as **hormone response elements** (HREs). Type II receptors are retained in the nucleus. In the absence of **ligand**, type II nuclear receptors often form a complex with co-repressor proteins. Hormone binding to the nuclear receptor results in dissociation of the co-repressor and the recruitment of co-activator proteins. For lipophobic hormones that cannot pass the cellular membrane, activity is mediated and amplified within a cell by the action of second messenger mechanisms (molecules that relay signals from receptors on the cell surface to target molecules inside the cell in the cytoplasm or nucleus). Most hormone receptors are G protein-coupled receptors. Upon hormone binding, the receptor undergoes a conformational change and exposes a binding site for a G-protein. The G-protein is bound to the inner membrane of the cell and consists of three sub-units: alpha, beta, and gamma. Upon binding to the receptor, it releases a GTP molecule, at which point the alpha sub-unit of the G-protein breaks free from the beta and gamma sub-units and is able to move along the inner membrane until it contacts another membrane-bound protein: the **primary effector**. The primary effector then has an action, which creates a signal that can diffuse within the cell. This signal is called the **secondary messenger**. The secondary messenger may then activate a **secondary effector**, whose effects depend on the particular secondary messenger system.

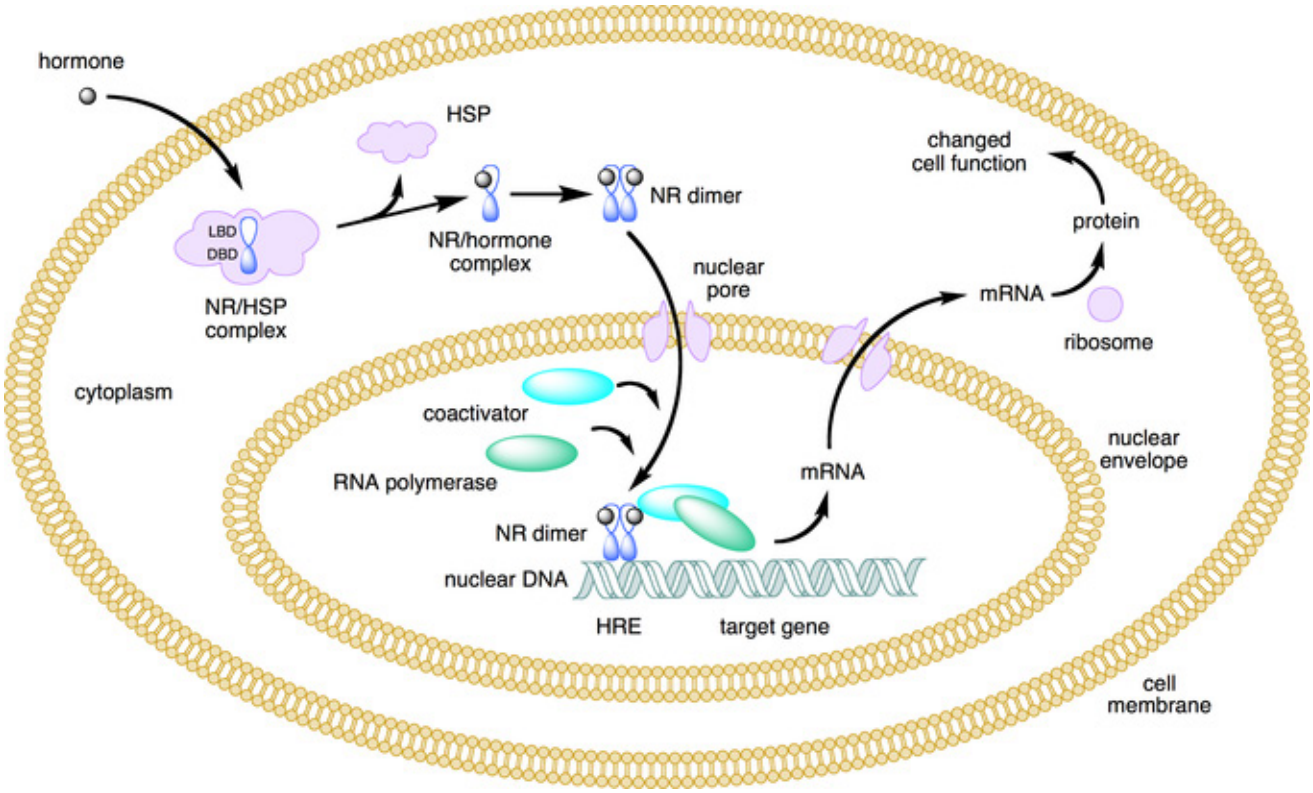


Figure UIV-2. This figure depicts the mechanism of a type I nuclear receptor (NR) that, in the absence of ligand, is located in the cytosol. Hormone binding to the NR triggers translocation to the nucleus, where the NR binds to a specific sequence of DNA known as a hormone response element (HRE).

Table UIV-1. The primary endocrine system organs called glands and some secondary organs as well as some of the hormones the glands produce, the class of molecule the hormone is composed of, and the hormone's target.

Gland	Hormone produced	Molecule	Target; Function
Primary - primary function is the secretion of hormones:			
Hypothalamus	Hypothalamic releasing & inhibiting hormones	peptide	anterior pituitary; regulate anterior pituitary hormone.
	Gonadotropin releasing hormone (GnRH)	peptide	released every one to three hours to control the reproductive system
Posterior Pituitary	Antidiuretic	peptide	kidneys; stimulate water reabsorption by kidney.
	Oxytocin	peptide	uterus and mammary glands; stimulate uterine muscle contractions and release of milk by mammary glands
Anterior Pituitary	Growth hormone (GH)	protein	**soft tissues and bones; cell division, protein synthesis, and bone growth**
	Gonadotropic hormones (FSH & LH)	glycoproteins	**gonads; regulates gametogenesis (egg and sperm production) and instigates sex hormone production**
	Thyroid stimulating (TSH)	glycoproteins	thyroid; stimulate the thyroid
	Adrenocorticotropic (ACTH)	peptide	adrenal cortex; stimulate adrenal cortex
	Prolactin (PRL)	protein	mammary glands; milk production
Thyroid	Thyroxine (T4; iodinated amino acid derivative) & Triiodothyronine (T3)	iodinated amino acid derivative	all tissues; increase metabolic rate and regulate growth and development. **T3 stimulates the production of RNA polymerase I and II and increasing the rate of protein synthesis**
	Calcitonin (peptide)	peptide	bones, kidneys, and intestine; lowering blood calcium level
Parathyroids	Parathyroid (PTH; peptide)	peptide	bones, kidneys, and intestine; raising blood calcium level
Adrenal Medulla	Epinephrine and Norepinephrine	modified amino acid	cardiac and other muscles; released in emergency situations; raises blood glucose level, "fight or flight" response
Adrenal Cortex	Glucocorticoids (cortisol)	lipid steroids	all tissue to raise; blood glucose level and stimulate the breakdown of protein
	Mineralocorticoids (aldosterone)	lipid steroids	kidneys; to reabsorb sodium and excrete potassium
	Sex Hormones	lipid steroids	gonads, skin, muscles, and bones; stimulate reproductive organs and bring on sex characteristics
Pineal Gland	Melatonin	modified amino acid	brain; control the circadian and circannual rhythms, possibly involved in maturation of sexual organs
Secondary - have a non-endocrine primary function but also have hormone-producing activities:			
Pancreas	Insulin	protein	liver, muscles, and adipose tissues; lower blood glucose levels and promote formation of glycogen
	Glucagon	protein	liver, muscles, and adipose tissues; raise blood glucose levels
Testes	Androgens	lipid steroids	gonads, skin, muscles, and bone; stimulates male sex characteristics
Ovaries	Estrogen & progesterone	lipid steroids	gonads, skin, muscles, and bone; stimulate female sex characteristics
Thymus	Thymosin	peptide	T lymphocytes; stimulate the production and maturation of T-lymphocytes (T-cells)
Kidney	Erythropoietin	glycoprotein	rythroid precursor cells; stimulate production and maintenance of crucial red blood cells
Integument, Heart, Skeleton, Gastrointestinal Tract, Adipose Tissue, Liver			

Name:_____ Team #:_____ Section #:_____

1. In Figure UIV-1:
   A. Color the endocrine glands.
   B. Draw in the Hypothalamus
   C. Label the male and female organs
   D. Label the hormones found in Table UIV-1 next to the name of the gland below that produces them.
   E. Circle the hormones that control:
      - Cell division (mitosis) in red
      - Gametogenesis (meiosis) in purple
      - Protein synthesis in yellow

1. Heart
2. Thymus (red)
3. Parathyroid (orange)
4. Thyroid (yellow)
5. Pituitary (pink)
6. Pineal (green)
7. Adrenal medulla & cortex (purple)
8. Pancreas (pink)
9. Ovaries (gray)
10. Testes (brown)
11. Brain & Spinal cord
12. Trachea & Bronchus
13. Lungs
14. Stomach
15. Kidneys
16. Uterus & Fallopian tubes
17. Scrotum

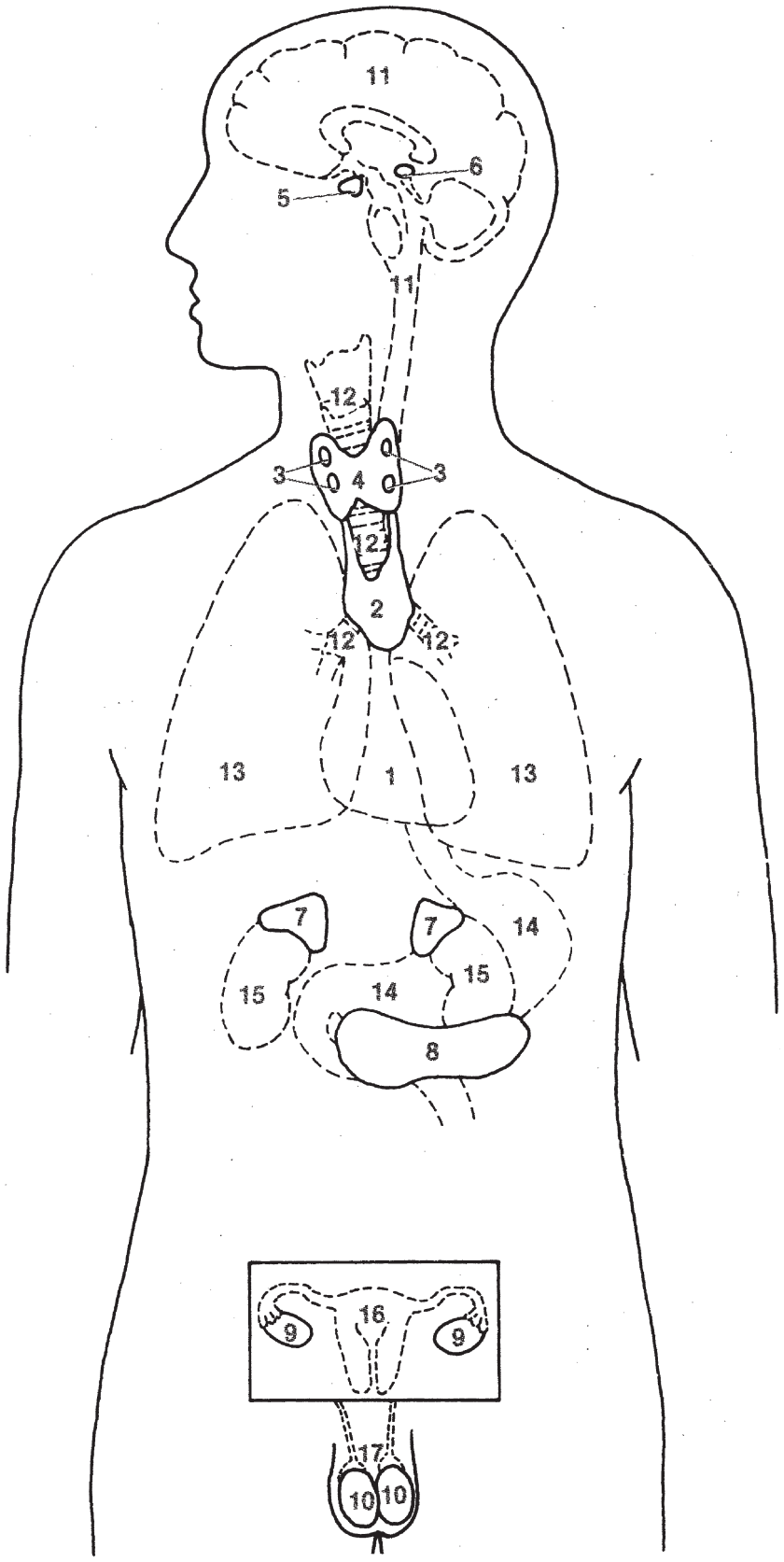


Figure UIV-1. The major endocrine glands and some of the major hormones of the endocrine system. The male is shown on the bottom and the female in the box above the male. Dashed lines indicate an organ that is not fully part of the endocrine system.

2. Match diagrams in Figures UIV-3, 4 & 5 to the slides you observe in lab, color, label accordingly, and include total magnification.

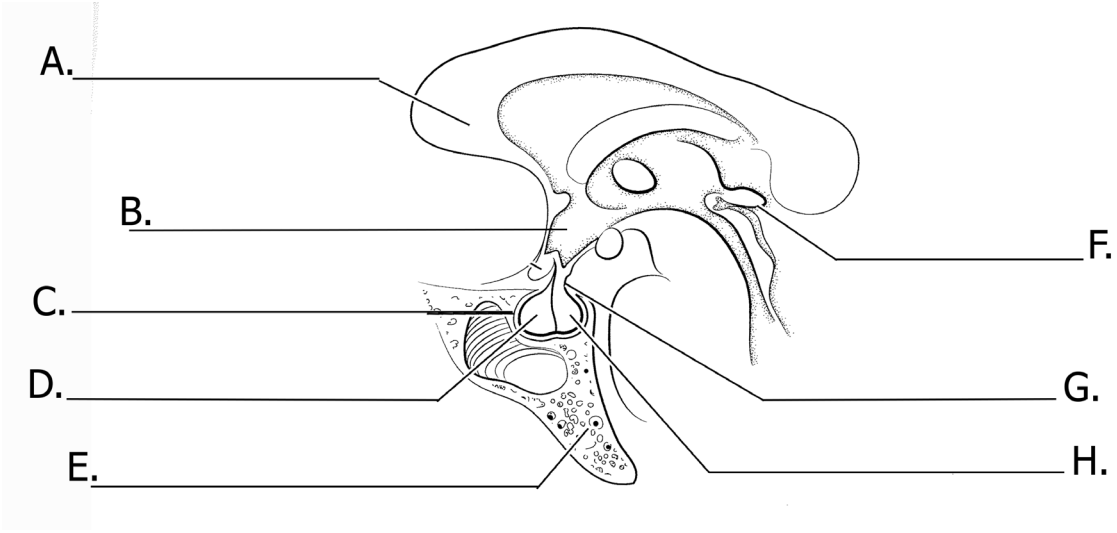


Figure UIV-3. Endocrine system cross-sections with the following structures; A. Corpus callosum (yellow), B. Hypothalamus (pink) C. Hypophyseal fossa, D. Anterior Pituitary (red), E. Sphenoid bone, F. Pineal gland (green), G. Infundibulum (light purple), H. Posterior Pituitary (blue), I. Basophilic cells, and J. Acidophilic cells.

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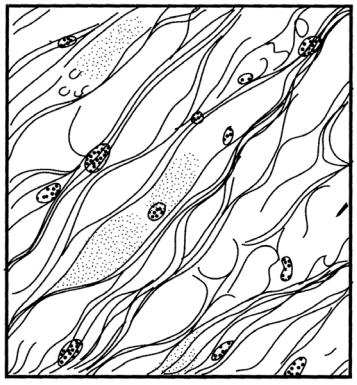

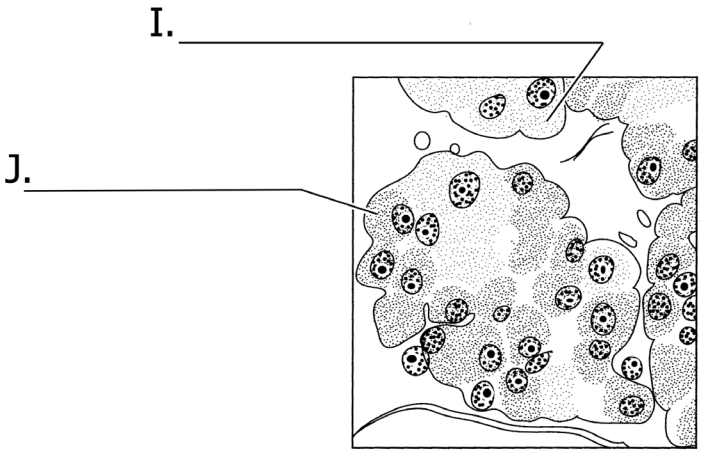

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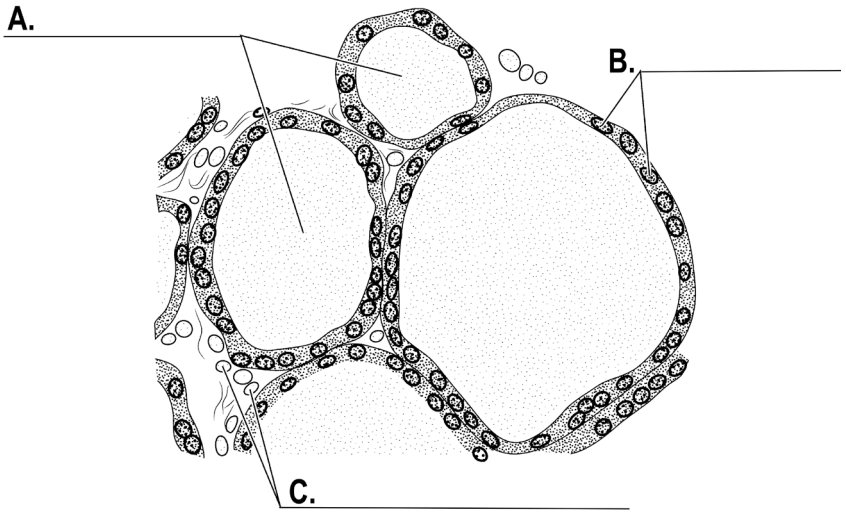

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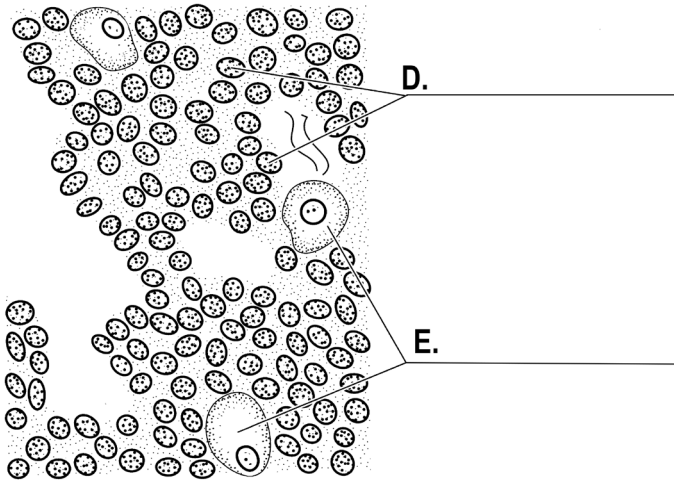

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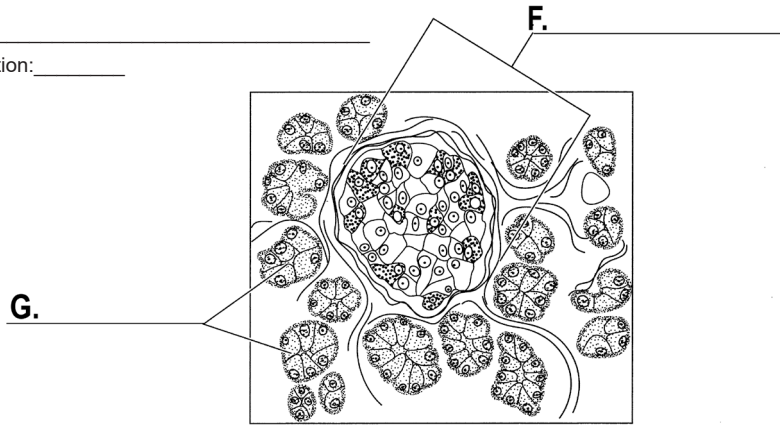

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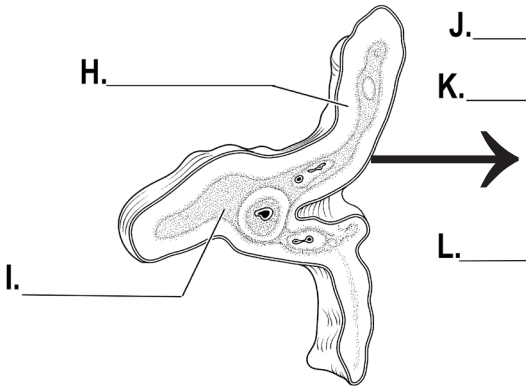

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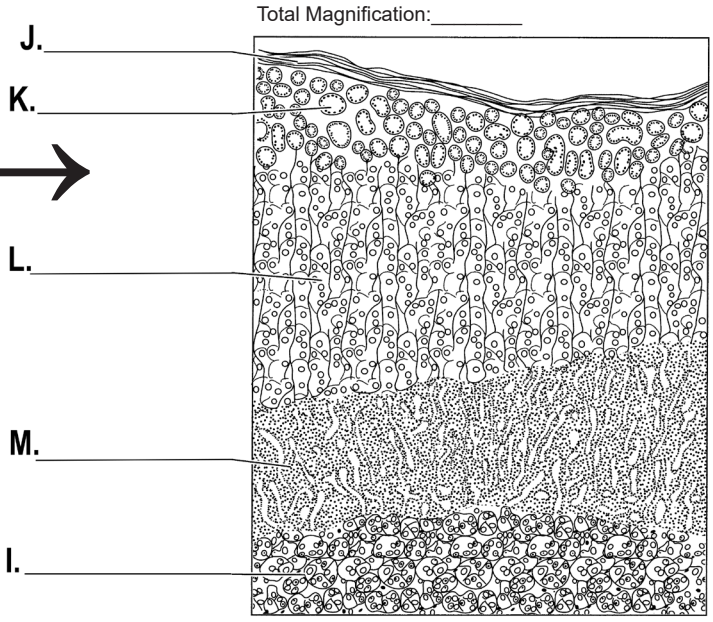
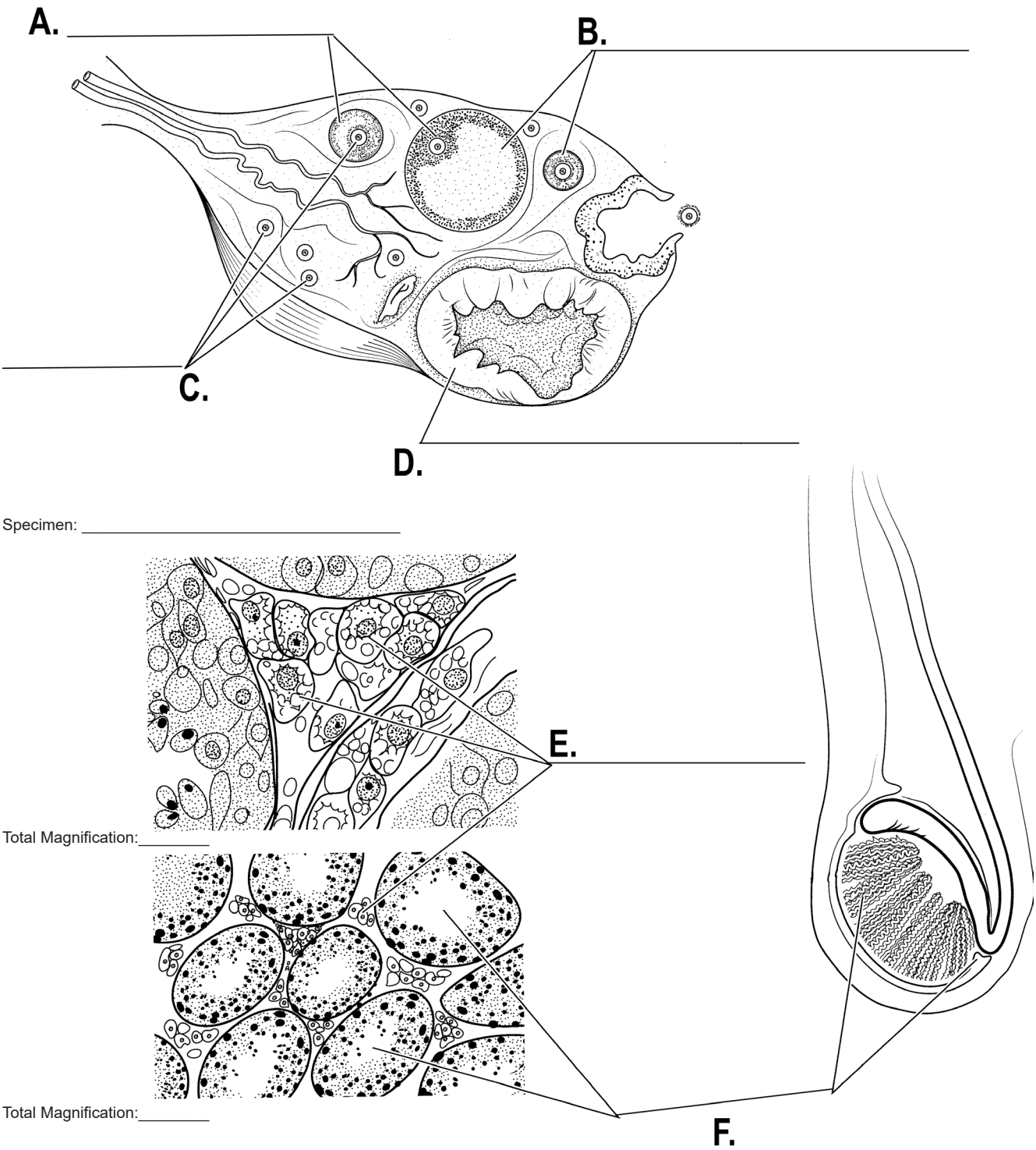


Figure UIV-4. Endocrine system cross-sections with the following structures; A. Colloid (red), B. Follicular cells, C. Parafollicular cells, D. Principal Chief cells(yellow), E. Oxyphilic cells, F. Pancreatic islets G. Acinar cells (light purple), H. Cortex, I. Medulla, J. Capsule, K. Zona glomerulosa, L. Zona fasciculata, and M. Zona reticularis.

Specimen: _____

Total Magnification:_____



Specimen: _____

Total Magnification:_____

Total Magnification:_____

Figure UIV-5. Cross sections of the reproductive glands of the endocrine system with the following structures; A. Granulosa cell (red), B. Ovarian follicles, C. Oocytes, D. Corpus luteum (yellow), E. Interstitial cells, and F. Seminiferous tubules (light purple).

Part 1: Deoxyribonucleic acid

Deoxyribonucleic acid (DNA) contains the genetic instructions specifying the biological development of all cellular forms of life, and most viruses. DNA is a long polymer of nucleotides (a polynucleotide) and encodes the sequence of the amino acid residues in proteins using the genetic code, which consists of a triplet code of nucleotides. In organisms that belong to the domains Archaea and Bacteria, DNA is not separated from the cytoplasm by a nuclear envelope. This is one of the characteristics that distinguish these organisms as prokaryotes. In complex eukaryotic organisms comprised of plants, animals, fungi and protists, most of the DNA is located in the cell nucleus. Plant chloroplasts and the mitochondria of eukaryotic organisms also carry DNA. DNA is often referred to as the molecule of heredity because it is responsible for the genetic propagation of most inherited traits. In humans, these traits can range from hair color to disease susceptibility. Every person's DNA, their genome, is inherited from both parents. The mother's mitochondrial DNA, together with twenty-three chromosomes from each parent, combine to form the genome of a zygote, the fertilized egg. As a result, with certain exceptions such as red blood cells, most human cells contain 23 pairs of chromosomes, together with mitochondrial DNA inherited from the mother. Studies of relatedness in humans are based on the fact that a) mitochondrial DNA is inherited only from one's mother, and b) the male Y chromosome is inherited only from one's father. Ribonucleic acid (RNA) is a polynucleotide that "reads" the information encoded in DNA and directs the synthesis of proteins, which have a variety of functions. Both DNA and RNA consist of nucleotides comprised of a phosphate molecule, a nitrogenous base and a five-carbon sugar, ribose. The nitrogenous bases that form nucleotides in DNA include the purines, adenine (A) and guanine (G), and the pyrimidines, cytosine (C) and thymine (T). In RNA, the pyrimidine uracil (U) replaces thymine.

A. Replication

DNA replication is the process by which a double-stranded DNA molecule is copied to produce two identical DNA molecules. Replication is an essential process because, whenever a cell divides, the two new daughter cells must contain the same genetic information, or DNA, as the parent cell.
The replication process relies on the fact that each strand of DNA can serve as a template for duplication. DNA replication initiates at specific points, called origins, where the DNA double helix is unwound. A short segment of RNA, called a primer, is then synthesized and acts as a starting point for new DNA synthesis. An enzyme called DNA polymerase next begins replicating the DNA by matching bases to the original strand. Once synthesis is complete, the RNA primers are replaced with DNA, and any gaps between newly synthesized DNA segments are sealed together with enzymes.

DNA replication is a crucial process; therefore, to ensure that mistakes, or mutations, are not introduced, the cell proofreads the newly synthesized DNA. Once the DNA in a cell is replicated, the cell can divide into two cells, each of which has an identical copy of the original DNA.

☐ Construct a DNA segment model using 10 nucleotides (5 base-pairs) and their corresponding bonds following the instructions on sheets A-B of the DNA modeling manual.

☐ Replicate 4 base-pairs of the DNA model you constructed by following the instructions on sheets D-E.

3. In Figure UIV-6 label the following:
   A. 3' and 5' ends (boxes)
   B. Helicase
   C. Lagging strand (template strand and synthesized strand)
   D. Leading strand (template strand and synthesized strand)
   E. Ligase
   F. Okazaki fragments (OF)
   G. DNA Polymerase
   H. DNA Primase
   I. RNA primer
   J. Topoisomerase

4. Once you have completed your model and Q3 you must demonstrate the replication process to your TA. **Instructor initials** _____

☐ Reverse your replication and save the resulting DNA molecule model for Part 3.

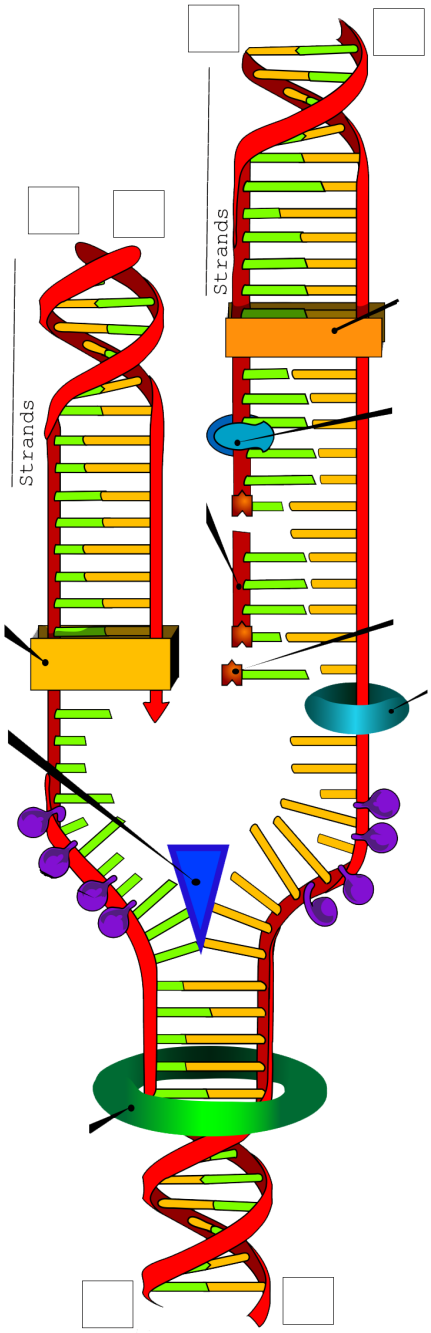


Figure UIV-6. DNA structure and replication.

B. Transcription & Translation

Transcription is the process by which the information in a strand of DNA is copied into a new molecule of messenger RNA (mRNA). DNA safely and stably stores genetic material in the nuclei of cells as a reference, or template. Meanwhile,

mRNA is comparable to a copy from a reference book because it carries the same information as DNA but is not used for long-term storage and can freely exit the nucleus. Although the mRNA contains the same information, it is not an identical copy of the DNA segment, because its sequence is complementary to the DNA template.

Transcription is carried out by an enzyme called RNA polymerase and a number of accessory proteins called transcription factors. Transcription factors can bind to specific DNA sequences called enhancer and promoter sequences in order to recruit RNA polymerase to an appropriate transcription site. Together, the transcription factors and RNA polymerase form a complex called the transcription initiation complex. This complex initiates transcription, and the RNA polymerase begins mRNA synthesis by matching complementary bases to the original DNA strand. The mRNA molecule is elongated and, once the strand is completely synthesized, transcription is terminated. The newly formed mRNA copies of the gene then serve as blueprints for protein synthesis during the process of translation.

Translation is the process by which a protein is synthesized from the information contained in a molecule of messenger RNA (mRNA). During translation, an mRNA sequence is read using the genetic code, which is a set of rules that defines how an mRNA sequence is to be translated into the 20-letter code of amino acids, which are the building blocks of proteins. The genetic code is a set of three-letter combinations of nucleotides called codons, each of which corresponds with a specific amino acid or stop signal. Translation occurs in a structure called the ribosome, which is a factory for the synthesis of proteins. The ribosome has a small and a large subunit and is a complex molecule composed of several ribosomal RNA molecules and a number of proteins. Translation of an mRNA molecule by the ribosome occurs in three stages: initiation, elongation, and termination. During initiation, the small ribosomal subunit binds to the start of the mRNA sequence. Then a transfer RNA (tRNA) molecule carrying the amino acid methionine binds to what is called the start codon of the mRNA sequence. The start codon in all mRNA molecules has the sequence AUG and codes for methionine. Next, the large ribosomal subunit binds to form the complete initiation complex. During the elongation stage, the ribosome continues to translate each codon in turn. Each corresponding amino acid is added to the growing chain and linked via a bond called a peptide bond. Elongation continues until all of the codons are read. Lastly, termination occurs when the ribosome reaches a stop codon (UAA, UAG, and UGA). Since there are no tRNA molecules that can recognize these codons, the ribosome recognizes that translation is complete. The new protein is then released, and the translation complex comes apart.

☐ Perform a transcription on 4 base-pairs of DNA model you built during Part 2 by following the instructions on sheets F-G.

5. Once you have completed your model you must demonstrate transcription to your TA. **TA initials** _____

☐ Deconstruct the DNA model into its individual pieces.

6. Given the coding strand 5'-ATG ATG GTT CCC CTA ACA CCG GAC CAA TAG TAG-3'.
   A. Determine the template strand.
   B. Transcribe the template strand.
   C. Translate the transcription to protein.

Part 2: RFLP Analysis of DNA

Restriction Fragment Length Polymorphism (RFLP) is widely used in genotyping, DNA fingerprinting, mapping of genes, and diagnosis of genetic disorders. Individuals, species, or organisms can be distinguished on the basis of their RFLP pattern. RFLP refers to differences (or variations) in DNA sequences at sites recognized by restriction endonucleases (restriction enzymes). Such variation results in different sized (or length) DNA fragments produced by digesting the DNA with a restriction enzyme. It is a commonly employed molecular tool to check the small but specific variations in a sequence of double-stranded DNA. Restriction endonucleases recognize a set of nucleotides at a restriction site and cleave the DNA at those sites. A specific RFLP pattern emerges during electrophoresis separation of the digested DNA, producing variable lengths of cleavage fragments which are characteristic of a sequence of DNA.

A. PCR

RFLP requires the use of a molecular laboratory technique called **polymerase chain reaction** (PCR). PCR (Fig. UIV-7) rapidly produces/amplifies millions to billions of copies of a specific segment of DNA. The amplified DNA segment can then be studied in greater detail. PCR involves using short synthetic DNA fragments called **primers** to select a segment of the genome allowing multiple rounds of DNA synthesis to amplify that segment. Primers are designed by researchers to target/bind to a specific DNA sequence which then directs the ***Taq* polymerase** to build new DNA starting at the primer location and continuing along the sequence. After the initial elongation the sample is heated again to denature the newly formed DNA duplex, cooled to allow primer binding and extension to happen again. Each time the sample cycles through the different temperatures the amount of DNA doubles. This simple cycle (anneal, extend, denature) is the basis of PCR. By repeating this sequence of heating and cooling many times billions of a specific DNA sequence in a sample are produced in a matter of minutes.

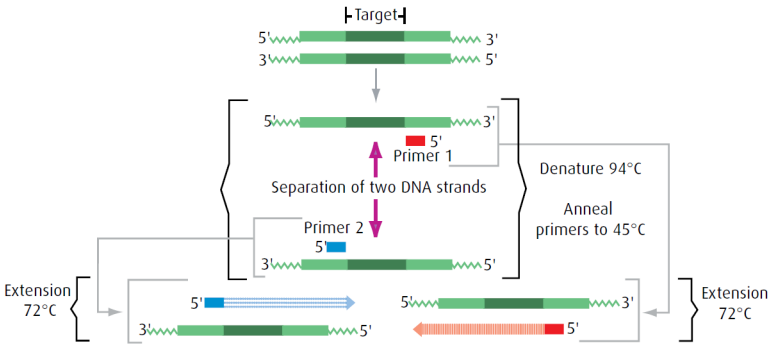


Figure UIV-7. Diagram of the polymerase chain reaction.

B. Restriction Enzymes

After amplification, the DNA is digested with restriction enzymes that cut double-stranded DNA in a sequence-specific manner. Most restriction endonucleases recognize palindromic or partially palindromic sites. For example, EcoRI (pronounced "eco R one") is a 377 amino acid restriction endonuclease enzyme isolated from the bacteria, *Escherichia coli*. It cleaves DNA double helices into fragments at specific sites. EcoRI cuts DNA after G forming sticky ends with AATT (Figure UIV-8).The triangle denotes the site where the phosphodiester bond is broken.

5′... G▼A A T T C ...3′
3′... C T T A A▲G ...5′

Figure UIV-8. A palindrome is defined as dyad symmetry around an axis. The recognition site of EcoRI with triangle indicating the cut site.

C. Electrophoresis

Electrophoresis describes the migration of charged particles under the influence of an electric field. Gel electrophoresis refers to the technique in which molecules are forced by an electric current to move through a gel. Most DNA electrophoresis requires a gel made from agarose. The majority of agar used in culinary and commercial applications is extracted from the cell wall of the red seaweed, *Gelidium amansii*. Agar consists of a mixture of two polysaccharides: agarose and agaropectin. Agarose is a linear polymer, made up of repeating units of agarobiose, a disaccharide made up of D-galactose and 3,6-anhydro-L-galactopyranose. **Because the phosphate groups in the backbone of DNA are negatively charged**, **the entire DNA molecule has a negative charge.** During gel electrophoresis, the **negatively charged DNA molecules move toward the positive pole of the electrophoresis chamber**.

Agarose gel electrophoresis separates molecules into discrete bands, each comprising molecules of the same size. Movement of the molecules is inversely proportional to the size/length of the molecule. ($\log_{10}$ of the molecules length). Larger fragments of DNA move through the gel slowly because they frequently collide with particles in the gel matrix. Smaller fragments of DNA are less likely to collide with particles in the matrix, and therefore move through the gel more quickly. Once the fragments of DNA have been separated by molecular size, their locations within the gel can be determined using a stain. The pattern of stained bands is compared to the pattern produced by other DNA samples. DNA standard markers (molecular weight standards in base pairs) can be used to determine the size of the unknowns running on the gel. It some cases, a standard curve will need to be created to extrapolate the size of each unknown.

7. Why do DNA molecules move towards the negative or positive electrode?

8. What is RFLP analysis and explain how restriction endonucleases function in RFLP analysis?

9. What is PCR, how are primers used in PCR, and explain the basic cycle of PCR? ?

10. What is *Taq* polymerase and what is its function?

11. What does recognition site mean with respect to restriction endonucleases?

12. Explain the movement of DNA fragments during gel electrophoresis?

13. What are DNA standard markers?

Name:_____ Team #:_____ Section #:_____

Part 3: Molecular Analysis of Cancer

Most cancers result from somatic (body cell) mutation during a person's life (Figure UIV-9A). These changes occur either though exposure to mutagens that change the DNA sequence or through errors in DNA replication. Not all change will cause cancer because some changes may occur in non-coding DNA sections or they may be recessive to the function of a gene. Hereditary germline mutations are directly inherited through DNA changes that can be passed form one generation to another as they are present in the gametes (Figure UIV-9B).

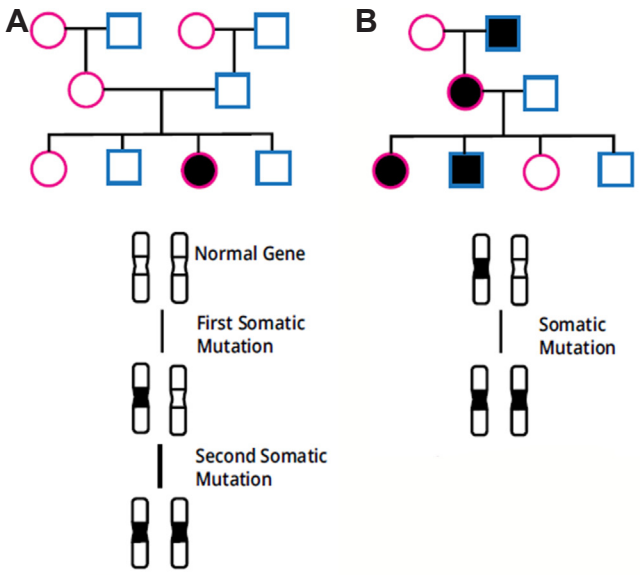


Figure UIV-9. Pedigree models of *TP53* gene inactivation: A) Sporadic somatic mutation that produces single tumors that are usually unilateral and occur after the age of 45, and B) Hereditary germline mutation that produces multiple tumors that can be bilateral and occur before the age of 45.

Cancer genomes or the whole DNA sequence from cancer cells can be analyzed using techniques like RFLP analysis. The *TP53* gene is a tumor suppressor gene that acts as a master regulator for the genes responsible for cell division and death by coding for the p53 protein that functions as a transcriptional regulator. Specific amino acids in the p53 protein allow it to bind to specific DNA sites activating the transcriptions of genes necessary for cell division and cell death. This gene is often called the "Guardians of the Genome" as it plays a major role in preventing changes to the genomic DNA. Just a single mutation in the gene greatly increases a person's susceptibility of cancer and *TP53* is the most commonly mutated gene in cancer.

Figure UIV-10. A palindrome is defined as dyad symmetry around an axis. The recognition site of *Pvu*II with triangle indicating the cut site.

We will determine if an individual has mutations in one or both copies of the *TP53* gene (both *TP53* alleles). Breast cancer is the second most common form of cancer in women and most often occurs in the milk ducts although they can develop in other breast tissue. Besides breast tissue, mutations in the *TP53* gene can also cause cancer in other tissues of the body. *Pvu*II restriction endonuclease enzyme recognizes the *TP53* gene mutant sequence at codon position 165 (Figure UIV-10). The normal DNA at this site can not be cut because it is not recognized by the enzyme. In this exercise; 1) a blood sample, 2) a breast tumor sample, and 3) a healthy breast tissue sample were collected from a female patient that had a precancerous breast lump biopsy. DNA was extracted from the sample, amplified using PCR, and then the DNA was digested with *Pvu*II restriction endonuclease enzyme.

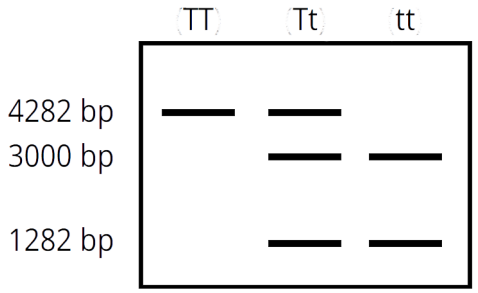


Figure UIV-11. *Pvu*II restriction enzyme. Columns; 1) normal TT p53 alleles 2) Heterozygous Tt p53 alleles, 3) Mutated tt p53 alleles.

When a person is homozygous recessive (tt) for the *TP53* gene mutation it means a mutation has occurred in both alleles (UIV-11 column 3) by either pathways (UIV-9). If the person is heterozygous (Tt) it means that the mutation is a sporadic somatic mutation in a single allele of the gene. The homozygous dominate (TT) indicates that no *TP53* gene mutation has occurred in either alleles (UIV-11 column 1).

A. Gel Preparation

☐ Write you Team number on the top of the first page next to your name.

☐ Place the gel casting tray in the gel chamber so that the rubber gaskets seal against the edges of the unit.

☐ Place the six-tooth well comb in the first set of notches at the end of the tray.

☐ Add the following ingredients to an Erlenmeyer flask: 30ml **Tris Acetate EDTA buffer, Ph 7.8** (1X TAE buffer)

and 0.3 g agarose.

☐ Swirl the mixture to disperse clumps of agarose powder.

☐ Place the flask in the microwave, heat for 10 seconds. Use a hot glove and gently swirl the solution.

☐ Repeat the process two more times to dissolve the agarose particles.

☐ Once the flask has cooled for 4 minutes, pour the agarose mixture into the center of the casting tray.

Caution: Flask and solution will be HOT! Be sure to use protective heat gloves or oven mitts. Use caution when handling the hot agarose solution to avoid boiling over.

☐ Allow the gel to solidify until opaque (approximately 15 minutes).

☐ As you wait for the gel to solidify, practice loading samples into the DNA DuraGel (permanent polymer gel) that is found in the square Petri dish using micropipettes and tips.

☐ After the gel has solidified, gently remove the casting tray (with gel) from the center platform of the electrophoresis chamber, rotate 90 degrees, and place back on the center platform of the chamber.

☐ Pour running buffer (1X TAE buffer) into the chamber. Make sure the gel is covered with 5 mm of buffer.

☐ Carefully remove comb from the gel by pulling it straight up. Do not tear the wells created by the teeth of the comb.

B. Electrophoresis

☐ Position the gel wells farthest from the positive electrode (closest to the black).

☐ Remove the comb.

☐ Using a micropipette:

- Pipette 30 µL of sample your assigned sample into the correct well (Figure UIII-7).
- Discard the pipette tips into the plastic beaker between each sample that is loaded.
- Repeat by pipetting 30 µL of the other assigned samples into the appropriate wells.

☐ After the DNA samples are loaded:

- Insert the plug of the black wire into the black input of the power source (negative input) and insert the plug of the red wire into the red input of the power source (positive input).

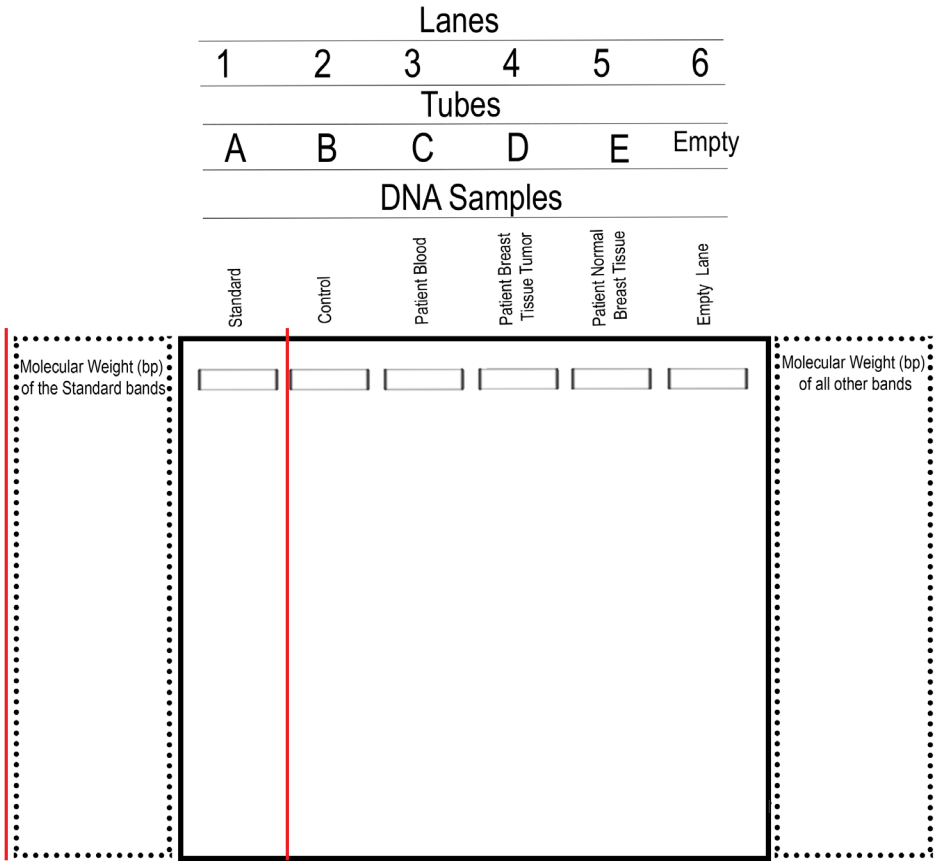


Figure UIV-12. Comparisons of bands produced from RFLP aanlysis of patient DNA samples using PCR, *Pvu*II restriction enzyme, and separated by gel electrophoresis.

- Turn on the electrophoresis power supply and check that it is set at ~134 volts (both blue and white supplies), low (blue supply only), and 25 minutes (white supply only).
- Press the run button (white supply only).

☐ Check that current is flowing by observing bubble formation at the thin wire electrodes.

C. Staining

☐ Remove the casting tray (with the gel) from the electrophoresis chamber and slide the gel off the tray into the round Petri dish lid (wider but not as deep as the dish base).

☐ Pour the 1X TAE buffer from the gel chamber into the labeled 'Reuse Running Buffer' pitcher.

☐ Place the blue dye side of an InstaStain MetBlue card on the gel and run your fingers over the surface several times to ensure good contact between the card and the gel.

☐ Place the Petri dish base on the card and then place a beaker on the base.

☐ After one (1) minute, remove the staining card and move the gel into the Petri dish base.

☐ Destain for 5 minutes by adding 37ºC water from the hot water baths to the Petri dish, submerging the gel. Replace the water in the Petri dish every minute. Discard the used blue water in the sink.

☐ Place the Petri dish with gel in it on the light box and observe the position of the bands.

1. In Figure UIII-7 **sketch in the bands.** The six small boxes at the top of the figure correspond to the wells in which the DNA samples were placed.

☐ Measure the distance the bands traveled using a ruler.

2. In Figure UIII-7 write in the molecular weight (Table UIII-1 columns 4 & 5) of the DNA standard marker bands (lane 1) and then the other bands (lanes 2-5).

3. **Measure the distance** in millimeters (mm) of each band from the well in which the DNA sample was placed. Write the distance on Table UIII-1 (column 6).

☐ Discard the gel in the garbage.

Table UIV-2. Comparisons of bands produced from RFLP analysis of patient DNA samples using PCR, *Pvu*II restriction enzyme, and separated by gel electrophoresis.

Lane	Tube	Sample	Band	Molecular weight in base pairs (bp)	Distance band migrated (mm)
1	A	DNA standard markers	1 2 3 4 5 6 7	6751 3652 2827 1568 1118 825 630	
2	B	Control DNA is unmutated p53 allele	1	4282	
3	C	Patient Peripheral Blood DNA	1 2 3	4282 3000 1282	
4	D	Patient Breast Tumor DNA	1 2	3000 1282	
5	E	Patient Normal Breast Tissue DNA	1 2 3	4282 3000 1282	
6	Empty				

D. Interpretation

4. Open a Word document and at the top right of the document type:
   - Biol 2227L
   - Unit IV - Endocrine System (DNA)
   - Your section #
   - Your team #
   - Names of everyone on your team
5. Type the answers to the following questions into the document:
   A. In detail, describe what *TP53* and p53 are and what each do?
   B. In detail, describe what *Pvu*II is and what it does?
   C. What is the correct order of steps when adding sample to a gel well after you initially draw up sample into the micropipette tip?.
   D. What information did Lane 1 give you. Explain?
   E. What information did Lane 2 give you. Explain?
   F. Describe the location and number of bands in Lanes 3, 4, and 5.
   G. What does the comparison of Lanes 3 and 5 and Lanes 4 and 5 tell you in reference to Fig. UIII-6
   H. In your own words, describe the connection between the endocrine system, hormones, DNA replication, transcription, and translation. Make sure to name specific glands and hormones.

☐ **Send the document to your lab instructor using your ISU Google Account Gmail.**

Name:_____ Team #:_____ Section #:_____

Part 4: The Cell Cycle

In **diploid** organisms each body (**somatic**) cell contains two copies of the genome. Thus, each somatic cell contains two copies of each chromosome, and two copies of each gene. The exceptions to this rule are the sex chromosomes that determine sex in a given species. For example, in the XY system that is found in most mammals males have one X chromosome and one Y chromosome (XY) and females have two X chromosomes (XX). The paired chromosomes that are not involved in sex determination are called **autosomes**, to distinguish them from the sex chromosomes. Human beings have 46 chromosomes: 22 pairs of autosomes and one pair of sex chromosomes (X and Y). A cell cycle is a series of events that takes place in a cell as it grows and divides. A cell spends most of its time in what is called interphase (G_1, S, and G_2 stages of interphase), and during this time it grows, replicates its chromosomes, and prepares for cell division. The cell then leaves interphase, undergoes mitosis, and completes its division. The resulting cells, known as daughter cells, each enter their own interphase and begin a new round of the cell cycle. Cell cycle has different stages called G_1, S, G_2, and M. G_1 is the stage where the cell is preparing to divide. To do this, it then moves into the S phase where the cell copies all the DNA. So, S stands for DNA synthesis. After the DNA is copied and there's a complete extra set of all the genetic material, the cell moves into the G_2 stage, where it organizes and condenses the genetic material, or starts to condense the genetic material, and prepares to divide. The next stage is M. M stands for mitosis. This is where the cell actually partitions the two copies of the genetic material into the two daughter cells. After M phase completes, cytokinesis occurs resulting in two identical cells.

1. Fill in Table UIV-3 with the correct information.

Table UIV-3. Distinguishing characteristics of **mitotic** cellular division.

How many chromosomes does each human cell contain?_____	
DNA replication occurs during the _____ phase of _____.	
Characteristic	**Mitosis**
Location of the dividing cells?	
How many cellular divisions?	
Homologous chromosome bivalents are formed?	
Sister chromatids pair up?	
Crossing over occurs?	
Sister chromatids line up on the metaphase plate?	
Bivalents line up on either side of the metaphase plate?	
Sister chromatids separate?	
Number of daughter cells?	
Ploidy (haploid or diploid) level of daughter cells?	

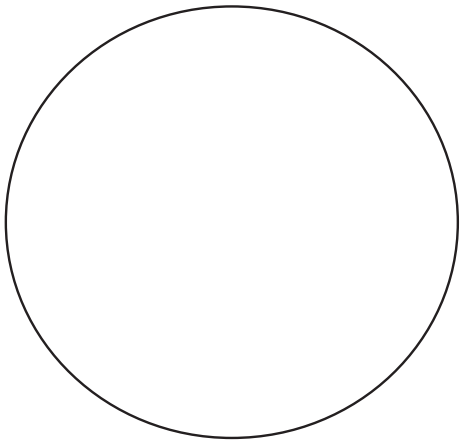
☐ Working in your assigned team, match each image to the correct description sheet.

2. Turn your task sheets into you instructor as a team before you present your matching. Each group member must participate in the following:
   A. Describe what each phase is.
   B. Point out the structures listed on the image sheets.
   C. **Instructor initials**_____.

A. Onion Root Tips & Whitefish Cells

☐ View the onion and crayfish/white fish specimen slides using the 4X, 10X, then 40X objective lenses. NOTE: on the onion slide the **apical meristem** is near the tip of the root behind the root cap.

3. Sketch each at the **40X** objective and label the following:
   A. The stages of mitosis and look carefully for evidence of cytokinesis.
   B. Specimen, Species, Domain, and Kingdom names.
   C. Total magnification.



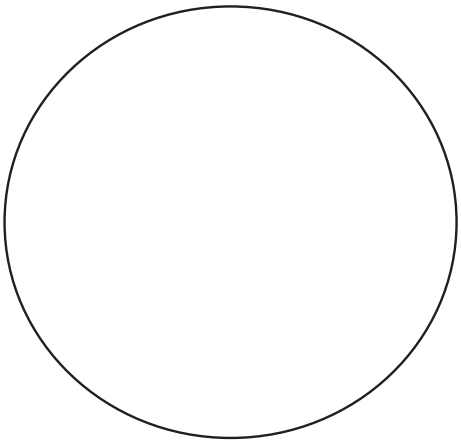
Specimen:_____

Species::_____

Domain:_____

Kingdom: _____

Total Magnification _____



Specimen:_____

Species::_____

Domain:_____

Kingdom: _____

Total Magnification _____

B. Duration of the Cell Cycle

The way in which biologists know the amount of time a cell spends in various stages of the cell cycle is by determining the frequency of cells at each stage in a cell population. You will determine the lengths of the phases of the onion root tip cell cycle, which is on average 16 hours (960 minutes) long.

☐ Obtain a prepared slide of and onion root tip.

☐ Select a field of view in the apical meristem region and focus using the 40X objective.

☐ **Count the number of cells in each phase of the cell cycle and enter these numbers into the correct column of Table UIV-4 and in Cell Cycle workbook on Canvas. Repeat for three non-overlapping fields of view. Save the workbook.**

Table UIV-4. Number of cells from three different fields of view in each cell cycle phase.

	field 1	field 2	field 3	total
interphase				
prophase				
prometaphase				
metaphase				
anaphase				
telophase				

D. Interpretation

4. Open a Word document and at the top right of the document type:
   - Biol 2227 Unit IV - Endocrine System (Cell Cycle)
   - Your section #
   - Your team #
   - Names of everyone on your team
5. Insert the graph into the document.
6. Create a figure caption BELOW the graph.
7. Type the answers to the following questions into the Word document:

   A. Why is mitosis important to both unicellular and multicellular eukaryotic organisms?
   B. Do chromosomes exist during interphase? Explain.
   C. Why are the activities of G1 important for a cell preparing to divide?
   D. The centrosome is the major microtubule-organizing centre in eukaryotic cells, being comprised of two centrioles surrounded by an electron-dense matrix. Why does it need to self-replicate during the cell cycle?
   E. Indicate if each statement is true of the G1, S, G2, or M phase of the cell cycle. A given statement may be true of one, several or none of the phases.
      - The amount of nuclear DNA in the cell doubles.
      - The nuclear envelope breaks into fragments.
      - Sister chromatids separate from each other.
      - Chromosomes are present as diffuse, extended chromatin.
      - This phase is part of interphase.
   F. Type this sentence with the missing words included: During the S phase a chromosome is duplicated to produce two sister _____ (identical copies formed by the DNA replication of a chromosome), each is called a _____ and they are connected at the _____. They are still in a loose less-condensed _____ form.
   G. In your own words, describe the connection between the endocrine system, hormones, and the cell cycle. Make sure to name specific glands and hormones.

☐ **Send the document to your lab instructor using your ISU Google Account Gmail.**.

Part 5: Blood Cell Cycle

Stem have the potential to develop into many different types of cells in the body. They have asymmetric cell division that results in daughter cells with their own unique life course. One of the daughter cells has a *finite capacity* for mitosis and begins to differentiate, whereas the other daughter cell remains a stem cell with *unlimited proliferative* ability. Progenitor cells are early descendants of stem cells that can divide and differentiate to form one or more kinds of cells, but cannot divide indefinitely. Mammalian blood cells are produced in the bone marrow by multipotent stem cells called hematopoietic cells that can go through mitosis to produce more hematopoietic cells or progenitor cells that will differentiate into a variety of blood cells. Both bone marrow and blood are considered connective tissue. The whole blood of humans (*Homo sapiens*) contain red blood cells, white blood cells, and platelets (~45% of volume) suspended in plasma (~55% of volume). **Red blood cells (RBCs, erythrocytes)** carry oxygen using a complex iron containing protein called **hemoglobin**. RBCs carry oxygen from the lungs to your body's tissue and also take carbon dioxide back to your lungs to be exhaled. Once mammalian hematopoietic cells (including those of humans) differentiate into RBCs the organelles disintegrate. This means that the RBCs do not have a nucleus or mitochondria, whereas all other vertebrates do have those organelles. Because of this, mature mammalian RBCs do not go through mitosis and cannot use the oxygen they transport. Instead, RBCs produce ATP through glycolysis and lactic acid fermentation. **White blood cells (WBCs, leukocytes)** are the cells of the immune system that are involved in protecting the body against both infectious disease and foreign invaders. Leukocytes are found throughout the body, including the blood and lymphatic system. **Platelets** (a type of **thrombocyte**) are small, colorless cell fragments in the blood whose main function is to interact with clotting proteins to stop or prevent bleeding. **Plasma** is a fluid, composed of about 92% water, 7% vital proteins, clotting factors, and 1% mineral salts, sugars, fats, hormones and vitamins. **Serum** is the blood plasma not including the clotting factors.

8. Observe the blood model:
   A. Sketch a representative of each of the seven types of blood cells depicted.
   B. Circle the ones that are leukocytes (white blood cells).
   C. Which ones produce B cells.

Erythrocyte (red blood cell) Lymphocyte Monocyte Neutrophil Eosinophil Basophil Thrombocyte

9. View each blood slide using the **40X** objective lens. Sketch each and label the following:
   A. RBC, WBC, RBC nucleus where possible.
   B. Specimen, Species, Class, Kingdom, and Domain names.
   C. Total magnification.

10. What class of tissue do both bone marrow and blood belong? _____

Specimen: _____

Species: _____

Class: _____

Kingdom:_____

Domain:_____ Total Magnification _____

Specimen: _____

Species: _____

Class: _____

Kingdom:_____

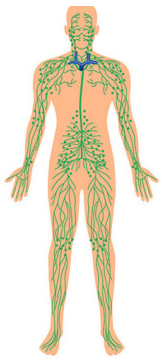
Domain:_____ Total Magnification _____

☐ Each group will be given a set of hematopoiesis cards to learn the order of blood cell division and maturation.

☐ Arrange your cards left to right with all the cards branching from the stem and progenitor cells.

11. Turn your task sheets into you instructor as a group before your arrangement. Each group member must participate in the following:
   A. Describe what each step is.
   B. Point out the structures listed on the image sheets.
   C. Instructor initials_____.

Unit V - Immune & Lymphatic Systems



Lymphatic System

Objectives

- Learn about the immune and lymphatic systems.
- Learn about meiosis and genetics.
- Connect the Rh and ABO blood types to genetics and the immune and lymphatic systems.
- Explain simple genetic dominance using Punnett squares of blood types.
- Use statistical null hypothesis testing for monohybrid and dihybrid crosses of blood types

Terms & Definitions

Agglutinate - the process that occurs if an antigen is mixed with its corresponding antibody.

Appendix - a narrow, finger-shaped pouch that projects out from the colon.

Chi-square test - a statistical hypothesis test where the sampling distribution of the test statistic is a chi-squared distribution when the null hypothesis is true.

Cross:
A. Monohybrid - A cross that studies one pair of alleles at one genetic locus that is used to determine the dominance relationship between two alleles.
B. Dihybrid - A cross that studies two pairs of alleles at two genetic loci on two separate chromosomes that is used to determine the dominance relationship between the four alleles.

Dendritic Cells - a type of antigen-presenting cell (APC) that form an important role in the adaptive immune system.

Frequency - the number, proportion, or percentage of items in a particular category in a set of data:
A. Allele - the number of copies of a particular allele in a population divided by the total number of alleles in that population; the relative proportion of all alleles that are of a designated type.
B. Genotype - the number of copies of a particular genotype in a population divided by the total number of individuals in that population; the proportion of individuals within a population that are of a specific genotype.

Gene - a unit of heredity that contributes to the characteristics or traits of an organism. At the molecular level, a gene is composed of organized sequences of DNA. Every person has two copies of each gene, one inherited from each parent:
A. Equilibrium - is a condition where a gene pool is not changing in frequency across generations.
B. Expression - gene function both at the level of traits and at the molecular level.
C. Flow - occurs when individuals migrate between different populations and results in changes in the genetic composition of the resulting populations.
D. Pool - all of the genes found in a population.
E. Sex-linked - refers to genes that are found on one sex chromosome but not the other.
F. X-linked - a gene found on the x chromosome but not on the y.
G. Y-linked - a gene found on the y chromosome but not on the x.

Genotype - the alleles or variants an individual carries for a particular gene:
A. Allele - one of two or more versions of DNA sequence (a single base or a segment of bases) at a given gene locus. An individual inherits two alleles, one from each parent, for any given gene where such variation exists. If the two alleles are the same, the individual is homozygous for that allele. If the alleles are different, the individual is heterozygous.
B. Heterozygous - two different alleles at the same gene.
C. Homozygous genotype - two identical alleles at the same gene.

Genetic drift - the random changes in a population's allele frequencies from one generation to the next that is attributed to chance. It occurs more quickly in small populations:
A. Bottleneck - an effect caused by adverse environmental conditions.
B. Founder - an effect caused by geographic separation of a subset of the population.

Dominant - a term that describes the displayed trait in a heterozygote:
A. Co - an allele from each parent is expressed in the offspring and the phenotypes of both parents are simultaneously expressed in the same offspring.
B. Complete - the effect of one allele in a heterozygous genotype completely masks the effect of the other. The allele that masks the other is said to be dominant to the latter, and the allele that is masked is said to be recessive to the former.

Immune system - a complex network of cells and proteins that defends the body against infection.
A. Cell-mediated immunity - A form of adaptive immunity that involves the destruction of infected cells by T lymphocyte cells, or the destruction of intracellular pathogens by macrophages.
B. Adaptive "acquired' immunity - One subsystem (of two) of the immune system that is composed of specialized, systemic B & T lymphocyte cells, and processes that eliminate pathogens or prevent their growth. Creates a stronger immune

response that the immune system remembers by recognizing antigens.
C. Antibody - a protein secreted by plasma cells that is part of the immune system response; antibodies travel all over the body to reach antigens identical to those that stimulated their production, combine with these antigens, then guide the attack that eliminates the antigens or the cells bearing them.
D. Antigen - any foreign molecule that the host does not recognize as self and that triggers a specific immune system.
E. Antibody-mediated immunity - A form of adaptive immunity that involves the activation of B lymphocyte cells and secretion of antibodies when in contact with a pathogen.
F. Immunity - the ability of an animal to ward off internal threats, including microorganisms, foreign molecules, and abnormal cells such as cancer cells.
G. Innate “nonspecific” immunity - One subsystem (of two) of the immune system that is inborn and does not depend upon previous exposure to an antigen. Immunoglobulin - a y-shaped protein with two heavy chains and two light chains that provides immunity to foreign substances: antibodies are a type of immunoglobulin.

Lymphatic System - a network of tissues, vessels and organs that work together to move a colorless, watery fluid called lymph back into your circulatory system (your bloodstream):
A. Lacteals - the lymphatic vessels of the small intestine which absorb digested fats.
B. Lymph - a colorless fluid containing white blood cells, which bathes the tissues and drains through the lymphatic system into the bloodstream.
C. Lymph Nodes - a kidney-shaped organ of the lymphatic system, and the adaptive immune system
D. Lymph Nodule - small, localized collection of lymphoid tissue.
E. Lymphatic Vessels - the network of capillaries (microvessels) and a large network of tubes located throughout your body that transport lymph away from tissues. Lymphatic vessels collect and filter lymph (at the nodes) as it continues to move toward larger vessels called collecting ducts.
F. Lymphoid - relating to or denoting the tissue responsible for producing lymphocytes and antibodies. This tissue occurs in the lymph nodes, thymus, tonsils, and spleen, and dispersed elsewhere in the body.
G. Lymphoid cells - any of the cells that mediate the production of immunity, including lymphocytes, lymphoblasts, and plasma

Meiosis - the process by which haploid (1n) cells are produced from a cell that was originally diploid (2n):
A. I - the separation of homologous chromosomes but the sister chromatids remain together resulting in two haploid cells (1n); occurs only in germ cells.
B. II - similar to a mitotic division but the connected sister chromatids remaining from meiosis I will separate to form four haploid cells.

Heritability - the amount of variation for a trait in the population that can be explained by differences in genes among individuals.

Phenotype - physical and physiological traits of an individual.

Population - a group of individuals of the same species that occupy the same environment and can interbreed with one another:
A. Biological - individuals of the same species that live and breed in the same geographic area.
B. Ecological - the study of how populations grow and what factors promote or limit growth.
C. Genetics - study of the factors in a population that determine allele frequencies and their change over time.

Polymorphism - the phenomenon that many traits or genes may display variation within a population.
A. Balanced - the phenomenon in which two or more alleles are kept in balance and maintained in a population over the course of many generations. The gene pools of most populations contain a number of deleterious alleles that reduce the overall fitness of a population.
B. Deleterious - a mutation that reduces the fitness of a variant.
C. Fitness - relative survival and reproduction of one variant compared to another in a population.

Principles of:
A. Independent assortment - states that the alleles of different genes assort independently of each other during gamete formation.
B. Segregation - separation of pairs of alleles during the production of gametes. Results in a 50% probability that a given gamete contains one allele rather that the other.
C. Uniformity - heterozygotes share a common phenotype due to dominance
D. Hardy-Weinberg - predicts an equilibrium if no new mutations are formed, no natural selection occurs, the population size is very large, the population does not migrate, and mating is random.

Punnett square - a common method for predicting the outcome of simple genetic crosses.

Recessive - applies to an allele with an effect that is not visible in a heterozygote.

Recombination - recombination occurs randomly in nature as a normal event of meiosis and is enhanced by the phenomenon of **crossing over**, in which gene sequences called linkage groups are disrupted, resulting in an exchange of segments between paired chromosomes that are undergoing separation.

Reproductive terms - the tissues and cells used in reproduction.
A. Egg cell - gamete produced by a female organism.
B. Embryo - the developmental stage commencing after the first mitotic divisions of the zygote and ending when body structures begin to appear.
C. Fertilization - the union of two gametes, such as an egg cell with a sperm cell, to form a zygote.
D. Gamete - a haploid cell that is involved with sexual reproduction, such as a sperm or egg cell.
E. Germ cells - any biological cell that gives rise to the gametes of an organism that reproduces sexually.

F. Gonad - a reproductive, sex, mixed gland that produces the gametes and sex hormones of an organism.
G. Oocytes - an immature ovum, or egg cell. An oocyte is produced in the ovary during female gametogenesis. The female germ cells produce a primordial germ cell (PGC), which then undergoes mitosis, forming oogonia. During oogenesis, the oogonia become primary oocytes.
H. Ovarian Follicles - a roughly spheroid cellular aggregation set found in the ovaries. It secretes hormones that influence stages of the menstrual cycle.
HI. Ovaries - an organ found in the female reproductive system that produces an ovum and secrete hormones that play a role in the menstrual cycle and fertility.
J. Ovum - the female reproductive cell, or gamete, egg
K.Sexual reproduction - a process that requires a fertilization event in which two gametes unite to produce a cell called a zygote.
L. I. Sperm cell - gamete produced by a male organism.
M. Seminiferous Tubules - located within the testes, and are the specific location of meiosis, and the subsequent creation of male gametes, namely spermatozoa.
N. Zygote - a diploid cell formed by the fusion of two haploid gametes.

Synapsis - the pairing of two chromosomes that occurs during meiosis. It allows matching-up of homologous pairs prior to their segregation, and possible chromosomal crossover between them. Synapsis takes place during prophase I of meiosis.

Tonsils - a set of lymphoid organs facing into the digestive tract, which is known as Waldeyer's tonsillar ring and consists of the adenoid tonsil, two tubal tonsils, two palatine tonsils, and the lingual tonsils. These organs play an important role in the immune system.

Variation - refers to the differences or deviations from the recognized norm or standard.
A. Continuous - a range of slightly different values for a trait in a population.
B. Genetic - differences in alleles that exist among individuals in a population.

White Blood Cells - also called leukocytes or leucocytes, are the cells of the immune system that are involved in protecting the body against both infectious disease and foreign invaders. All white blood cells are produced and derived from multipotent cells in the bone marrow known as hematopoietic stem cells. Leukocytes are found throughout the body, including the blood and lymphatic system.

Evolution - the phenomenon that populations of organisms change from one generation to the next. As a result, some organisms become more successful at survival and reproduction.
A. Convergent - the process whereby two different species from different lineages show similar characteristics because they occupy similar environments.
B. Divergent evolution - the process whereby two different species from the same lineages show different characteristics because they occupy different environments.

Natural selection - the process that eliminates those individuals that are less likely to survive and reproduce in a particular environment, while allowing other individuals with traits that confer greater reproductive success to increase in numbers.
A. Balancing - a type of natural selection that maintains genetic diversity in a population.
B. Diversifying - natural selection for individuals at both ends of a range of phenotype but against the "average" phenotype.
C. Sexual - a type of natural selection that is directed at certain traits of sexually reproducing species that make it more likely for individuals to find or choose a mate and/or engage in successful mating.
D. Stabilizing selection - a pattern of natural selection that favors survival of individuals with intermediate phenotypes.

Phylogeny - evolutionary history of a group of organisms:
A. Monophyletic – a group of species, a taxon, consisting of the most recent common ancestor and all of its descendants.
B. Paraphyletic – a group of organisms that contains a common ancestor and some, but not all, of its descendants.

Spatial isolation - a mechanism for reproductive isolation that depends on the geographic separation of populations

Trait/character - a characteristic of an organism, such as the appearance of seeds, flowers, or stems; an identifiable characteristic; refers to a variant:
A. Adaptive - a genetic trait that helps an organism to maximize its reproductive success
B. Homologous - a feature that is similar across species because of common decent. Homologous traits may begin to look different form one another over time.
C. Quantitative - a trait that shows continuous variation over a range of phenotypes.
D. Shared derived - a trait that is shared by a group of organisms but not by a distant common ancestor.
E. Shared ancestral - a trait shared with a distant ancestor.

Background

Immune System

The immune system (Table UV-1 and Figure UV-1) is a complex functional network of biological processes and reactions of the body that are closely connected to the endocrine and nervous systems. If an immune response cannot be activated when there is sufficient need, problems arise, like infection. On the other hand, when an immune response is activated without a real threat or is not turned off once the danger passes, different problems arise, such as allergic reactions and autoimmune disease. There are two general branches of the immune system; innate and adaptive immunity. The overall function of the **innate "nonspecific" immunity** is to prevent or limit infection by recognizing; **1)** danger-associated molecular patterns (DAMPs) that distinguish between healthy and unhealthy cells (cells may be unhealthy because of infection or because of cellular damage caused by non-infectious agents like sunburn or cancer), and **2)** pathogen-associated molecular patterns (PAMPs) release by infectious microbes such as viruses and bacteria. This branch of the immune system is dependent on; **a)** physical barriers of the skin and mucous membranes and their secretions, **b)** innate leukocytes (macrophages, neutrophils, and dendritic cells) that identify and eliminate pathogens, either by attacking larger pathogens through contact or by engulfing and then killing microorganisms, **c)** inflammation, and **d)** the complement of ~30 plasma proteins that are normally inactive, but in response to the recognition of molecular components of microorganisms they become sequentially activated in an enzyme cascade. **Adaptive immunity** (Figure UV-1) creates a stronger immune response that the immune system remembers by recognizing antigens. The major cells of the adaptive immune system are special types of leukocytes, called B and T lymphocytes (B-cells and T-cells). T-cells are involved in the **cell-mediated immunity** and B-cells in **antibody-mediated immunity**.

Lymphatic System

The lymphatic system (Table UV-1, Figures UV-2 & 3) has three primary functions; **1)** it returns excess interstitial fluid to the blood. Of the fluid that leaves the circulation system capillaries, about 90 percent is returned. The 10 percent that does not return becomes part of the interstitial fluid that surrounds the tissue cells. Small protein molecules may "leak" through the capillary wall and increase the osmotic pressure of the interstitial fluid. This further inhibits the return of fluid into the capillaries, and fluid tends to accumulate in the tissue spaces. If this continues, blood volume and blood pressure decrease significantly and the volume of tissue fluid increases, which results in edema (swelling). Lymph capillaries pick up the excess interstitial fluid and proteins and return them to the venous blood of the circulation system. After the fluid enters the lymph capillaries, it is called lymph. **2)** It absorbs fats and fat-soluble vitamins from the digestive system and the subsequent transport of these substances to the venous circulation. The mucosa that lines the small intestine is covered with fingerlike projections called villi. There are blood capillaries and special lymph capillaries, called lacteals, in the center of each villus. The blood capillaries absorb most nutrients, but the fats and fat-soluble vitamins are absorbed by the lacteals. The lymph in the lacteals has a milky appearance due to its high fat content and is called chyle. **3)** The most well known function is defense against invading microorganisms and disease. It is composed of **primary lymphoid organs**: bone marrow and the thymus. They create special immune system cells called lymphocytes. **Secondary lymphoid organs** include lymph nodes, spleen, tonsils, and mucosa associated lymphoid tissue arranged as a series of filters that monitor the contents of the interstitial fluid. Lymph fluid that is forced out of the bloodstream during normal circulation is filtered through lymph nodes to remove bacteria, abnormal cells, and other matter. This fluid is then transported back into the circulation system via the lymph vessels. Lymph only moves in one direction, toward the heart.

Table UV-1. Distinguishing characteristics of the immune and lymphatic systems and their components.

Component	Organ Type	Function
Red bone marrow	Primary	Major hematopoietic stem cell tissue in the bone marrow gives rise to two main types of cells: myeloid and lymphoid lineages. These include monocytes, macrophages, neutrophils, basophils, eosinophils, erythrocytes, dendritic cells, and megakaryocytes, or platelets, as well as T-lymphocytes (T-cells) and B-lymphocytes (B-cells), and natural killer(NK) cells.
Thymus gland	Primary	Programs the immune system to recognize self, and provides a site for maturation of the T-cells and produces thymosin hormone which stimulates the maturation of lymphocytes in other lymphatic organs.
Spleen	Secondary	Traps foreign material and remove damages and ages erythrocytes, while also acting as a reservoir for erythrocytes, leukocytes, and platelets
Lymph nodes	Secondary	Filter lymph, trapping foreign material such as bacterial and viral particles
Tonsils	Secondary	Lymphocytes and macrophages in the tonsils provide protection against harmful substances and pathogens that may enter the body through the nose or mouth.
Mucosa associated lymphoid tissue (MALT)	Secondary	Plays a role in regulating submucosal membrane immunity; gastrointestinal tract (ex. Peyer's patches), nasopharynx, thyroid, breast, lung, salivary glands, eye, and skin. Populated by lymphocytes such as T cells and B cells, plasma cells, and macrophages that situated to encounter antigens passing through the mucosal epithelium.
Lymphatic vessels		Carry fluid away from the tissues. The smallest lymphatic vessels are the lymph capillaries, which begin in the tissue spaces as blind-ended sacs. Lymph capillaries are found in all regions of the body except the bone marrow, central nervous system, and tissues, such as the epidermis, that lack blood vessels. The wall of the lymph capillary is composed of endothelium in which the simple squamous cells overlap to form a simple one-way valve. This arrangement permits fluid to enter the capillary but prevents lymph from leaving the vessel.
Lymph (Lymphatic fluid)		A connective tissue that is a transparent fluid found in the lymph vessels that is derived from the interstitial fluid.
Interstitial fluid		Found in the spaces around cells. It comes from substances that leak out of blood capillaries of the circulation system and removed via the lymphatic system.

Name:_____ Team #:_____ Section #:_____

1. Figure UV-1, label and color the structures listed in the figure caption.

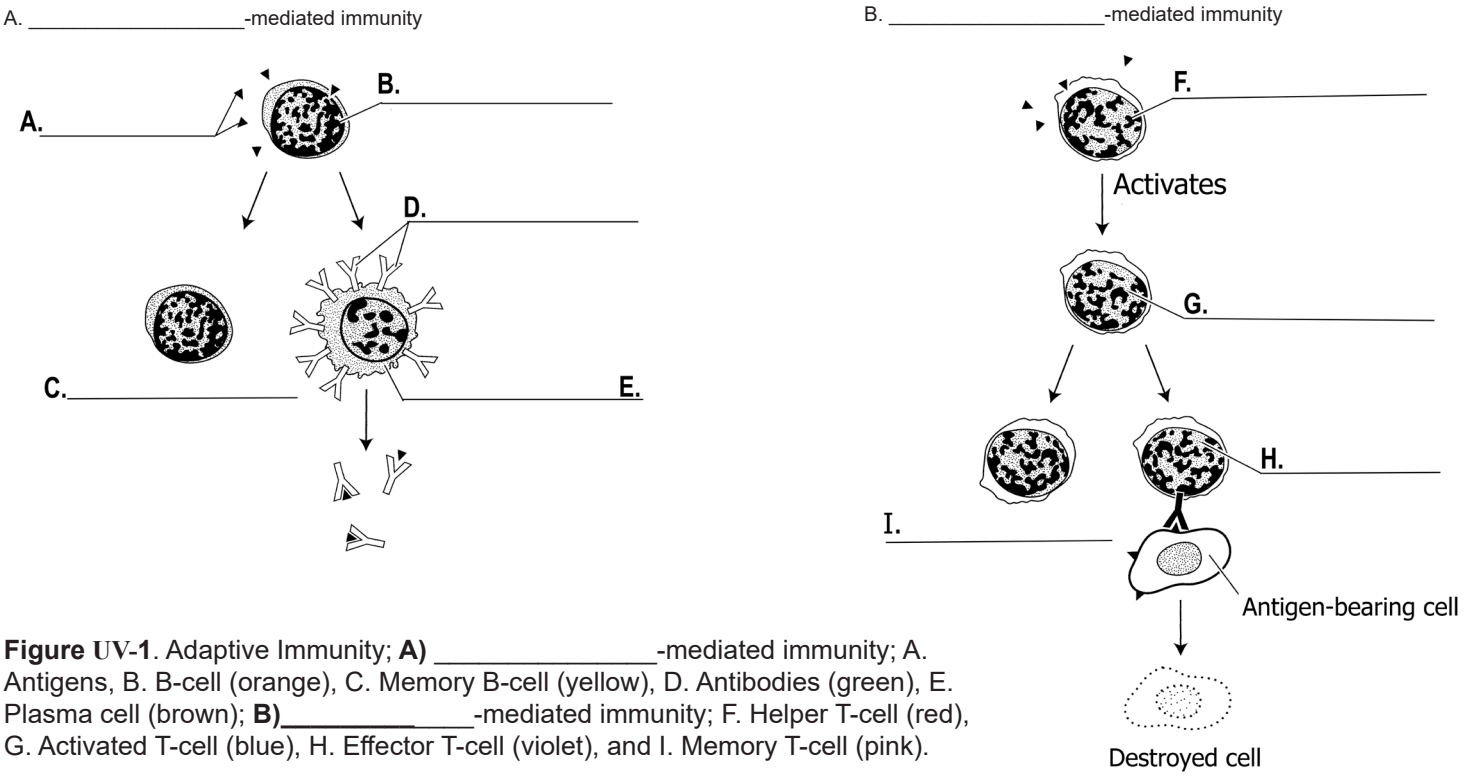


Figure UV-1. Adaptive Immunity; **A)** _____-mediated immunity; A. Antigens, B. B-cell (orange), C. Memory B-cell (yellow), D. Antibodies (green), E. Plasma cell (brown); **B)**_____-mediated immunity; F. Helper T-cell (red), G. Activated T-cell (blue), H. Effector T-cell (violet), and I. Memory T-cell (pink).

2. Figure UV-2:
   A. Label and color the structures listed in the figure caption
   B. Draw in and label the cisterna chyli using the model found in the lab.
   C. Define cisterna chyli.
3. Figure UV-3, label and color the structures listed in the figure caption.

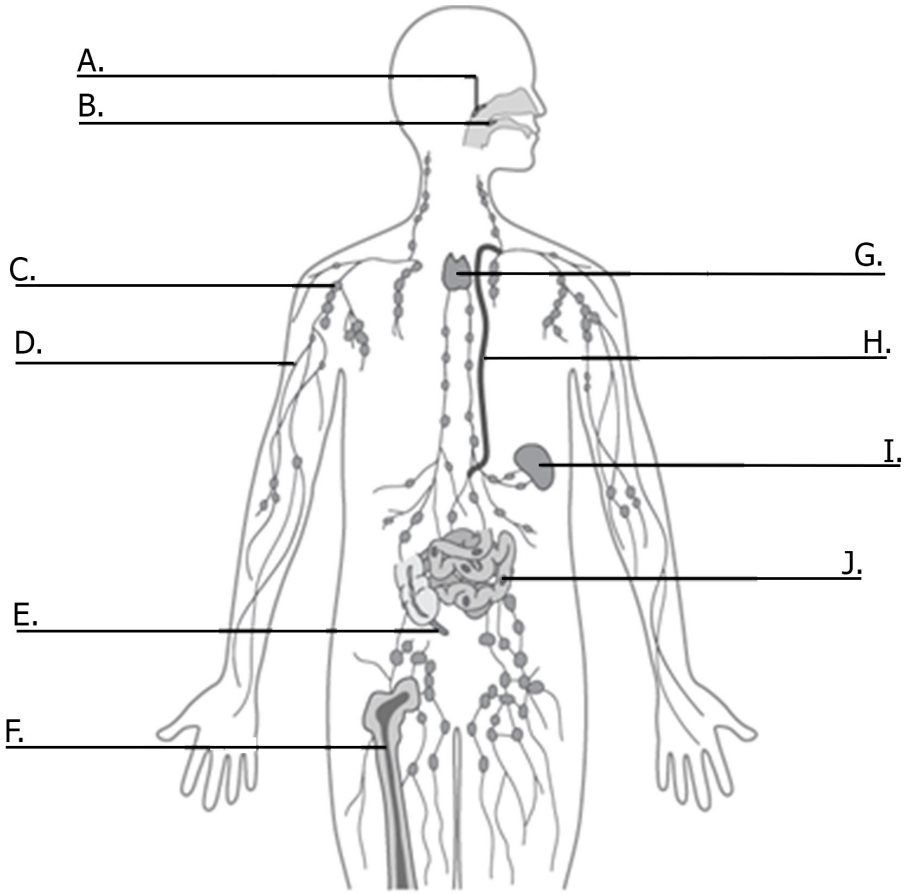
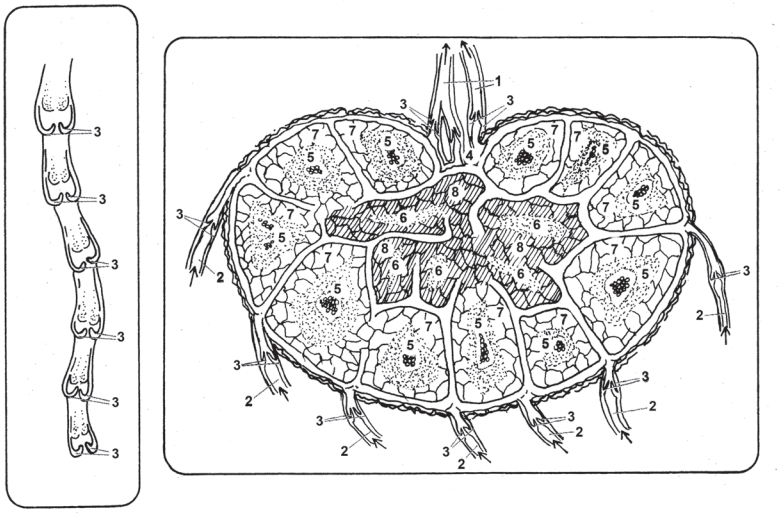


Figure UV-2. Lymphatic system; A. Adenoids, B. Tonsils, C. Lymph node, D. Lymph vessels, E. Appendix (green), F. Bone marrow (red), G. Thymus (blue), H. Thoracic duct, and I. Spleen (pink), and J. Peyer's patches.



1. Efferent lymphatic vessel (red)
2. Afferent lymphatic vessel (blue)
3. Lymphatic valve (pink)
4. Hilum (purple)
5. Primary follicle (green)
6. Medullary cord (orange)
7. Cortex (yellow)
8. Medulla (brown)

Figure UV-3 Lymph; **A)** vessels and **B)** node cross-section.

Part 1: Patterns of Inheritance

The process of sexual reproduction involves two parents, each contributing one gamete. Gametes are produced by a process called meiosis, which starts by the duplication of the chromosomes, followed by two rounds of cell divisions and halving of the chromosome number. Gametes have half the chromosome number of other adult cells of an organism. Sexual reproduction has two processes that maximize diversity in a species. The different forms of a **gene** that are found at a specific point (or locus) along a given chromosome are known as alleles. Diploid organisms have two alleles for each autosomal gene - one inherited from the mother, one inherited from the father. One crucial process is that diploid cells give rise to unique haploid cells through genetic recombination between homologous chromosomes during meiosis. Exchange of genetic material between maternally and paternally derived chromosomes markedly increases the genetic diversity of the resultant haploid cells. One theoretical advantage of sexual reproduction is that the process of meiosis permits the random recombination of genetic material, thereby increasing the range of traits displayed by members of the species. This diversity increases the chances of success of the species in adapting to an ever-changing environment. A second process of sexual reproduction is that the haploid cells fuse during fertilization to form a new and unique diploid cell. The single-cell diploid zygote has all the genetic information necessary to grow and develop into an adult organism.

Organisms that reproduce sexually are thought to have an advantage over organisms that reproduce asexually, because novel combinations of genes are possible in each generation. Furthermore, with few exceptions, each individual in a population of sexually reproducing organisms has a distinct genetic composition. We have meiosis to thank for this variety. **Recombination** is the formation of new gene combinations in a gamete. It results from two events in meiosis, independent assortment and crossing over. **Independent assortment** occurs in meiosis I when each pair of homologous chromosomes lines up on the metaphase plate. The pairs of **homologous chromosomes** line up independently of other pairs and the paternal chromosome may be on the left or right. The number of possible combinations of maternal and paternal chromosomes in the nuclei produced by meiosis equals 2 raised to the power of *n*, where *n* is the number of pairs of chromosomes. For the 23 pairs of human chromosomes, this amounts to over 8 million combinations. **Crossing over** occurs when homologous chromosomes undergo **synapsis** during prophase I, equivalent sections may be exchanged between non-sister chromatids. This adds further variability among the **gametes** produced during meiosis.

A. Meiosis

There are two types of nuclear division: mitosis and meiosis. Meiosis reduces the chromosome number in daughter nuclei to half that of the parent nucleus. Gametes (sex cells) in animals and spores in plants are produced by meiotic division. The mother's mitochondrial DNA, together with twenty-three chromosomes from each parent, combine to form the genome of a zygote, the fertilized egg. As a result, with certain exceptions such as red

blood cells, most human cells contain 23 pairs of chromosomes, together with mitochondrial DNA inherited from the mother. Studies of relatedness in humans are based on the fact that a) mitochondrial DNA is inherited only from one's mother, and b) the male Y chromosome is inherited only from one's father. Although the names given to various phases of meiosis are similar to those of mitosis, there are obviously important differences in what occurs during the phases.

Meiosis I

In early prophase I, the sister chromatids of homologous chromosomes undergo synapsis, organizing themselves in a formation known as a **tetrad**. This allows crossing over and the exchange of genetic material between segments of **homologous chromosomes** during late prophase I. In metaphase I, the tetrads migrate to the metaphase plate. In anaphase I, each homologous pair of sister chromatids is pulled to one **pole**. In telophase I, new nuclear membranes form around the daughter nuclei that consist of sister chromatids that are still attached by centromeres. Telophase I may or may not be followed by cytokinesis.

Meiosis II

Meiosis II begins with the formation of a spindle in prophase II. The sister chromatids, still attached with centromeres, move toward the metaphase plate. At metaphase II, the chromatids are lined up and attached to spindle fibers. Anaphase II begins when the centromeres separate and the sister chromatids, now considered chromosomes, begin moving in opposite directions. During telophase II the nuclear membrane re-forms, the spindle disappears and cytokinesis divides the cytoplasm. The result is four haploid cells, none of which are alike because of the **genetic recombination**.

4. Fill in Table UV-2 with the correct information.

Table UV-2. Distinguishing characteristics **meiotic** cellular division.

DNA replication occurs during the _____ phase of _____.		
How many chromosomes does each human cell contain?_____		
Characteristic	**Meiosis I**	**Meiosis II**
Location of the dividing cells?		
How many cellular divisions?		
Homologous chromosome bivalents are formed?		
Sister chromatids pair up?		
Crossing over occurs?		
Sister chromatids line up on the metaphase plate?		
Bivalents line up on either side of the metaphase plate?		
Sister chromatids separate?		
Number of daughter cells?		
Ploidy (haploid or diploid) level of daughter cells?		

B. Simple Inheritance

There may be a number of alleles for a given gene. Individuals that have two copies of the same allele are referred to as **homozygous** for that allele; individuals that have copies of different alleles are known as **heterozygous** for that allele. The inheritance patterns observed will depend on whether the allele is found on an autosomal chromosome or a sex chromosome, and on whether the allele is **dominant** or **recessive**. **Complete dominance** occurs when the phenotypes of heterozygous and dominant homozygous are indistinguishable. **Codominance** occurs when the phenotypes of both parents are simultaneously expressed in the same offspring. **Codominance** is the specific term for a system in which an allele from each homozygote parent combines in the offspring, and the offspring simultaneously demonstrates both phenotypes. An example of codominance occurs in the human ABO blood group system.

Observations of the way traits, or characteristics, are passed from one generation to the next in the form of identifiable phenotypic traits probably represents the oldest form of genetics. However, the scientific study of patterns of inheritance is conventionally said to have started with the work of the Austrian monk, Gregor Mendel, in the second half of the nineteenth century. Mendel described three principles of inheritance; **1) Uniformity**: heterozygotes share a common phenotype due to dominance, **2) Segregation**: pairs of gene variants (alleles) are separated into reproductive cells during meiosis producing gametes, and **3) Independent Assortment**: genes independently separate from one another when reproductive cells develop which means that the pairs of homologous chromosome are divided in half to form haploid cells and this assortment of homologous chromosomes is random.

Part 2: Human Blood Immunity & Genetics

Although all blood is made of the same basic elements, not all blood is alike. The International Society of Blood Transfusion recognizes 33 blood group systems that are described by the **presence or absence of certain antigens on the cell membranes of red blood cells (RBCs)**. The antigens that determine blood type can either be proteins, such as the D antigen, or carbohydrates; A and B antigens are polysaccharides. An **antibody** is a protein secreted by plasma B cells (differentiated from B lymphocytes) and is part of the immune response. The antibodies travel through the body to reach antigens identical to those that stimulated their production. Natural antibodies are antibodies produced in the absence of overt antigenic stimulation as in the ABO system. D antigen will illicit an immune response in people who are Rh- .

There are 33 blood group systems but we will focus on only two, ABO and Rh. In these two systems there are two independent genes that provide instructions for making specific blood antigens (Table UIV-2). The *ABO* gene is found on chromosome 9 and the *RHD* gene is found on chromosome 1 (Figure UIV-1). Both genes are inherited from your parents through a **Mendelian pattern of inheritance**. Rh and ABO alleles **segregate independently** of each other when gametes are formed during meiosis.

A. Blood Group Systems

The **Rh blood group system** is the second most clinically significant of the blood groups, second only to ABO. It has one genetic locus that exhibits two (2) alleles: D and d and two phenotypic traits: Rh+ and Rh-. The Rh blood groups are determined by the presence (+) or absence (-) of the D antigen, also known as the Rhesus (Rh) factor, on surface of red blood cells. There are two possible alleles for the Rh factor: a dominant allele (D) which encodes for the **D antigen protein** on red blood cells, and a recessive allele (d) which does not encode for the D antigen.

The **ABO blood group system** is the most clinically significant of the blood groups. It has one genetic locus that exhibits three (3) alleles: I^A, I^B, and i and four phenotypes (also known as blood groups or types): A, B, AB, and O. The four major blood groups are determined by the presence or absence of **two carbohydrate antigens (A and/or B)** on the surface of red blood cells. Not only does the ABO system have three alleles at one locus but I^A and I^B are dominant over i and **codominant** to each other.

Table UV-3. The genotypes and phenotypes of the ABO and Rh blood group systems and the antigens that determine the systems.

Genotypes	Alleles	Phenotypic Trait (Blood Groups or Types)	Antigen on Red Blood Cell		Antibodies in Plasma	
			A	B	A	B
I^AI^A, I^Ai	I^A, i	A	yes	no	no	yes
I^BI^B, I^Bi	I^B, i	B	no	yes	yes	no
I^AI^B	I^A, I^B	AB	yes	yes	no	no
ii	i	O	no	no	yes	yes
Genotypes	**Alleles**	**Phenotypic Trait**	**D Antigen on Red Blood Cell**		No Natural Antibodies Present in Plasma	
DD, Dd	D, d	Rh +	yes			
dd	d	Rh -	no			

5. Go to: https://ghr.nlm.nih.gov/chromosome/1 and fill in the blanks of the following sentence: Chromosome ____ is the largest human chromosome, spanning about _____ million DNA building blocks (base pairs) and representing approximately_____% of the total DNA in cells.

6. Go to: https://ghr.nlm.nih.gov/chromosome/9 and fill in the blanks of the following sentence. Chromosome _____ is made up of about _____ million DNA building blocks (base pairs) and represents approximately _____% of the total DNA in cells.

7. Red blood cells have surface antigens on their plasma membranes. Which macromolecule type is the main component of surface antigens for the:

   A. ABO blood system?

   _____

   B. Rh (D antigen) blood system?

   _____

8. What is the main macromolecule component of an antibody?

   _____

9. Which kind of blood cell makes antibodies?

   _____

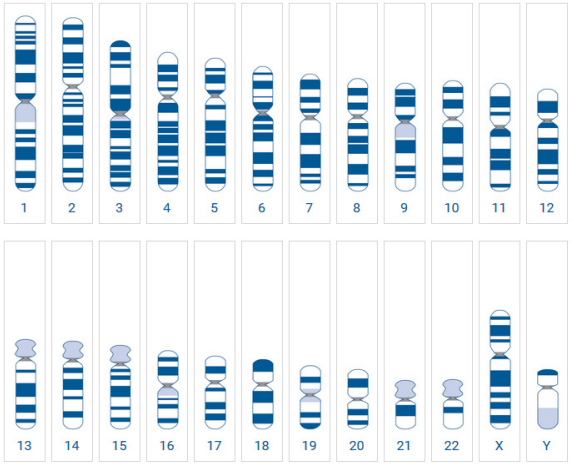


Figure UV-4. Humans normally have 23 pairs of chromosomes, for a total of 46. Twenty-two of these pairs, called autosomes, and look the same in both males and females. The 23rd pair, the sex chromosomes, differ between males and females. Females have two copies of the X chromosome, while males have one X and one Y chromosome. *RHD* gene is found on Chromosome 1 and the *ABO* gene on Chromosome 9.

B. Your Blood Groups

☐ Obtain four sections of paper towel, one blood typing card, one safety medium-flow lancet, four blood mixing sticks, and a bandage.

☐ Set everything on three layers of paper towel. Reserve the other for your fingertip.

☐ Fill in the date and your name on the card.

☐ Place one stick above each circle on the card.

☐ Add a drop of water to each of the circles.

IMPORTANT: Do not cross-contaminate samples. When mixing; 1) use the respective blood mixing sticks, 2) keep the blood and water drop inside its circle, and 3) mix until the colored antisera is fully dissolved in the water/blood.

☐ Use alcohol with a cotton ball to gently clean the fingertip you will use for testing.

☐ Remove the cap from the lancet. Place the lancet on the fingertip, and using firm pressure, press down until the lancet pierces the fingertip.

☐ Anti-A (antisera with anti-A anitbodies):
- Collect a droplet of blood on the concave surface of the Anti-A blood mixing stick.
- Swirl it on the circle labeled Anti-A(blue antisera).
- Place the stick above the Anti-A circle.

☐ Anti-B (antisera with anti-B anitbodies):
- Collect a droplet of blood on the concave surface of the Anti-B blood mixing stick.
- Swirl it on the circle labeled Anti-B (yellow antisera).
- Place the stick above the Anti-B circle.

☐ Anti-D (antisera with anti-D anitbodies):
- Collect a droplet of blood on the concave surface of the Anti-D blood mixing stick.
- Swirl it on the circle labeled Anti-D (pink antisera).
- Place the stick above the Anti-D circle.

☐ Control (no antibodies):
- Collect a droplet of blood on the concave surface of the Control blood mixing stick.
- Swirl it on the circle labeled Control (green antisera).
- Place the stick above the Control circle.

Table UV-4. Reaction to antisera.

Dried antisera	Reaction Occurred? (Y/N)
Anti-A	
Anti-B	
Anti-D (Rh)	
Control	

☐ Dispose of the lancet in the sharps container.

☐ Dispose of the paper towels, blood mixing sticks, and cotton ball in the biohazard container.

☐ Let your card air dry (you can skip ahead to Part 3 while you wait for it to dry) and then look at the agglutination under a stereo microscope.

☐ Carefully examine the thin film left behind and enter the reaction in Table UV-4:
- Film remains uniform in appearance, there is no agglutination.
- Film appears granular, agglutination has occurred. A positive agglutination reaction indicates the blood type

10. What was your phenotype for the ABO and Rh blood group systems? ABO _____ Rh _____

11. What are your possible genotypes? _____

12. Add your phenotype to the BloodType workbook on the instructor computer. Your ABO and Rh types need to be added to separate columns (D & E) but in the same subject row. What is your subject #_____.

☐ Place your card in the plastic bag, seal the bag, and take it home with you.

Part 3. Statistical Null Hypothesis Testing

For statisticians, a population contains all possible outcomes from a variable. Ideally we would measure the entire population. Usually, however, this is not possible. As a result, we sample some subset of the population, and draw inferences concerning the population from these data. One approach is to test whether or not the pattern indicated by the sample data coincides with a population that represents "no effect" or a "default effect". This population is often called a null distribution, and tests concerning this distribution are called null hypothesis tests. In a null hypothesis test we make one of two possible decisions; we either reject the null hypothesis (H_0) or we fail to reject the null hypothesis. There are four steps to null hypothesis testing:

1) Define the null (H_0) and alternative (H_A) hypotheses as well as the **alpha (α).** H_A is the mathematical opposite of the H_0 For instance, if we defined the null hypothesis as: H_0: X = 0, then we would use the alternative hypothesis H_A: X ≠ 0. Alpha is a probabilistic standard for limiting a particular kind of mistake: rejecting H_0 when H_0 is true. Reflecting conventional practice, we will use a probability of 0.05 for alpha. This means that, given an infinite number of tests, we would only reject the null hypothesis 5% of the time when the null is actually true. We can assess the validity of H_0 by comparing a test statistic outcome (see below) to an entity called a critical value. If the test statistic is greater than the critical value then we reject H_0, and conclude in favor of H_A. It should be emphasized that rejecting H_0 does not mean the null is false. It only means that the process we are making inference to is very poorly described by null . Conversely, failing to reject H_0 does not mean that the null is true, but we have insufficient data to reject it. In our work today, we will consider the following null and alternative hypotheses:

H_0 = 3:1 is the TRUE dominant to recessive phenotypic ratio for a monohybrid cross, and 9:3:3:1 is the TRUE ratio for a dihybrid cross.

H_A = 3:1 is NOT the TRUE dominant to recessive phenotypic ratio for a monohybrid cross, and 9:3:3:1 is NOT the TRUE ratio for a dihybrid cross.

2) Calculate a test statistic. We will use a chi-square test. This test is appropriate when data are counts with respect to categories. The chi-square test statistic is a relatively simple mathematical calculation that measures how well the observed experimental results (the observed count) correspond to the expected results under null. The formula for chi-square test statistic is:

$$X^2 = \sum_{i=1}^{c} \frac{(o_i - e_i)^2}{e_i}$$

where o_i and e_i are the observed and expected counts, respectively.

Table UV-5. Critical values for the chi-square test at three levels of alpha, 0.05, 0.01, and 0.001.

Degrees of Freedom (df)	Critical Values at alpha 0.05, 0.01, and 0.001		
	0.05	0.01	0.001
1	3.84	6.64	10.83
2	5.99	9.21	13.82
3	7.82	11.34	16.27
4	9.49	13.28	18.47
5	11.07	15.09	20.52
6	12.59	16.81	22.46
7	14.07	18.48	24.32
8	15.51	20.09	26.12
9	16.92	21.67	27.88
10	18.31	23.21	29.59

If the null hypothesis is true, then the test statistic, x^2, will be a random outcome from a chi-square distribution (Table UIV-4) with *c* - 1 degrees of freedom where *c* = the number of categories being considered.

3) Determine degrees of freedom and the critical value. Degrees of freedom (*df*) are the number of categories under consideration minus one. For the monohybrid example, there are two categories (Rh+ and Rh-). Thus, in this case, the degrees of freedom are 2 - 1 = 1. For the dihybrid example, there are four categories (A+ or B+, A- or B-, O+, O-). In this case, the degrees of freedom are 4 - 1 = 3. A critical value is the minimum value the test statistic must be in order to reject H_0 at a particular alpha.

4) Draw a conclusion. If the test statistic is greater than or equal to the critical value (Tablue UV-5), then H_0 is rejected. If the test statistic is less than the critical value we fail to reject H_0.

A. Monohybrid Cross

In a monohybrid cross, **one pair of alleles is studied**. If both parents (P) are homozygous, with one parent homozygous dominant and the other parent homozygous recessive, the first generation (F_1) will be heterozygous exhibiting the dominant phenotype. As Mendel demonstrated, 75% of the second generation (F_2) offspring will have the dominant phenotype and 25% the recessive phenotype (a 3:1 ratio). This prediction can be validated using the chi-square statistical test. In this exercise you will perform a chi-square test on the second-generation offspring of a monohybrid cross with 3:1 phenotypic ratio.

☐ Divide into twelve separate groups with **one-to-two people per group**.

☐ You will be given a deck of cards for a Rh F_1 monohybrid cross (Parental cross was DD X dd).

Figure UV-5. Punnett square F_1 cross resulting in the F_2 generation.

13. Determine what the phenotypic ratio is using the Punnett square (Figure UIV-5) for the Rh F_1 cross which will give rise to the F_2 generation.

14. Count the number of Rh+ and Rh- cards in your deck.

15. Fill in Table UIV-6 with the card counts.

16. Calculate (show calculations in space provided) the expected numbers (e_1 and e_2) for the cards.

You expect 3/4 of your sample to be Rh+.
e_1 = your sample (total #) multiplied by 3/4 (or 0.75) = (Round off to the nearest whole number.)

You expect 1/4 of your sample to be Rh-.
e_2 = your sample (total #) multiplied by 1/4 (or 0.25) = (Round off to the nearest whole number.)

Note: subscripts refer to the categories under consideration

Table UV-6. Number of F_2 offspring cards.

Phenotype	Count data
Rh+	
Rh-	
Total	

Table UV-7. e_1, e_2, and X^2.

e_1	
e_2	
X^2	

17. Add the values to Table UIV-7.

18. You can now test the validity of the card data using the chi-square test. Once you have calculated (show calculations in space provided) your expected numbers (e_1 and e_2) for your card sample, put your numbers in the chi-square formula and perform the calculations. Calculate the chi-square number (x^2) all class cards. The formula for the chi-square calculation is:

$$X^2 = \frac{(o_1 - e_1)^2}{e_1} + \frac{(o_2 - e_2)^2}{e_2}$$

x^2 = the symbol for the chi-square test statistic
o = observed count (number) (o_1 = the Rh+, o_2 = Rh-)
e = the expected number under H_0. Both e_1 and e_2 must be calculated for your particular sample.

19. Add the values to Table UIV-6.

20. Did you reject or fail to reject the H_0 for your deck? What does this mean?

B. Dihybrid Cross

In a dihybrid cross, **two genes, each on a separate chromosome, with each gene having at least two alleles are studied**. If both parents (P) are homozygous, with one parent homozygous dominant for both traits and the other parent homozygous recessive for both traits, the first generation (F_1) will be heterozygous expressing the dominant phenotype for both traits. As Mendel demonstrated, there will be four different phenotypes in the second generation (F_2) yielding a 9:3:3:1 ratio (9/16 with both dominant traits, 3/16 with one dominant and one recessive trait, 3/16 with the alternative dominant and recessive trait and 1/16 with both recessive traits). This predictions can be validated using the chi-square statistical test.

Figure UV-6. Punnett square of the F_1 cross resulting in the F_2 generation.

In this exercise we will create a dihybrid cross of the Rh and ABO blood systems. However, since the ABO system has three alleles with codominance, **we will adjust the exercise to look at the I^A and I^B alleles separately.**

☐ You will be given a deck of cards for the F_1 Dihybrid cross of A^+ or B^+ (parental cross was I^AI^ADD X *ii*dd **OR** I^BI^BDD X *ii*dd):

21. Were you assigned the A^+ (I^AiDd X I^AiDd) or B^+ (I^BiDd X I^BiDd) phenotypic trait?

_____

22. Using your textbook to help you, construct a Punnett square of the F_1 dihybrid cross (Figure UIV-6) for this phenotypic trait.

23. Fill in Table UIV-8 with the card counts.

24. Calculate the expected numbers (e_1, e_2, e_3, e_4) for all cards (show calculations in space provided); To calculate the expected numbers (e_1, e_2, e_3, e_4) for your 9:3:3:1 dihybrid cross:

    a. You expect **9/16 of your sample (total #) to be A^+ or B^+**

    e_1 = our sample (total #) multiplied by 9/16 (or 0.5625)

    b. You expect **3/16 of your sample (total #) to be A^- or B^-** .

    e_2= your sample (total #) multiplied by 3/16 (or 0.1875)

    c. You expect **3/16 of your sample (total #) to be O^+.**

    e_3 = our sample (total #) multiplied by 3/16 (or 0.1875)

    d. You expect **1/16 of your sample (total #) to be O^-.**

    e_4 = our sample (total #) multiplied by 1/16 (or 0.0625)

25. Add the values to Table UIV-9.

Table UV-8. Number of F_2 offspring cards.

Phenotype	Count data
_____$^+$	
_____$^-$	
_____$^+$	
_____$^-$	
Total	

Table UV-9. e_1, e_2, e_3, e_4 and X^2 .

e_1	
e_2	
e_3	
e_4	
X^2	

26. You can now test the validity of your data using the chi-square test. Calculate chi-square number (x^2) for all cards (show calculations in space provided). The formula for the chi-square calculation is:

$$X^2 = \frac{(o_1 - e_1)^2}{e_1} + \frac{(o_2 - e_2)^2}{e_2} + \frac{(o_3 - e_3)^2}{e_3} + \frac{(o_4 - e_4)^2}{e_4}$$

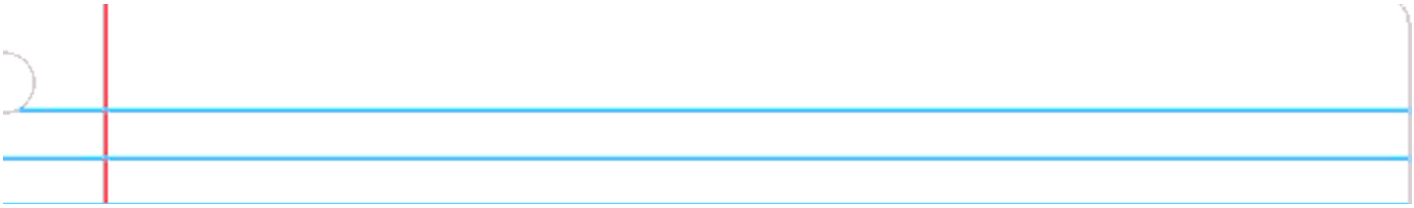
x^2 = the symbol for the chi-square test statistic

o = observed count (number) (o_1 = **A^+ or B^+**, o_2 = **A^- or B^-**, o_3 = **O^+**, and o_4 = **O**)

e = the expected number under H_0. All e_1, e_2, e_3, and e_4 must be calculated for your particular sample.

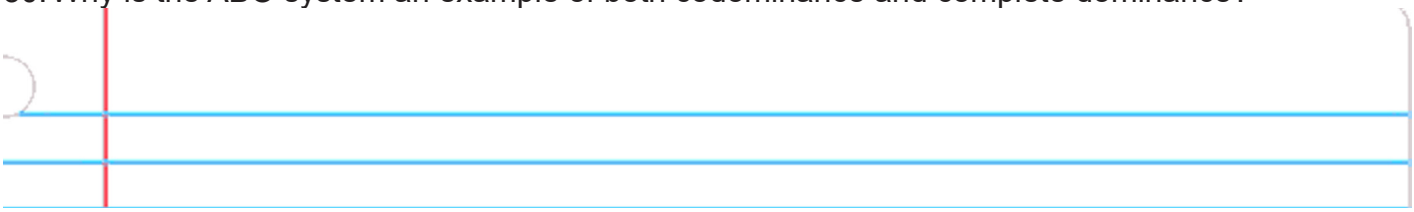
27. Add the values to Table UIV-9.

28. Did you reject or fail to reject the H_0 for your deck? What does this mean?



29. Calculations REQUIRED:

30. Why is the ABO system an example of both codominance and complete dominance?



31. A woman with blood type AB+ and a man with type O- decide to have a child. What are the possible genotypes of the child? **Construct two Punnett Squares to show the genotypes.**

Name:_____ Team #:_____ Section #:_____

Part 4: Population Genetics

Population genetics is the study of genetic variation within populations, and involves the examination and modeling of changes in the frequencies of genes and alleles in populations over space and time. A **population** is a group of organisms of the same species within a specific geographical location. Many of the genes found within a population will be polymorphic - that is, they will occur in a number of different forms (or alleles). How frequently a trait is observed in a population is not related to whether or not it is dominant or recessive. Instead, it is a reflection of how frequently the gene responsible for causing a trait is found. **Allele frequency** is the relative proportion of all alleles of a gene that are of a designated type and **genotype frequency** is the proportion of individuals within a population that are of a prescribed genotype.

Mathematical models are used to investigate and predict the occurrence of specific alleles or combinations of alleles in populations, based on developments in the molecular understanding of genetics, Mendel's laws of inheritance, and modern evolutionary theory. The focus is the population or the species - not the individual. The collection of all the alleles of all of the genes found within a freely interbreeding population is known as the **gene pool** of the population. Each member of the population receives its alleles from other members of the gene pool (its parents) and passes them on to other members of the gene pool (its offspring). Population genetics is the study of the variation in alleles and genotypes within the gene pool, and how this variation changes from one generation to the next.

In natural populations, however, the genetic composition of a population's gene pool may change over time. Mutation is the primary source of new alleles in a gene pool, but the other factors act to increase or decrease the occurrence of alleles. **Genetic drift** occurs as the result of random fluctuations in the transfer of alleles from one generation to the next, especially in small populations formed, say, as the result adverse environmental conditions (the **bottleneck effect**) or the geographical separation of a subset of the population (the **founder effect**). The result of genetic drift tends to be a reduction in the variation within the population, and an increase in the divergence between populations. If two populations of a given species become genetically distinct enough that they can no longer interbreed, they are regarded as new species (a process called **speciation**).

In many cases, the effects of natural selection on a given allele are directional. The allele either confers a selective advantage, and spreads throughout the gene pool, or it confers a selective disadvantage, and disappears from it. In other cases selection acts to preserve multiple alleles within the gene pool and a balanced equilibrium is observed. This situation, labeled balanced polymorphism, can arise because of a selective advantage for individuals heterozygous for a given allele. As a result of balanced polymorphism, the gene pools of most populations contain a number of deleterious alleles that reduce the overall fitness of the population (known as the **genetic load**).

A. Hardy-Weinberg Equations

The Hardy-Weinberg equation describes and predicts a balanced equilibrium in the frequencies of alleles and genotypes. It allows us to calculate the allele and genotype frequencies of Mendelian traits in a population. As we've already learned, Mendelian traits are controlled by one gene with two alternative alleles, one of which is dominant and one of which is recessive. The Hardy-Weinberg principle can be used to predict allele and genotype frequencies in populations that meet the following assumptions:

- ✓ The population is large enough that random statistical events do not affect genotypic frequencies (i.e., there is no genetic drift).
- ✓ Mutations do not occur.
- ✓ Migration into the population (immigration) or out of the population (emigration) does not occur.
- ✓ Natural selection does not occur.
- ✓ Mating is entirely random.

The Hardy-Weinberg equations use the letters *p* and *q* to represent alternative alleles for a particular gene. The letter ***p* represents the frequency of the dominant allele**, and ***q* represents the frequency of the recessive allele**. Because all diploid individuals possess two alleles, the sum of *p* plus *q* equals one, shown in Equation 1:

Equation 1: $p + q = 1$

The second Hardy-Weinberg equation can be used to calculate the frequencies of genotypes in a population that result from combinations of two alternative alleles, shown in Equation 2:

Equation 2: $p^2 + 2pq + q^2 = 1$

In this equation, $\boldsymbol{p^2}$ **represents the frequency of homozygous dominant genotypes**, $\boldsymbol{2pq}$ **represents the frequency of heterozygous genotypes**, and $\boldsymbol{q^2}$ **represents the frequency of homozygous recessive genotypes**. These are the three possible genotypic combinations with two alternative alleles.

These equations are useful because if the frequency of one of the alleles (*p* or *q*) within a population is known, or the frequency of one of the homozygous genotypes (either p^2 or q^2) within a population is known, the frequencies of the other allele and other genotype can be calculated.

For example, assume that coat color in cats is controlled by one gene locus with two possible alleles (B and b). If a population of cats ($n = 1000$) has 910 individuals with the dominant coat color (B_), and 90 individuals with the recessive coat color (bb), the frequency of the homozygous dominant genotype is 0.49, the heterozygous genotype is 0.42, and the homozygous genotype is 0.09. Calculate as follows:

Step A1 - determine q^2 of Equation 2 (90 cats have the recessive coat color, bb, out of a total 1000 cats:

$$p^2 + 2pq + 90/1000 = 1$$
$$p^2 + 2pq + 0.09 = 1$$

Step A2 - solve for *p* of Equation 1 by first taking the square root of q^2 from Equation 2 (Step 1):

$$p + \sqrt{0.09} = 1$$
$$p + 0.3 = 1$$
$$0.7 + 0.3 = 1$$

Step A3 - fill in Equation 2 with the *p* and *q* calculated from Equation 1 (Step 2):

$$0.7^2 + 2(0.7)(0.3) + 0.3^2 = 1$$
$$0.49 + 0.42 + 0.09 = 1$$

B. Using Hardy-Weinberg Equations with THREE Alleles

Hardy-Weinberg equations can also be used to understand frequencies in a population where three alleles exist for a single locus. In this case, ***p* represents the frequency of the dominant allele 1**, ***q* represents the frequency of dominant allele 2**, and ***r* represents the frequency of the recessive allele**, shown in Equation 3:

Equation 3: $p + q + r = 1$

The second Hardy-Weinberg equation for three alleles can be used to calculate the frequencies of genotypes in a population that result from the combinations of the three alleles, shown in Equation 4:

Equation 4: $p^2 + 2pr + 2pq + q^2 + 2qr + r^2 = 1$

In this equation, ***p^2 and q^2* represent the frequency of homozygous dominant genotypes**, ***2pr, 2pq, and 2qr* represent the frequency of heterozygous genotypes**, and ***r^2* represents the frequency of the homozygous recessive genotypes**. These are the six possible genotypic combinations with three alternative alleles.

Like the previous equations if the frequency of one of the alleles (*p*, *q* or *r*) within a population is known, or the frequency of one of the homozygous genotypes (either p^2, q^2 or r^2) within a population is known, the frequencies of the other alleles and other genotypes can be calculated.

For example, shell color in a species of *Helix* snail is controlled by three alleles at a single locus: C^B (brown), $C^P C^B$ (brown and pink), C^P (pink) and C^Y (yellow). The brown and pink alleles are codominant and dominant to yellow; yellow is completely recessive. A population of snails (*n*=1000) has 463 brown individuals, 289 pink individuals, 182 brown/pink individuals, and 66 yellow individuals. You can calculate the allele and genotype frequencies as follows:

Step B1 - If the population size is *n*, then we also know that:

$n(p^2 + 2pr)$ = the number of brown snails in the population,
$n(q^2 + 2qr)$ = the number of pink snails in the population,
$n(2pq)$ = the number of brown/pink snails in the population, and
$n(r^2)$ = the number of yellow snails in the population.

Notice that we fit both homozygote and heterozygote frequencies into the brown and pink equations but the brown/pink and yellow equations are due to either heterozygotes or homozygotes.

Step B2 - solve for *r* of Equation 3 by first taking the square root of r^2 from Equation 4 which will give us the allele frequency for yellow in a snail population:

$$r^2 = 66/1000 = 0.066$$
$$p + q + \sqrt{0.066} = 1$$
$$p + q + 0.257 = 1$$

Step B3 - set up a quadratic equation for the number of brown snails.

$$n(p^2 + 2pr) = 463 = 1000\ (p^2 + 2pr)$$
$$p^2 + 2pr = 463/1000$$
$$p^2 + 2pr = 0.463$$
$$p^2 + 2p(0.257) = 0.463$$
$$p^2 + 0.514p = 0.463$$
$$p^2 + 0.514p - 0.463 = 0$$

Quadratic Equation: $ax^2 + bx + c = 0$

Step B4 - using the quadratic formula solve the quadratic equation for *p* determined in step B3 ($p^2 + 0.514p - 0.463 = 0$ where $a = 1$, $b = 0.514$, and $c = -0.463$:

Quadratic Formula: $$x = \frac{-b \pm \sqrt{b^2 - 4ac}}{2a}$$

$$p = \frac{-b \pm \sqrt{b^2 - 4ac}}{2a} = \frac{-0.514 \pm \sqrt{(0.514)^2 - 4(1)(-0.463)}}{2(1)} = \frac{-0.514 \pm \sqrt{0.264 - (-1.85)}}{2}$$

$$p = \frac{-0.514 \pm \sqrt{2.114}}{2} = \frac{-0.514 \pm 1.454}{2} = -0.984, 0.47$$

There are two solutions for *p*. Since *p* cannot be negative, we know that $p = 0.47$.

Step B5 - solve for *q* using *p* and *r*:

$$p + q + r = 1$$
$$q = 1 - (p + r)$$
$$q = 1 - (0.47 + 0.257)$$
$$q = 1 - (0.727) = 0.273$$

Step B6 - determine the allele and genotype frequencies and population estimates (Table UV-8A and B) now that we have solved for:

$p = 0.47$
$q = 0.273$
$r = 0.257$

Table UV-10. A) Allele and **B)** Genotype frequencies and population estimates for the color of *Helix* shells (*n*=1000).

A	Alleles	Dominance	Allele Frequencies
p	C^B	dominant	0.47
q	C^P	dominant	0.273
r	C^Y	recessive	0.257
		Total =	1

B	Genotype	Phenotypic Trait	Genotype Frequencies	Population Estimates
p^2	C^BC^B	brown homozygous dominant	$(0.47)^2$	221
$2pr$	C^BC^Y	brown heterozygous dominant	2(0.47)(0.257)	241
$2pq$	C^BC^P	brown/pink heterozygous codominant	2(0.47)(0.273)	257
q^2	C^PC^P	pink homozygous dominant	$(0.273)^2$	75
$2qr$	C^PC^Y	pink heterozygous dominant	2(0.273)(0.257)	140
r^2	C^YC^Y	yellow homozygous recessive	$(0.257)^2$	66
		Total =	1	1000

Part 5: Calculating Frequencies

Over several semesters, a population of ISU students from the Anatomy and Physiology II Laboratory typed their blood. In lab today, we will use their results to predict the allele and genotype frequencies in the Biol 3302L population.

☐ Divide into 12 separate groups with **one to two people per group**. Write you groups number on the top of page UV:17.

☐ Your instructor will project the data.

1. Determine the total number of students that typed their blood; *n* =_____.
2. Fill in Table UV-11 with the number of students expressing the different phenotypic traits.

Table UV-11. ABO and Rh phenotypes expressed by the students in Biol 3302L.

ABO Phenotype	Number of Students
A	
B	
AB	
O	
Rh Phenotype	**Number of Students**
Rh +	
Rh -	

A. Rh (D antigen) Frequencies

Remember the Rh Blood Group System has one genetic locus that exhibits two (2) alleles: D and d and two phenotypes: Rh+ and Rh-. The Rh blood groups are determined by the presence (+) or absence (-) of the D antigen on surface of red blood cells. There are two possible alleles for the Rh factor: a dominant allele (D) which encodes for the D antigen protein on red blood cells, and a recessive allele (d) which does not encode for the D antigen.

3. What are the possible genotype(s) for the dominant Rh trait:_____ _____
4. What are the possible genotype(s) for the recessive Rh trait:_____

5. Calculate the allele and genotype frequencies and the population estimates for D antigen (Rh factor) and show the calculations in the space provided below.

6. Fill in Table UV-12 with the frequencies and estimates calculated using the Hardy-Weinberg Equations 1 and 2.

Table UV-12. A) Allele and **B)** Genotype frequencies and population estimates for D antigen (*n* =_____).

A	Alleles	Dominance	Allele Frequencies
p	D		
q	d		
		Total =	

B	Genotype	Phenotypic Trait	Genotype Frequencies	Population Estimates
p^2	DD			
$2pq$	Dd			
q^2	dd			
		Total =		

Calculations REQUIRED:

Step A1:

Step A2:

Step A3:

B. ABO Frequencies

The ABO Blood Group System has one genetic locus that exhibits three (3) alleles: I^A, I^B, and i and four phenotypic traits: A, B, AB, and O. Because there are three alleles on one locus and the I^A and I^B are **codominant** over i, we will use the Hardy-Weinberg equations for three alleles.

7. What is the codominant phenotypic trait? _____ What is the possible genotype for this trait?_____

8. What is the recessive phenotypic trait? _____ What is the possible genotype for this trait?_____

9. What are the other possible phenotypic traits? _____ _____ What are the other possible genotypes for these traits?_____ _____ _____ _____

10. Calculate the allele and genotype frequencies and the population estimates for the A and B antigens and show the calculations in the space provided.

11. Fill in Table UV-13 with the frequencies and estimates calculated using the Hardy-Weinberg Equations 3 and 4 along with the quadratic equation and formula.

Table UV-13. A) Allele and **B)** Genotype frequencies and population estimates for the A and B antigens (n =_____).

A	Alleles	Dominance	Allele Frequencies
p	I^A		
q	I^B		
r	i		
		Total =	

B	Genotype	Phenotypic Trait	Genotype Frequencies	Population Estimates
p^2	I^AI^A			
$2pr$	I^Ai			
$2pq$	I^AI^B			
q^2	I^BI^B			
$2qr$	I^Bi			
r^2	ii			
		Total =		

Calculations REQUIRED:

Step B1:

Step B2:

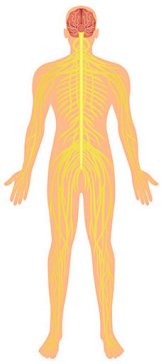
Step B3:

Step B4:

Step B5:

Step B6:

Unit VI - Nervous System



Nervous System

Objectives

- Name, identify, and define the various structures of the nervous system.
- Study the potential of hydrogen (pH) and electrical conductivity of a variety of common beverages using Vernier computer interfaces (Go!Link) and software (Logger Pro).
- Calculate the mean and standard deviation of pH and conductivity.
- Study the electrical activity of a muscle activated by a reflex arc through nerves to and from the spinal cord. using Vernier computer interfaces (Go!Link) and software (Logger Pro).
- Compare the relative speeds of voluntary and reflex muscle activation.
- Associate muscle activity with involuntary activation.
- Observe the effect of central nervous system influence on reflex amplitude.
- Calculate the approximate speed of a nerve impulse.
- Compare reflex response and electrical amplitude in different subjects.
- Create tables and figures with corresponding APA captions using Microsoft® Office Excel and Word.

Terms & Definitions

Action potential - an electrical signal along a cell's plasma membrane typically in muscle cells and along the axon of a neuron.

Bipolar - a type of neuron having one axon and one dendrite extending from the soma.

Cerebrospinal fluid (CSF) - an ultrafiltrate of plasma contained within the ventricles of the brain and the subarachnoid spaces of the cranium and spine.

Cranial - nerves of the PNS that are directly connect to the brain.

Cranial vault - the space in the skull within the neurocranium.

Cranium - a protective bony housing that encases the brain.

Effector - a peripheral tissue or organ that response to ervous or hormonal stimulation.

Extracellular fluid (ECF) - fluids found outside of cells. The most abundant electrolyte in extracellular fluid is sodium. The body regulates sodium levels to control the movement of water into and out of the extracellular space due to osmosis

Hyper-, Hypocalcemia - blood calcium level is above or below normal.

Hyper-, Hypochloremia - blood chloride level is above or below normal.

Hyper-, Hypokalemia - blood potassium level is above or below normal.

Hyper-, Hypomagnesemia - blood magnesium level is above or below normal.

Hyper-, Hyponatremia - blood sodium level is above or below normal.

Hyper-, Hypophosphatemia - blood phosphorous level is above or below norm

Hypophyseal Fossa - an indentation in the roof of the body of the sphenoid bone in the middle cranial fossa

Interstitial fluid - fluid found in the spaces around cells. It comes from substances that leak out of blood capillaries (the smallest type of blood vessel). It helps bring oxygen and nutrients to cells and to remove waste products from them. As new interstitial fluid is made, it replaces older fluid, which drains towards lymph vessels. When it enters the lymph vessels, it is called lymph. Also called tissue fluid.

Intracellular fluid (ICF) - the fluid inside cells made up of protein, water, electrolytes, and solutes.

Foramen magnum - a large, oval-shaped opening in the occipital bone of the skull. Infundibulum - the hollow stalk which connects the hypothalamus and the posterior pituitary gland

Mesaxon - a pair of parallel plasma membranes of a neurolemmocyte (Schwann cell)

Multipolar - a type of neuron having one axon and multiple dendrites extending from the soma.

Myenteric Plexus - provides motor innervation to both layers of the muscular layer of the gut, having both parasympathetic and sympathetic input (although present ganglion cell bodies belong to parasympathetic innervation, fibers from sympathetic innervation also reach the plexus), whereas the submucous plexus has only parasympathetic fibers and provides secretomotor innervation to the mucosa nearest the lumen of the gut.

Polarization (de-, hyper-) - de- the change in the membrane potential that occurs when a cell becomes less polarized that is less negative to the surrounding fluid; hyper- the change in the membrane potential that occurs when the cell becomes more polarized.

Postganglionic - extends from the cell body to an effector.

Preganglionic - extends from the cell body and exits the CNS in either a cranial nerve or a spinal nerve.

Pseudounipolar - a type of neuron with a single structure that extends from the soma that later branches into two distinct structures. They develop from bipolar cells but the processes that extend from the cell body fuse to form a single neurite.

Nervous system - groups of cells that sense the internal and external environmental changes and transmit signals that enable an animal to respond in an appropriate way.

A. Central (CNS) - the central nervous system (CNS) consists of the brain and spinal cord organs. Because they are so vitally important, the brain and spinal cord, located in the dorsal body cavity, are encased in bone for protection. The brain is in the cranial vault, and the spinal cord is in the vertebral canal of the vertebral column. Although considered to be two separate organs, the brain and spinal cord are continuous at the foramen magnum.

- Brain - organ of the central nervous system of animals that functions to process and integrate information:
- Cerebrum - a region of the forebrain that is responsible for the higher functions of conscious thought, planning, and emotions as well as control of motor function.
- Corpus Callosum - a large bundle of more than 200 million myelinated nerve fibers that connect the two brain hemispheres.
- Cerebellum - the hindbrain along with the pons, responsible for monitoring and coordinating body movements.
- Hypothalamus - a region of the forebrain below the thalamus which coordinates both the autonomic nervous system and the activity of the pituitary, controlling body temperature, thirst, hunger, and other homeostatic systems, and involved in sleep and emotional activity.
- Medulla oblongata - a region of the hindbrain that coordinates many basic reflexes and bodily functions, such as breathing.
- Midbrain - one of three major divisions of the brain; the other two being hindbrain and forebrain.
- Pons - the region of the hindbrain, along with the cerebellum that is responsible for monitoring and coordinating body movements.
- Thalamus - a region of the forebrain that plays a major role in relaying sensory information to appropriate parts of the cerebrum and, in turn, sending outputs from the cerebrum to other parts of the brain. Parasympathetic - Postganglionic parasympathetic fibers (oculomotor, facial, glossopharyngeal, and vagus cranial nerves) release acetylcholine that regulates resting response.
- Spinal cord - the structure that connects the brain to all areas of the body and together with the brain constitutes the CNS.
- Cerebrospinal fluid (CSF) - fluid that exists in ventricles within the CNS and surrounds the exterior of the brain and spinal cord; it absorbs physical shocks to the brain resulting from sudden movements or blows to the head.

B. Glia cells - cells that surround the neurons; a major class of cells in nervous systems that perform various functions.

C. Neuron - a highly specialized cell found in the nervous system that communicates with other cells by electrical or chemical signals.

- Axon - an extension of the plasma membrane of a neuron that is involved in sending signals to neighboring cells.
- Axon hillock - the part of the axon closest to the body; typically where an action potential begins.
- Axon terminal - the end of the axon that sends the electrical or chemical messages to other cells
- Cell body (soma) - a part of a neuron that contains the cell nucleus and other organelles.
- Dendrites - a treelike extension of the plasma membrane of a neuron that receives electrical signals from other neurons.
- Telodendria - the end branches of an axon.

D. Perineurium - a protective sheath that surrounds a nerve fascicle.

E. Peripheral (PNS) - The peripheral nervous system (PNS) consists of nerves and ganglia. Nerves are bundles of nerve fibers, much like muscles are bundles of muscle fibers. Cranial nerves (ventral/inferior from brain) and spinal nerves (lateral from spine) extend from the CNS to peripheral organs such as muscles and glands. Ganglia are collections, or small knots, of nerve cell bodies outside the CNS.

- Autonomic - motor impulses to cardiac muscle, smooth muscle, and glandular epithelium (involuntary or automatic functions).
- Enteric (ENS) - control of; 1) movements of the gastrointestinal tract (GIT) by detecting chemical and mechanical stimuli from ingestion coordinating peristalsis, 2) gastric acid secretion, 3) GIT hormone release, and local blood flow. It also interacts with the GIT immune system. Primary afferent neurons.
- Sympathetic - postganglionic sympathetic fibers release norepinephrine producing an acute response that activates the blood flow in skeletal muscles and lungs, dilates lungs and blood vessels, and raises the heart rate.
- Ganglion - a group of neuronal cell bodies in the PNS that is involved in a similar function.
- Neurolemmocyte - the principal glia of the peripheral nervous system (PNS).
- Neurilemma - the outermost nucleated cytoplasmic layer of the neurilemmocytes that surrounds the axon of the neuron

F. Myelin fatty sheath - an insulating layer made up of specialized glial cells wrapped around axons.

G. Nerve - a structure found in the PNS that is composed of multiple myelinated neurons bound by connective tissue; carries information to or from the CNS.

H. Nerve impulse - a signal transmitted along a nerve fiber. It consists of a wave of electrical depolarization that reverses the potential difference across the nerve cell membranes.

I. Net - interconnected neurons with no central organ.

J. Neurocranium - the upper and back part of the skull, which forms a protective case around the brain

K. Neurotransmitter - a small signaling molecule that is released form an axon terminal and diffuse to a postsynaptic cell where it elicits a response.

Reflex arc - a simply circuit that allows an organism to respond rapidly to inputs from sensory neurons and consists of only a few neurons. A sensory neuron sends a signal straight to the spinal cord (bypassing the brain) which in turn generates a response that may be subconscious.

Resting potential - the difference in charges across the plasma membrane in an unstimulated neuron.

Sphenoid bone - an unpaired bone of the neurocranium. It is situated in the middle of the skull towards the front, in front of the basilar part of the occipital bone.

Viscerocranium - elements of the skull that are not part of the neurocranium.

Background - Nervous System

The nervous system (Table UVI-1) can be divided into central (CNS) and peripheral (PNS). Both the CNS and PNS are composed of nervous tissue which consists of **neurons** (nerve cells) and **glia cells**. The CNS is composed of the nervous tissue of the brain and spinal cord whereas the PNS is composed of all other nervous tissue outside of the CNS. The PNS collects information from the integumentary system and the other sense organs and transmits the signals to the CNS. The nervous system depends on neurons and synaptic contacts to convey information both electrically and chemically. A neuron is composed of a **cell body** and protoplasmic extensions (**dendrites** and **axons**). Each neuron can have one or more **dendrites** (receives impulses) but only one myelin sheath covered **axon** (transmit impulses). **Glia cells** are more numerous than neurons and support, protect, and nourish the neurons. Spinal cord neurons can be classified based on their function as either sensory, motor, or inter- neurons. Sensory (afferent) neurons are typically **pseudounipolar** (one axon split into two branches). Motor (efferent) neurons and interneurons are **multipolar** with one axon and several dendrites from the cell body). Pseudounipolar neurons develop from bipolar cells but the processes that extend from the cell body fuse to form a single neurite. The classification of brain neurons is more complex but they can **bipolar** (one axon and dendritic process), pseudounipolar, or multipolar. Neurons process and transmit information by the movement of an electrical charge and chemical neurotransmitters (acetylcholine, glutamate, dopamine, noradrenaline serotonin, histamine,derivative γ-aminobutyric acid GABA, glycine). A neurotransmitter is a small signalling molecule released from a neuron axon terminal and diffuses to a postsynaptic cell where it may illicit and excitatory or inhibitory response. The **action potential** is an electrical signal along a cell's plasma membrane typically in muscle cells and along the axon of a neuron. The **resting potential** is the difference in charge across the plasma membrane of an unstimulated neuron. The charge across the membrane is dependent on the ions that are found in the ICF relative to the ECF. Passive and active transport of specific ions (Table. UVI-2) maintain the concentration gradients between the ICF and ECF. The force of electrical movement is measured in volts (V). The resting potential of a neuron is usually -0.07 V or -70 mV meaning that the force of electrical movement within the intracellular fluid is 70 mV less than the extracellular fluid. At a synapse (the junction between two neurons), an **action potential** causes one neuron to release a chemical neurotransmitter. The neurotransmitter will either excite or inhibit the other neuron from firing its own action potential. An action potential occurs when the ICF reaches a threshold of -55 mV.

Table UVI-1. Organization of the Nervous System.

Central		The central nervous system (CNS) consists of the brain and spinal cord organs. Because they are so vitally important, the brain and spinal cord, located in the dorsal body cavity, are encased in bone for protection. The brain is in the cranial vault, and the spinal cord is in the vertebral canal of the vertebral column. Although considered to be two separate organs, the brain and spinal cord are continuous at the foramen magnum.	
Peripheral		The peripheral nervous system (PNS) consists of nerves and ganglia. Nerves are bundles of nerve fibers, much like muscles are bundles of muscle fibers. Cranial nerves (ventral/inferior from brain) and spinal nerves (lateral from spine) extend from the CNS to peripheral organs such as muscles and glands. Ganglia are collections, or small knots, of nerve cell bodies outside the CNS.	
	Afferent	Sensory nerves that transmit impulses from peripheral organs to the CNS.	
	Efferent	Motor nerves that transmit impulses from the CNS out to the peripheral organs to cause an effect or action.	
	Somatic	Motor impulses to the skeletal muscles (voluntary nervous system)	
	Autonomic	Motor impulses to cardiac muscle, smooth muscle, and glandular epithelium (involuntary or automatic functions).	
		Sympathetic	Postganglionic sympathetic fibers release norepinephrine producing an acute response that activates the blood flow in skeletal muscles and lungs, dilates lungs and blood vessels, and raises the heart rate.
		Parasympathetic	Postganglionic parasympathetic fibers (oculomotor, facial, glossopharyngeal, and vagus cranial nerves) release acetylcholine that regulates resting response.
		Enteric	Control of; 1) movements of the gastrointestinal tract (GIT) by detecting chemical and mechanical stimuli from ingestion coordinating peristalsis, 2) gastric acid secretion, 3) GIT hormone release, and local blood flow. It also interacts with the GIT immune system. Primary afferent neurons.

Table UVI-2. Important electrolytes. Recommended daily allowances (RDA) for adults.

Ion	Location/Absorption/Secretion	Function
Sodium (Na^+) RDA <2,300 mg	Extracellular fluid (ECF): plasma, interstitial fluid transcellular fluid Cerebrospinal fluid (CSF) Sodium-potassium pumps in cell membranes Reabsorbed by active transport and then transported to the peritubular capillaries. Because Na+ is actively transported out of the tubule, water follows it to even out the osmotic pressure	ECF volume Resting membrane potential in neurons and muscle fibers. Osmotic pressure gradient
Potassium (K^+) RDA 4700mg	Intracellular fluid (ICF), CSF Sodium-potassium pumps in cell membranes Aldosterone increases potassium secretion from the distal convoluted tubule. Excreted, actively and passively through the renal distal convoluted tubules and collecting ducts.	Homeostasis between sodium and potassium Resting membrane potential (neurons, muscle fibers)
Calcium (Ca^+) RDA 18-17mg	ECF 1,25-dihydroxy vitamin D3 controls the absorption of calcium in the intestine. Parathyroid hormone (PTH) is responsible for calcium secretion in the distal tubule of kidneys whereas calcitonin acts on bone cells to increase calcium levels in the blood.	Skeletal mineralization Contraction of muscles Release of neurotransmitters Blood clotting Enzyme activity Endocrine gland hormone release
Magnesium (Mg^+) RDA 400-420 mg	ICF Muscle contracts, calcium re-uptake by the calcium-activated ATPase of the sarcoplasmic reticulum is brought about by magnesium.	ATP metabolism Contraction and relaxation of muscles Proper neurological functioning Neurotransmitter release
Chloride (Cl^-) RDA 2,300 mg	ECF, CSF Serum chloride levels regulated by kidneys. Filtered by the glomerulus and reabsorbed by both proximal and distal tubules by both active and passive transport.	Osmotic pressure gradient between the ICF and ECF Proper hydration Electrical neutrality of ECF (balance cations)
Phosphate (HPO_4^{2-}) RDA (as phosphorous) 1,250 mg	ECF 1,25-dihydroxy vitamin D3 controls the absorption of phosphate in the intestine. PTH is responsible for phosphate secretion in the distal tubule of kidneys whereas calcitonin acts on bone cells to increase phosphate levels in the blood.	Bones and teeth ATP Nucleotides
Bicorbonate (HCO_3^-)	Blood The kidneys excrete, reabsorb the filtered bicarbonate, and generate new bicarbonate by net acid excretion. Buffering system to regulate pH.	Carbon dioxide is converted to carbonic acid which is quickly turned to bicarbonate in the cytoplasm of red blood cells by carbonic anhydrase Acid-base balance buffering system

Name:_____ Team #:_____ Section #:_____

1. On Figure VI-1 label and color the:
   - organs that make up the CNS (green).
   - nerves and ganglia that make up the PNS (red)

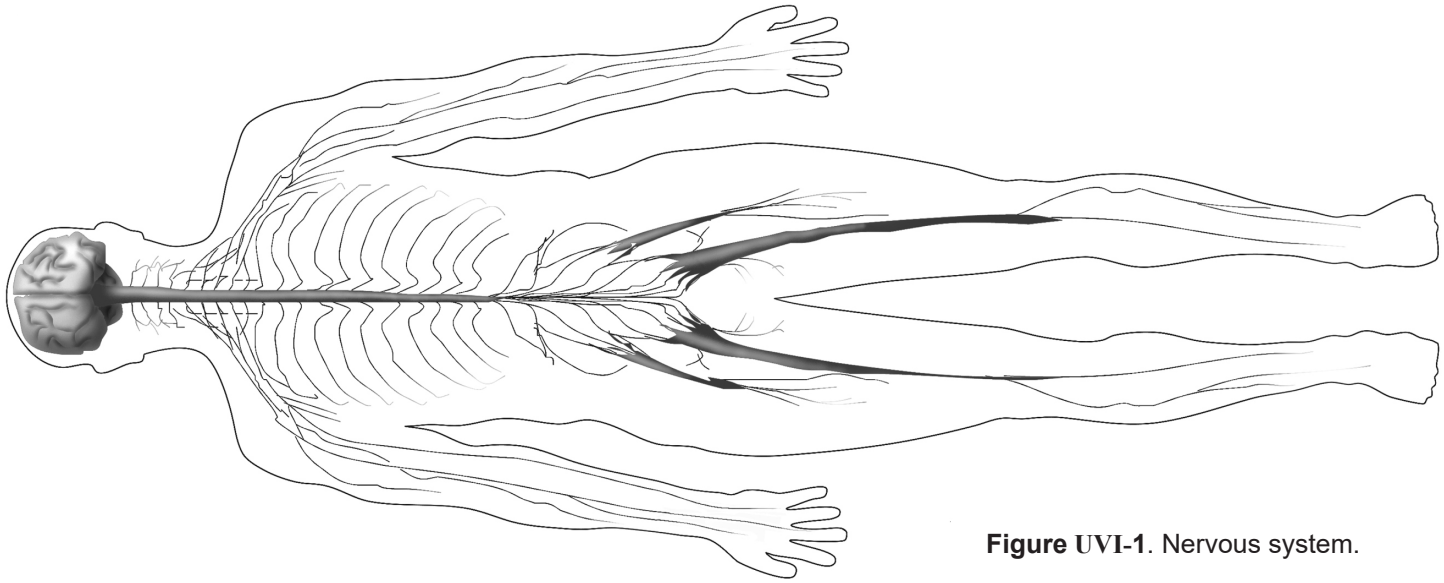


Figure UVI-1. Nervous system.

2. On Figure VI-2:
   - Label each neuron type.
   - Label the structure of a neuron A-C.
   - Draw arrows in red on each neuron showing the transmission of signals to and from the cell body

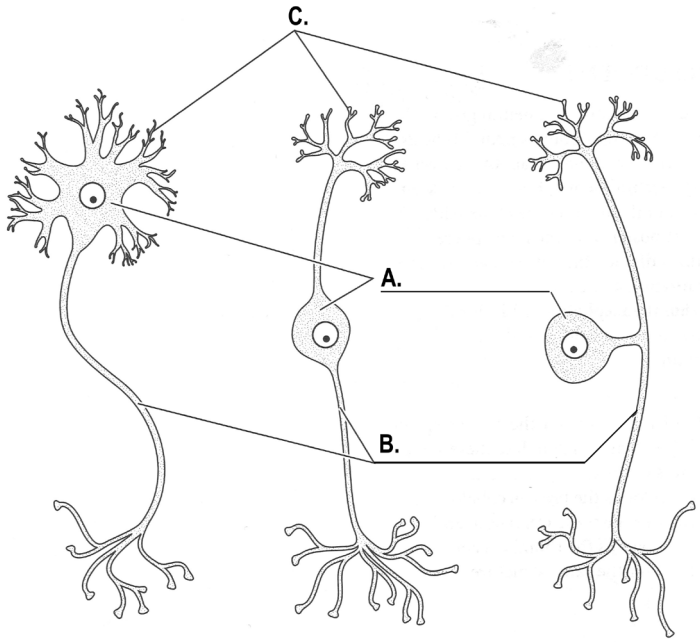


Figure UVI-2. Neuron types found in vertebrates.

3. Observe the models in lab:

A. Sketch the axon of a neuron model A and label 1-5, 14.

- Define 5

B. Sketch the axon of a neuron model B and label 7-13.

- Define 7

- Define 9

C. Sketch the brain model and label A-E and 46.

- What are the targets of 46 (Table UIV-1; endocrine)?
- Label the location of the hypothalamus?

D. On Figure VI-1 label the:

- cerebrum
- cerebellum
- sciatic nerve
- lumbar plexus

A

B

C

Targets of the pituitary:

Figure UVI-3. A) Magnified neuron B) axon of neuron, and C) Simplistic CNS brain anatomy.

Part 1. Electrolytes

Reread the content from Unit I B filtration and Unit I Part 1. pH & Conductivity about electrolytes. Reabsorption of water and key electrolytes are regulated and influenced by hormones (Table and Figure UIV-1). Sodium, potassium, and chloride are some of the key electrolytes along with magnesium, calcium, phosphate, and bicarbonates (UVI-2). Electrolytes come from our diet.

We will be measuring electrical conductance and admittance in siemens (S). Volts and siemens are converted in a 1-to-1 ratio, meaning that 1 volt equals 1 siemen. We will convert are data to mV to give us a better idea of the electrolyte ability of the beverages that many of us often consume and then relate that to the different electrolyte disorders.

A. Experimental Design

4. At the top right of the document type:
   - Biol 2227L
   - Unit VI - Nervous System (Electrolytes)
   - Your section #
   - Your team #
   - Names of everyone on your team

5. Type the answers to the following questions into the Word document.
   A. Background: Describe what an electrolyte is and how they can affect the nervous system.
   B. Question?
   C. Determine your variables: Which is your response (dependent) variable? Which is your predictor (independent) variable? Are the variables categorical and/or quantitative?
   D. Describe the control and experimental groups for your experiment.
   E. Describe the type of relationship between the variables (positive, negative, or neutral).
   F. Develop a hypothesis.
   G. What is your prediction as to the outcome of your experiment.

B. Data Collection

☐ Your instructor will project the Electrolytes spreadsheet that will indicate which control and beverages your group (indicated on the random assigned seating spreadsheet) is responsible for testing.

☐ Record the names of your group's assigned control solutions and beverages in Table UVI-3.

☐ Check that the; a) Go!Links are connected to the computer, b) pH and conductivity sensors are connected to the Go!Links, and c) sensors are connected to the ring stand clamp (they can fit in the clamp together).

☐ Open the Electrolyte.cmbl file found in the Documents > 2227L folder on the lab computer. You will see two graphs with the horizontal axis (x axis) Time(s) scaled from 0-10. pH graph - vertical axis (y axis) pH scaled from 0-14; EC graph - vertical axis (y axis) EC(dS/m) scaled from 0-14.

☐ Set the conductivity sensor range to 20,000 µS using the switch on the sensor.

☐ A window will open. Click on the 'Use Sensor Settings' button.

☐ Put on goggles and gloves.

☐ Obtain a clean and dry glass beaker.

Before use and between each use, rinse the tips of the pH and conductivity sensors thoroughly using the wash bottle and plastic beaker, then dry with a KimWipe. Do not let the pH electrode dry out. Keep the tip covered in the buffer of the storage vial between tests and after use.

Controls

☐ Add 30 ml of a control solution to the beaker.

☐ Insert the sensors into the beaker solution by lowering the clamp on the ring stand.

☐ Briefly swirl the solution in the beaker. Let the sensors sit in the solution for at least 1 minute before taking a recording.

☐ Click the 'Collect' button. The pH and EC will be tested in a 10 second run by the software.

☐ Once collection is complete, click the ½ button.

☐ Record the mean pH and EC of the 10 second runs in the 'pH' and 'conductivity' section of Table UVI-3.

☐ Wash and dry the sensors.

☐ Empty the control beaker into the waste bottle.

☐ Rinse and dry the beaker.

☐ Repeat for the second control.

☐ Enter your data into the Electrolyte spreadsheet on the instructor computer.

Table UVI-3. The pH and conductivity of the controls and solutions you tested.

Solutions	pH	Conductivity
Control 1 _____		
Control 2 _____		
Beverage 1_____		
Beverage 2_____		

Test Solutions

☐ Repeat the steps used to test the pH and conductivity of the control solutions for the beverages.

☐ Record the mean pH and EC of the 10 second runs in the 'pH' and 'conductivity' section of Table UVI-3.

☐ Enter your data into the Electrolyte spreadsheet on the instructor computer.

C. Interpretation

☐ Your instructor will send your lab section's completed workbook to each student's ISU Google Account Gmail.

6. Insert the tables and graphs into the document.
7. Create a table caption ABOVE each table.
8. Create a figure caption BELOW each figure.
9. Type the answers to the following questions into the document:
   H. What were the controls for the experiment? Why were they chosen?
   I. Which beverages (of all tested in lab today, not just the ones you tested) did you find were the strongest and weakest Acid, Base, and Conductor.
   J. Did you reject or support your hypothesis? Explain in DETAIL why it was supported or rejected?
   K. Why were some beverages good conductors but were not very acidic or basic and vice versa?
   L. Explain how resting potential and action potential depend on electrolytes?
   M. What are some neurological conditions caused by electrolyte disorders?

☐ **Send the document to your lab instructor using your ISU Google Account Gmail.**

Name:_____ Team #:_____ Section #:_____

Part 2. Neuromuscular Reflexes

The somatic nervous system (SNS) contains both afferent nerves and efferent nerves. The twelve pairs of cranial nerves send information to the brain stem or from the brain stem to the periphery and the 31 pairs of spinal nerves send sensory information from the periphery to the spinal cord and muscle commands from the spinal cord to the skeletal muscle. The SNS is responsible for both voluntary muscle responses as well as involuntary muscle responses known as reflexes (reflex arc neural pathway known). A reflex arc is a simply circuit that allows an organism to respond rapidly to inputs from sensory neurons and consists of only a few neurons. A sensory neuron sends a signal straight to the spinal cord (bypassing the brain) which in turn generates a response that may be subconscious. One common example is the knee reflex: hitting the patellar tendon just below the knee cap with a reflex hammer leads to an automatic contraction of the quadriceps – which results in the lower leg kicking out (Figure UVI-4). Electromyography (EMG) measures electrical signals produced during muscle contractions in response to a nerve's stimulation of the muscle. In this experiment, you will use an Vernier EKG Sensor to compare the speed of a voluntary vs. a reflex muscle action and to measure the relative strength (amplitude) of the impulse generated by a stimulus with and without reinforcement. You will make a rough calculation of nerve impulse speed using data generated by an accelerometer used in conjunction with the EKG sensor.

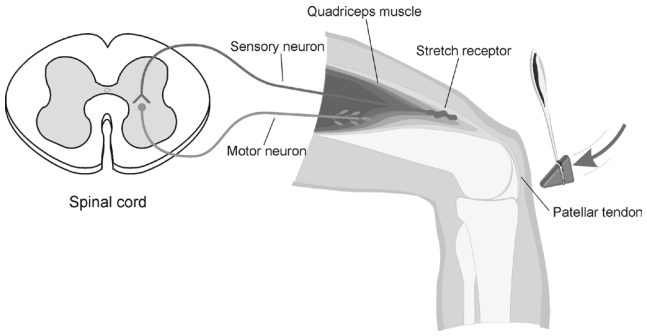


Figure UVI-4. Patellar tendon knee reflex.

Important: Do not attempt this experiment if you have pain in or around the knee. Inform your instructor of any possible health problems that might be exacerbated if you participate in this exercise.

☐ Open the Reflexes.cmbl file found in the Documents > 2227L folder on the lab computer. The .cmbl file will be opened by the Logger Pro software. You will see two graphs each with the horizontal axis (x axis) Time(s) scaled from 0-40. Neuromuscular Reflexes: EMG - vertical axis (y axis) Potential (mV) from -2 to 2; Acceleration - vertical axis (y axis) Acceleration (m/s^2) scaled from 0-200.

☐ Check that the; a) Go!Links are connected to the computer and b) EKG Sensor and the Accelerometer are connected to the Go!Links.

NOTE: the Accelerometer should be connected to the reflex hammer with the cable tie.

☐ Have the subject sit on the bench top which will allow their legs to dangle freely above the floor.

☐ Attach two electrode tabs above one knee along the line of the quadriceps muscle between the knee and the hip. The tabs should be 5 cm and 13 cm from the middle of the patella (see Figure UVI-5). Place a third electrode tab on the lower leg.

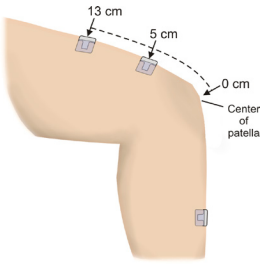


Figure UVI-5. Electrode attachment.

☐ Attach the red and green leads to the electrode tabs above the knee with the red electrode closest to the knee. Attach the black lead (ground) to the electrode tab on the lower leg.

A. Voluntary Activation of the Quadriceps Muscle

☐ Setting up a **stable baseline**: click the 'Collect' button.

- If the graph has a stable baseline (Figure UVI-6), click 'Stop' and continue to to next step.
- If your graph has an unstable baseline, click 'Stop' and collect a new set of data by clicking 'Collect' again. Repeat data collection until you have obtained a stable baseline for 5 seconds.

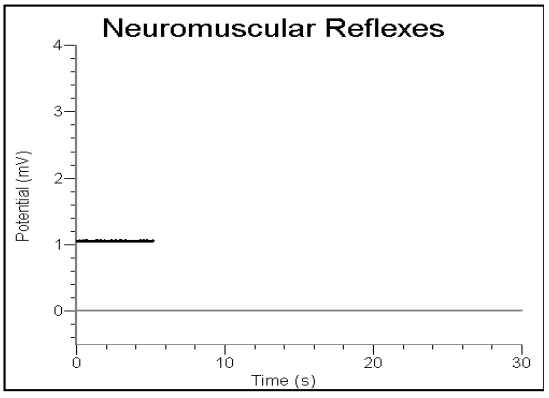


Figure UVI-6. EMG graph; baseline.

☐ Collecting **voluntary activation** data: have the **subject close his/her eyes, or avert them from the screen**:

A. Click the 'Collect' button.

B. After recording 5 seconds of stable baseline, swing the reflex hammer briskly to contact the table or other surface that generates a sound.

C. The subject should **voluntary kick his/her leg out (muscle contraction) immediately upon hearing the sound (stimulus)**.

D. Collect for six (6) kicks during the data-collection period.

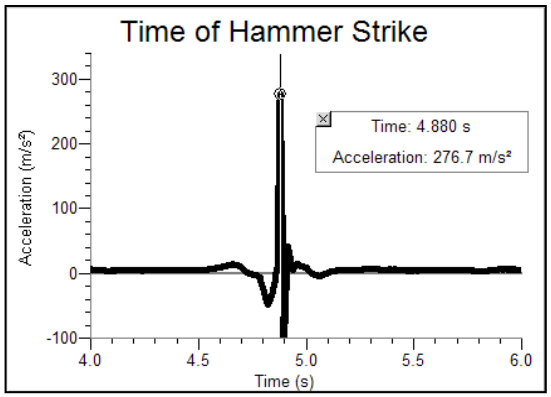


Figure UVI-7. Accelerometer graph; peak indicates the time when the table surface was struck.

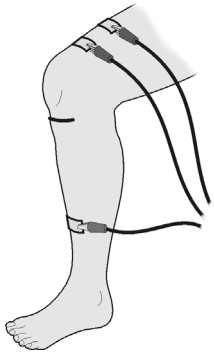


Figure UVI-8. Location of patellar tendon.

Table UVI-4. Voluntary stimulus-contraction pairs and mean change in time.

Kick	Time of muscle contraction (s)	Time of stimulus (s)	Δt (s)
1			
2			
3			
4			
5			
		Mean	

☐ Time elapsed between striking the table surface with the reflex hammer and the contraction of the quadriceps muscle:

A. Click the Examine button, , and place your cursor somewhere on the Acceleration graph.

B. Align the Examine line with the first high peak (which corresponds to the first kick) in the Accelerometer graph (Figure UVI-7). This peak indicates the time at which the table surface was struck (Time of stimulus).

C. Move the cursor to the EMG graph and align the Examine line with the first high peak (Kick 1) in the graph. This peak indicates the Time of muscle contraction.

D. Repeat this process of determining the time of the hammer strike and reflex for a total of five stimulus-contraction pairs.

E. Close the Examine box.

☐ **Record these times in Table UVI-4.**

☐ Calculate the change in time between the hammer strike and reflex for the five stimulus-contraction pairs and then calculate the **mean change in time for all five pairs**.

☐ **Record the values in Table UVI-4.**

B. Involuntary Activation of the Quadriceps Muscle

☐ Locate the subject's patellar tendon by feeling for the narrow band of tissue that connects the lower aspect of the patella to the tibia. Place a pen mark in the center of the tendon, which can be identified by its softness compared with the bones above and below (**Figure UVI-8**).

☐ Setting up a **stable baseline**: click the 'Collect' button.

- If the graph has a stable baseline (Figure UVI-6), click 'Stop' and continue to the next step.
- If your graph has an unstable baseline, click 'Stop' and collect a new set of data by clicking 'Collect' again. Repeat data collection until you have obtained a stable baseline for 5 seconds and then click 'Stop'.

☐ Collecting **patellar reflex** data: have the **subject close his/her eyes, or avert them from the screen**:

A. Click the 'Collect' button.

B. After recording 5 seconds of stable baseline, swing the reflex hammer briskly to contact the mark on the subject's tendon. If this does not result in a visible reflex, aim toward other areas of the tendon until the reflex is obtained.

C. Collect for 5-10 reflexes during the data-collection period and then click 'Stop'.

☐ Time elapsed between striking the patellar tendon with the reflex hammer and the contraction of the quadriceps muscle:

A. Click the Examine button, , and place your cursor somewhere on the Acceleration graph.

B. Align the Examine line with the first high peak (which corresponds to the first reflex) in the Accelerometer graph (Figure UVI-7). This peak indicates the time at which the tendon was struck (stimulus).

C. Move the cursor to the EMG graph and align the Examine line with the first high peak (Reflex 1) in the graph. This peak indicates the time at which the quadriceps muscle contracted (contraction).

D. Repeat this process of determining the time of the hammer strike and reflex for a total of five stimulus-reflex pairs.

E. Close the Examine box.

☐ **Record these times in Table UVI-5.**

☐ **Calculate the change in time between the hammer strike and reflex for the five stimulus-kick pairs and then calculate the mean change in time for all five pairs. Record the values in Table UVI-5.**

Table UVI-5. Patellar stimulus-reflex pairs and mean change in time.

Reflex	Time of muscle contraction (s)	Time of stimulus (s)	Δt (s)
1			
2			
3			
4			
5			
		Mean	

C. Reflex Reinforcement

☐ Locate the subject's patellar tendon by feeling for the narrow band of tissue that connects the lower aspect of the patella to the tibia. Place a pen mark in the center of the tendon, which can be identified by its softness compared with the bones above and below (**Figure UVI-8**).

☐ Setting up a **stable baseline**: click the 'Collect' button.

- If the graph has a stable baseline (Figure UVI-6), click 'Stop' and continue to the next step.
- If your graph has an unstable baseline, click 'Stop' and collect a new set of data by clicking 'Collect' again. Repeat data collection until you have obtained a stable baseline for 5 seconds and then click 'Stop'.

☐ Collecting **patellar reflex data without/with reinforcement** data: have the subject close his/her eyes, or avert them from the screen:

A. Click the 'Collect' button.

B. After recording 5 seconds of stable baseline, swing the reflex hammer briskly to contact the mark on the subject's tendon. If this does not result in a visible reflex, aim toward other areas of the tendon until the reflex is obtained.

C. Continuing collecting data for five to six (5-6) **reflexes** during the data-collection period.

D. Have the subject reinforce the reflex by hooking together his/her flexed fingers and pulling apart at chest level, with elbows extending outward (Figure UVI-9).

E. Continuing collecting data for five to six (5-6) **reinforced reflexes** during the data-collection period.

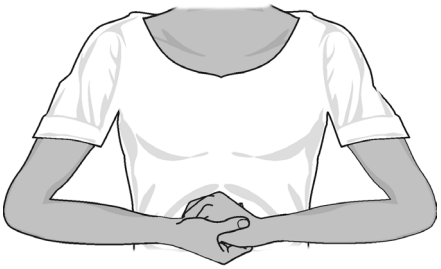


Figure UVI-9. Reinforce the reflex by hooking together flexed fingers and pulling apart at chest level, with elbows extending outward

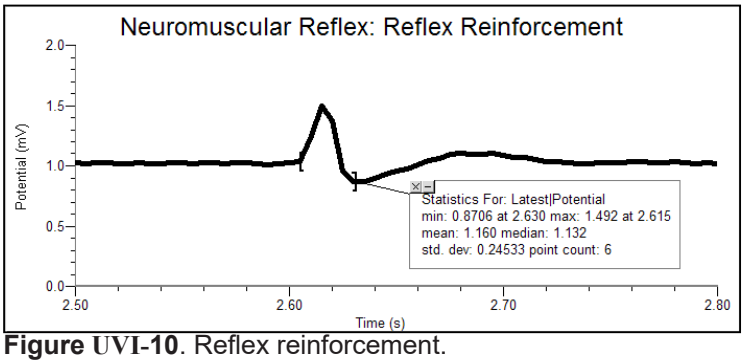


Figure UVI-10. Reflex reinforcement.

F. A total of ten to twelve (10-12) reflexes should appear on the graph and then click 'Stop'.

☐ Click the Statistics button . Move the brackets to frame the first area of increased amplitude (depolarization) in this run (Figure UVI-10).

☐ **Record the maximum, minimum, and Δy value (amplitude) for this depolarization in Table UVI-6, rounding to the nearest 0.01 mV.**

☐ Repeat this process for 5 unreinforced and 5 reinforced depolarization events, **using the Accelerometer to identify each primary reflex**. Ignore rebound responses. Record the appropriate values in Table UVI-6.

☐ Determine the average amplitude of the reinforced and unreinforced depolarization events examined. Record these values in Table UVI-6.

Table UVI-6. Patellar reflex reinforcement.

	Reflex without reinforcement			Reflex with reinforcement		
Reflex response	Max (mV)	Min (mV)	ΔmV	Max (mV)	Min (mV)	ΔmV
1						
2						
3						
4						
5						
		Mean			Mean	

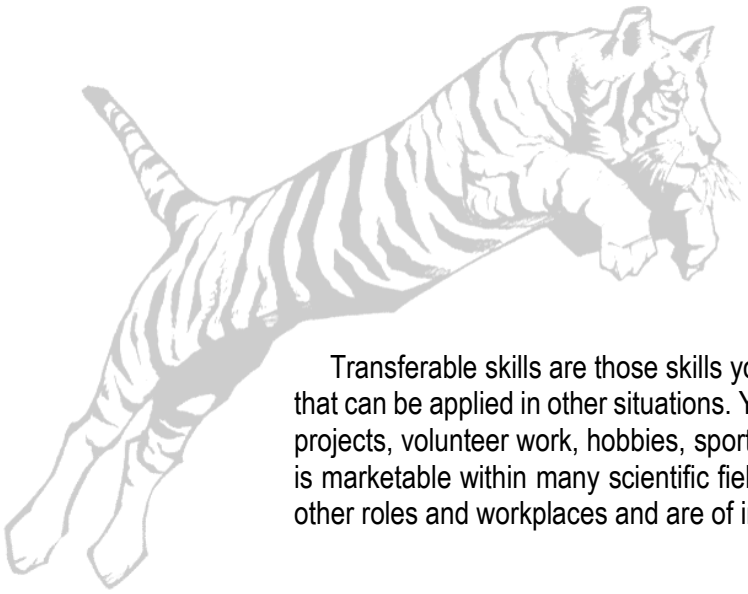
D. Data Analysis & Interpretation

☐ Open the 15_Reflexes workbook file found in the Documents > 2227L folder on the lab computers. Add your group's data to the spreadsheet tables. The graph will automatically populate.

1. Open a Word document and at the top right of the document type:
   - Biol 2227
   - Unit VI - Nervous System (Reflexes)
   - Your section #
   - Your team #
   - Names of everyone on your team
2. Insert the tables and graphs into the document.
3. Create a table caption ABOVE each table.
4. Create a figure caption BELOW each figure.
5. Type the answers to the following questions into the document:
   A. Compare the reaction times for voluntary vs. involuntary activation of the quadriceps muscle. What might account for the observed differences in reaction times?
   B. Using data from Table UVI-5, calculate speed at which a stimulus traveled from the patellar tendon to the spinal cord and back to the quadriceps muscle (a complete reflex arc):
      - Measure the distance in cm from the mark on the patellar tendon to the spinal cord at waist level.
      - Multiply the distance by two to obtain the total distance traveled in the reflex arc.
      - Divide by the mean Δt from Table UVI-5 and divide by 100 to obtain the speed, in m/s, at which the stimulus traveled.

   C. Nerve impulses have been found to travel as fast as 100 m/s. What could account for the difference between your answer to 5B and the value obtained by researchers?
   D. Assume the speed of a nerve impulse is 100 m/s. How does this compare to the speed of electricity in a copper wire (approx. 3.00×10^8 m/s)?
   E. Compare the data you obtained in this experiment with other members of your group/class. Can individual differences be attributed to any physical differences (body shape/size, muscle mass, physical fitness level)?

☐ **Send the document to your lab instructor using your ISU Google Account Gmail.**



TRANSFERABLE SKILLS

Transferable skills are those skills you acquire during any activity in your life - not just your studies - that can be applied in other situations. You can acquire skills through all sorts of activities: employment, projects, volunteer work, hobbies, sports, virtually anything. The knowledge you will develop in biology is marketable within many scientific fields. You will also gain skills that are transferable to a variety of other roles and workplaces and are of interest to a wide variety of employers.

Four types of skills that **all undergraduates** (regardless of major) are expected to develop:

- ✓ **Intellectual** – comprehension, critical reasoning, analytical, evaluation, planning and information-gathering, report writing.
- ✓ **Communication** – clarity of writing, layout and presentation of oral and written material, referencing, use of appendices, bibliographies, glossaries, indexes, and figures/tables.
- ✓ **Organizational** – prepare for exams, organize and complete assignments, time management, working under pressure.
- ✓ **Interpersonal** – negotiation, diplomacy, flexibility, adaptability, teamwork as well as independent work, delegation, and self-motivation.

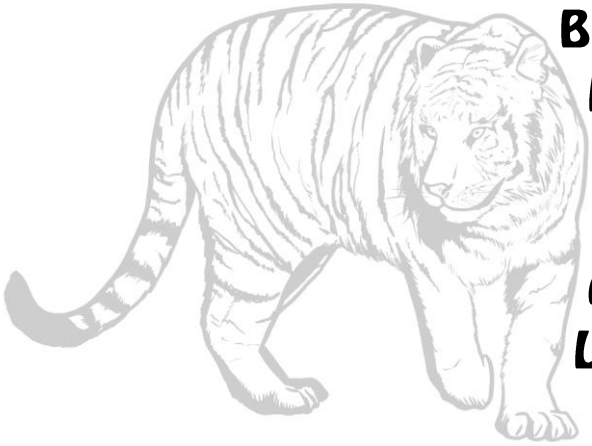
Four types of skills (in addition to those above) that **all biology students** are expected to develop:

Foundational knowledge – structure/anatomy, function, physiology, reproduction, growth/development, origin, ecology, evolution, and distribution of organisms.

Applied knowledge

- ✓ **Research** – use primary sources (read, understand, and cite scientific literature) to develop questions that are innovative, novel, and creative; use the scientific method to answer these questions by constructing hypotheses and predictions, design experiments to test the hypotheses and predictions; monitor, record, and manage data; statistical analysis of the data; and conduct a critical analysis of the results.
- ✓ **Numeracy** – mathematical ability is necessary in most fields, and it is important that all students maintain at least a rudimentary comprehension of numeracy.
- ✓ **Computer literacy** – typing speed and accuracy, text formatting, spreadsheet use, formal presentation construction, academic and professional use of search engines, email, and other types of software and web applications.

BENGAL SURVIVAL SKILLS



BE PREPARED AND RESPONSIBLE
EMBRACE POSITIVE CHOICES
NURTURE A POSITIVE ATTITUDE
GIVE RESPECT TO SELF AND OTHERS
ACT ON TIME AND ON TASK
LABOR FOR SUCCESS