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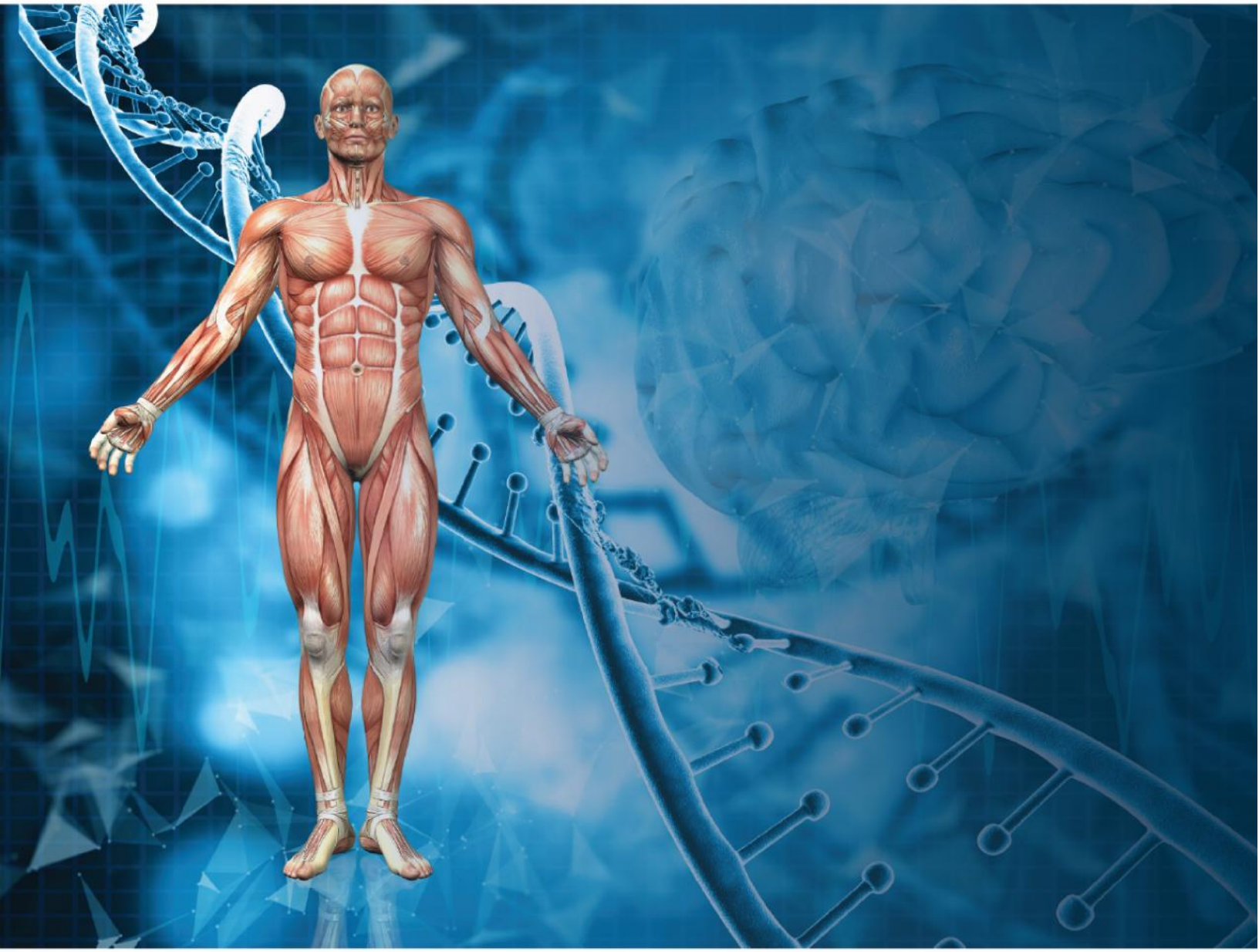
HUMAN STRUCTURE AND FUNCTION LABORATORY



Wided Kouidhi, Ph.D.

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Preface

This textbook is an essential resource for students and lecturers, providing a comprehensive yet accessible exploration of the human body's structure and function. It covers key biological systems through a combination of theoretical insights and practical applications.

Anatomy and Dissection: Students gain hands-on experience in mammalian dissection, using rodent models to explore anatomical structures that are foundational to human biology.

Histology: The textbook details the microscopic study of tissues, guiding students through tissue processing, sectioning, and staining techniques that reveal cellular structures.

Integumentary and Skeletal Systems: It examines the skin's role in protection and temperature regulation, alongside the skeletal system's function in support and movement.

Nervous System: This section explores the nervous system's response to stimuli, including reaction time and sensory perception exercises, highlighting the system's critical functions.

Lymphatic and Immune Systems: The textbook introduces carbon clearance assays to study the immune response, focusing on how the body handles foreign substances.

Endocrine System: Students learn about hormonal regulation, particularly glucose homeostasis, through practical exercises like glucose tolerance tests.

Cardiorespiratory System: The textbook covers the interplay between the cardiovascular and respiratory systems, emphasizing their roles during physical exercise and stress.

Digestive System: It provides insights into the chemical digestion of food, focusing on the enzymatic breakdown of proteins and carbohydrates.

Urinary System: The textbook explores the mechanisms of water and electrolyte balance, detailing the kidney’s role in urine formation and the effects of different substances on this process.

Reproductive System: The final chapter discusses the female reproductive cycle, offering a case study to clarify common misconceptions and explain hormonal regulation.

This textbook serves as a comprehensive guide, blending theoretical knowledge with practical skills, making it an invaluable tool for both students and instructors in the study of human biology.

Author,

Wided Kouidhi, Ph.D.

Acknowledgments

I would like to extend my deepest gratitude to the following individuals for their invaluable contributions to the "Human Structure and Function Laboratory" textbook.

To Sanjaya Waani, Head of Laboratory, for his unwavering support and assistance in organizing the lab sessions. His expertise and dedication were instrumental in ensuring the quality and effectiveness of the practical components included in this textbook.

To Elizabeth Sidhartha, Program Manager of Biomedicine, for her guidance and approval of the sessions. Her insights and leadership played a crucial role in shaping the content and ensuring that it meets the highest standards of academic excellence.

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Chapter I : Introduction to Mammalian Dissection and Anatomy

Overview

Rodent models have been crucial in the advancement of knowledge in biological and medical areas.

Although it does not represent the human structure and function precisely, it allows the thorough anatomical studies, physiology, immunological function, and provide information about the body as a whole; which then contribute to the discovery of prophylactic measures and disease treatments that affect human. Among the rodent group, the Asian mice specie *Mus musculus* are the most used animals in experimental studies. They are small, very fertile, short gestation period, easy domestication and maintenance. In addition to that, mice share 99% genetic similarity with humans, which again allow the investigation of human genetic disorder. For this reason, here we utilize mice as a medium to understand the general biology, anatomy, and histology of mammalian structures and functions.

Procedure

1. Measure the mice body weight
2. Anesthetize the mice using Ketamine or Xylazine, to calculate the volume needed to anesthetize the mice, use the following formula:

$$\text{volume (mL)} = \frac{Weight * Dose\ (100mg/Kg\ Body\ Weight)}{Ketamine\ Concentration\ (50mg/mL)}$$

3. Once the mouse loses equilibrium, using forceps and surgical scissor, dissect the mice from the lower abdomen up to the mice lower neck (**Be careful not to cut too deeply keep the tip of your scissors pointed upward. Do not**

damage the underlying structures):

a. Pin the mouse down by placing it ventral side up (Figure 1A).
b. Spray the entire chest and abdomen area with 70% EtOH
c. Lift the abdominal skin with a forceps, and cut through it with the scissors (Figure 1B).
d. Close the scissor blades and insert them under the skin. Moving in a cephalic direction, open and close the blades to loosen the skin from the underlying connective tissue and muscle (Figure 1B).
e. Once this skin-freeing procedure has been completed, cut the mouse along the body midline, from the pubic region to the lower jaw.
f. Make a lateral cut about halfway down the ventral surface of each limb.
g. Complete the job of freeing the skin with the scissor tips.
h. Pin the flaps to the tray (Figure 1C)
i. Notice that the muscles are packaged in sheets of pearly white connective tissue called fascia, which protect the muscles and bind them together (Figure 1D).
j. Carefully cut through the muscles of the abdominal wall in the pubic region, avoiding the underlying organs. To do this, hold and lift the muscle layer with a forcep and cut through the muscle layer from the pubic region to the bottom of the rib cage, in a similar way you did with the skin (Figure 1E).

4. Complete removal of blood from the cardiac chamber will be done by intracardiac puncture
5. Perform necropsy for the removal of organs: heart, lung, liver, and kidney; additionally, isolate the brain from the skull and isolate the muscle from the lower thigh of the mice
6. Wash each organ **immediately** in physiological buffer.

7. Dry organs using filter paper and tissue
8. Evaluate the organs in relation to the anatomical form, weight, coloring, length, and width.
9. Directly fixate the organs by submerging it in 10% formaldehyde or 4% paraformaldehyde for
   ~48hrs (Max 1 week)

Figure 1. Steps of the mouse dissection.

Result and Observation

Table 1. Absolute and relative weight values.

Organs	Absolute Weight (g)		Relative Weight (%)	
	Mice 1	Mice 2	Mice 1	Mice 2
Total Body Weight				
Left Lung				
Right Lung				
Heart				
Liver				
Left Kidney				
Right Kidney				
Spleen				

Table 2. Length and Width values.

Organs	**Length (mm)**		**Width (mm)**	
	Mice 1	**Mice 2**	**Mice 1**	**Mice 2**
Brain				
Left Lung				
Right Lung				
Heart				
Liver				
Left Kidney				
Right Kidney				

Analysis and Discussion

Please answer the following questions:

1. Why does the size of left and right lung different?

2. What are the different between mice anatomy and human anatomy? (state min. 3 points)

Overview

Histology is the microscopic study of animal and plant tissues through staining and sectioning and

examining them under a microscope. This technique has a lot of variation to cater to the tissue observed as well as their application. The process of histological staining generally involves 5 different steps: fixation to preserve the natural tissue structure and maintain the cell structure from degradation, dehydration processing to solidify tissue by removing their water content, embedding to enhance easier extraction of cellular structure, sectioning to allow mounting to the microscopic slide for examination and staining to highlight important features of the tissue and to increase tissue contrast.

Procedure

A. Tissue Processing: Dehydration and Paraffin Embedding

1. Position organ in cassettes
2. Do serial dehydration:
   - 90% EtOH for 3 x 1hr
   - 100% EtOH for 3 x 1hr
3. Clearing in Toluene for 2 x 75min
4. Embedding in warm paraffin (55-60^{o}C) overnight, followed by 2 more time for 2 hours each
5. After the embedding process, the organ samples are placed according to the plane of the section (transversal/longitudinal) in molds to prepare the paraffin blocks.

B. Histological Slide Preparation: Microtome Cutting

1. Chill paraffin-embedded tissue blocks on ice before sectioning. Cold wax

allows thinner sections to be obtained by providing support for harder elements within the tissue specimen. The small amount of moisture that penetrates the block from the melting ice will also make the tissue easier to cut.

2. Fill a water-bath with ultrapure water and heat to 40-45ºC.
3. Place the blade in the holder, ensure it is secure and set the clearance angle. The clearance angle prevents contact between the knife facet and the face of the block. Follow the microtome manufacturer's instructions for guidance on setting the clearance angle. For Leica blades this is normally between 1º and 5º
4. Insert the paraffin block and orientate so the blade will cut straight across the block.
5. Carefully, approach the block with the blade and cut a few thin sections to ensure the positioning is correct. Adjust if necessary.
6. Trim the block to expose the tissue surface to a level where a representative section can be cut. Trimming is normally done at a thickness of 10-30 µm.
7. Cut sections at a thickness of about 4-5 µm (you will probably need to discard the first few sections as they are likely to contain holes caused by trimming).
8. Using tweezers, pick up the ribbons of sections and float them on the surface of the water in the water bath so they flatten out. Use the tweezers to separate the sections.
9. Use microscope slides to pick the sections out of the water bath and store upright in a slide rack.
10. Place the slide rack into an oven and allow sections to dry overnight at 37ºC.
11. Proceed to Staining

C. Hematoxylin and Eosin Staining

1. Arrange the slides in the proper rack

2. Deparafinize
    - 2X toluene baths of 5 min each

3. Rehydration
    - 1X bath 100% alcohol for 5 min
    - 95% alcohol bath for 5 min
    - Rinse in distilled water

4. Staining
    - Harris Hematoxylin staining for 5 min (nuclei staining)
    - Rinse in running tap water
    - Immerse in Lithium carbonate (fast pass)
    - Rinsing with tap water
    - Eosin staining for 1 to 2 min (cytoplasm staining)
    - Rinsing with water

5. Dehydration
    - 1 X 95% alcohol bath for 5 min
    - 1 X 100% alcohol bath for 5min
    - 1 X toluene bath for 5 min

Finally the slides are covered with coverslips by Leukitt. Let them dry for few hours before observing through the microscope.

Results and Observations

1. Collect the staining result of every tissue samples and label it properly!
2. Prepare the following table for each

tissues observed! Overall Quality (10x

Objective)

YES	NO	
		Is the staining even?
		Are the nuclei standing out darker than the background?
		Is there absence of splotches (e.g. water droplets)?

Hematoxylin Staining (40x Objective)

YES	NO	
		Is the nuclear wall dark and crisp?
		Is the chromatin pattern stippled, not smudgy?
		Is there any cells that stain blush color?

Eosin Staining (40x Objective)

YES	NO	
		Can you observe RBC? Are they the darkest red?
		Can you see any blood vessels?

Analysis and Discussions

1. What does H&E stain and what are the colors?
2. Is there any problem faced during the experiment?
3. How can you improve the quality of your H&E staining?

Chapter III : Analysis of Integument and Skeletal System

Overview

The skin, or integument, is considered as an organ system due to its extent and complexity. It is tough yet pliable, making it possible to withstand constant insult from external factors. The skin has many functions, this includes as an insulation and cushion to the underlying body tissues and protects the entire body from mechanical damage, chemical damage, thermal damage, and bacterial invasion. The skin also contributes in regulating heat loss from the body surface via its capillary networks and sweat glands.

Another system that plays a crucial role for human body is the skeletal system. The skeletal system supports and protects the body as an internal framework, provides a system of levers with which the skeletal muscles work to move the body, stores lipids and many minerals and provides a site for hematopoiesis. The skeleton is made up of bones that are connected at joints, or articulations. Although the bones are relatively light, they are one of the hardest materials in our body. The hardness of bone comes from the inorganic calcium salts deposited in its ground
substance while its flexibility comes from the organic elements of the matrix, particularly the collagen fibers.

This session consists of two experiments, where you will learn how the integument plays a role in regulation of body temperature and examine the effects of acid solution on bones.

Procedure

A. Role of The Integument System in Regulating Body Temperature

1. Record initial observation based on table 1. Use thermometer to measure

body temperature.

2. Do a 5 minutes stretching, followed by a burpee exercise with a combination of 45 seconds burpee and 15 seconds break. Do the exercise for 10 minutes.

Figure 2. How to perform Burpees

3. Immediately after completing 10 minutes of exercise, record the observations again

Result and Observation

Name	Parameters	Before exercise	After exercise
1.	Body temperature (in ^{o}C)		
	Skin appearance (color, wet/dry)		
	Perspiration (none/little/moderate/profuse)		
2.	Body temperature (in ^{o}C)		
	Skin appearance (color, wet/dry)		
	Perspiration (none/little/moderate/profuse)		
Summary of changes observed after exercise:			

Analysis and Discussion

Please answer the following questions:

1. Why does 10-minute exercise result in changes in body temperature, skin appearance, and perspiration?

2. How does the integument system contribute to body temperature regulation in a cold environment?

B. Effect of Vinegar and Coke Towards Bone

1. Apply moderate pressure at the middle of chicken bone and observe whether the bone can be bent. Record your observation.
2. Pour around 250 ml of vinegar, coke, and water into separate food containers
3. Soak each bone into the liquid in the food container, making sure that the whole bone is immersed.
4. Close the lid of the container and leave it for 1 week.
5. After 1 week, take out the bone and rinse it with water.

6. Observe and record any visible changes on the physical appearance of the bone.
7. Feel if there is a change on the hardness of the bone. Observe what happen when you apply pressure. Does the bone bend or snap?

Result and Observation

Bone	**Immersing solution**	**Observation (visual and tactile)**	
		Before soaking	**After soaking**
1	Coke		
2	Vinegar		
3	Water		

Analysis and Discussion

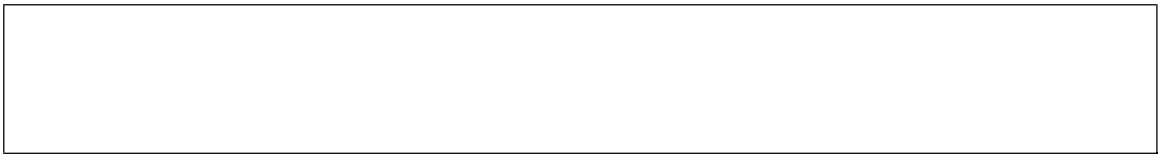
Please answer the following questions:

1. Why does soaking bones in the different solutions for 6 days result in the observed changes? Relate the changes to bone composition.

2. Which solution does coke resemble more, vinegar or water? What does that suggest about the properties of coke? If the pH of vinegar is around 2.5, and the pH of water is around 7, estimate the pH of coke.

3. What factors may have influenced the results? Suggest 2 improvements on the

experimental design.



Chapter IV : Analysis of Human Nervous System

Overview

Nervous tissue possesses two fundamentals properties; irritability and conductivity. Irritability if the

potential of a nerve to respond to some kind of stimulus (chemical, mechanical, electrical), and conductivity is the movement of a nerve impulse along the length of the neuron. Nerves receive information from a particular area (sensory organ, regions in the CNS, etc) and transmit impulse to either the brain for interpretation or an effector for some kind of action.

Reaction time is a measure of how quickly an organism can perform a response to a particular stimulus. Reaction time has been widely studied, as its practical implications may be of great consequence, e.g. a slower than normal reaction time while driving can have grave results. Many factors have been shown to affect reaction times, including age, gender, physical fitness, fatigue, distraction, alcohol, personality type, and whether the stimulus is auditory or visual.

In this session, we will investigate the tactile acuity of certain areas of the skin, as well as perform reaction time measurement and the effect of music distraction on reaction time.

Procedure

A. Two-Point Discrimination Test

1. Untwist the paperclip and make a U-shaped apparatus, with the initial distance of 5 cm between the points.
2. Touch both ends of the U-shaped paper clip on the tip of the forefinger, and determine if you could feel two contact points touching you. Do this on the upper arm as well.
3. Shorten the distance between the two tips by 0.5 cm, and repeat step (2). Keep shortening the distance until the two-point touch feels like it comes

from a single point, either on your finger or your upper arm.

4. Record the maximum distance between the two points that feel like a single point touch.

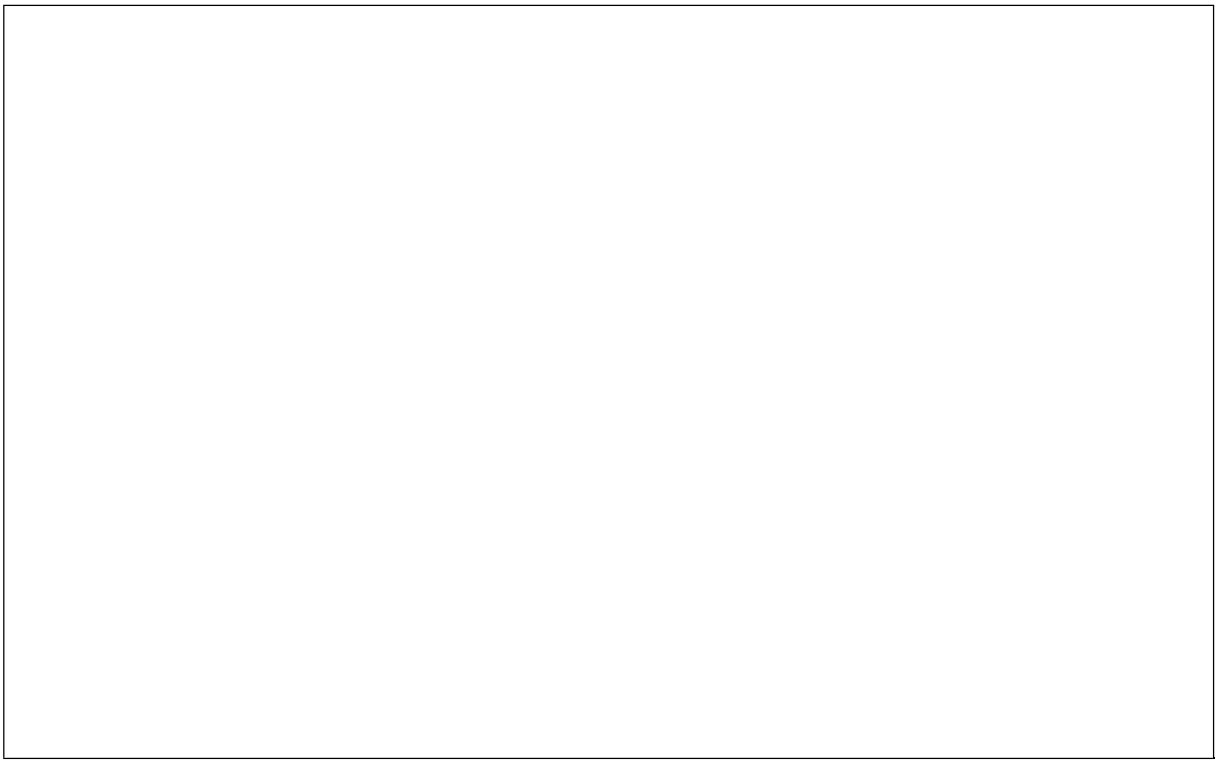
Result and Observation

Name	**Distance between two tips that feels like single point touch (cm)**	
	Fingertip	**Upper Arm**
1.		
2.		
3.		
4.		
5.		
6.		
Average		

Analysis and Discussion

1. Summarize your findings below.

2. What affects the ability to perceive separate contact points? How would you explain the difference between the results of the fingertip and upper arm?



B.Reaction Time Test

1. Perform the reaction time test by clicking on the highlighted button as quickly as you can. Record your score in the table.
2. Perform the reaction time test again but with music playing in the background. Record your score in the table.
3. Repeat the above steps 5 times, alternating between no music and with music.
4. Obtain the average score without music and the average score without music.

Result and Observation

Subject Name		**1.**	**2.**	**3.**	**4.**	**5.**
Score without music	**1st**					
	2nd					
	3rd					
	4th					
	5th					
	Average					
Score with music 1	**1st**					
	2nd					
	3rd					
	4th					
	5th					
	Average					
	1st					
	2nd					
	3rd					

Score with music 2	**4th**					
	5th					
	Average					
Score with music 3	**1st**					
	2nd					
	3rd					
	4th					
	5th					
	Average					

Analysis and Discussion

1. Summarize your findings below.

2. Explain what processes are happening in your nervous system in order to perform the reaction test, from perceiving the highlighted point on your screen up to the clicking action of your hand.

3. In general, how and why does background music affect reaction time? Suggest one way in which someone might achieve the opposite effect.

Chapter V : Investigation of Mammalian Lymphatic System (Carbon Clearance Assay)

Overview

Carbon clearance assay is a method to measure the activity of the immune system. It relies of the simple

principle that carbon is a foreign substance that need to be removed from our body through the process of phagocytosis. When carbon first introduced to the blood, the carbon pigment will turn the blood color darker. The immune reaction will then be reflected in the in-clearance of carbon particles from blood as indicated with lower blood absorbance value. To best illustrate the immune response, the obtained value need to be compared with mice liver, spleen, and total body weight.

In this session, we will investigate the ability of the lymphatic system to secrete immune cells to blood vessel and clear up the carbon we injected.

Procedure

1. Measure the mice body weight
2. Inject 0.3 mL 2xdiluted carbon ink to mice intravenously (Figure 2).
3. Using 28G lancet, collect the mice blood from submandibular vein (Figure 3) at 1, 15, and 30 min time points
4. Mix 2.5 µL of each blood samples with 200 µL of 0.1% Sodium Carbonate.
5. Measure the absorbance using UV Spectrophotometer at 675 nm
6. Calculate the carbon clearance rate at 15 min and 30 min using the following formula"

$$\text{Carbon clearance rate (K)} = \frac{logOD1 - logOD2}{}$$

$duration\ (min)$

7. Sacrifice the mice
8. Isolate the mice liver and spleen, wash it thoroughly in PBS, and gently pet it down to filter paper to dry.
9. Weight both the liver and spleen.
10. Calculate the Phagocytic Index using the following formula:

$$\text{Phagocytic Index}(\alpha) = \frac{K \times \text{Body weight of animal}}{\text{Liver weight} + \text{Spleen weight}}$$

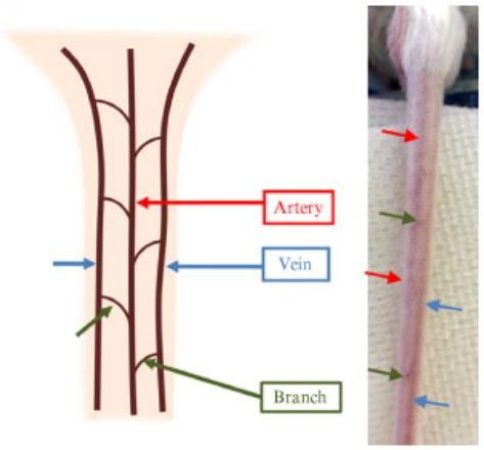


Figure 3. Location of mice tail vein.

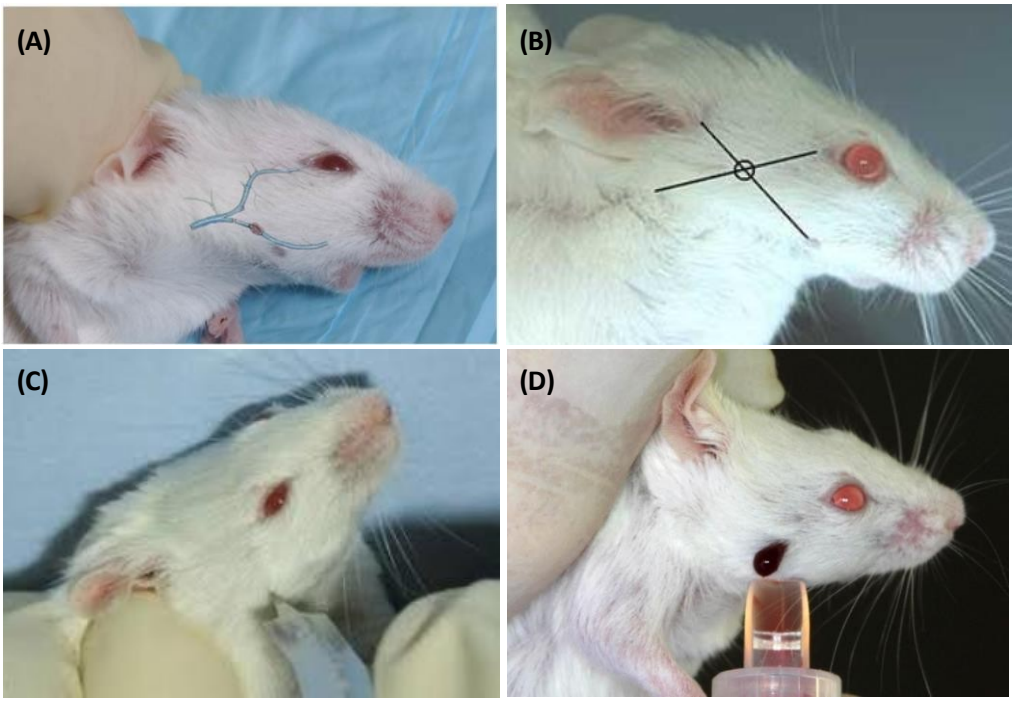


Figure 4. Mice blood collection from submandibular vein. (A) the position of mice submandibular vein, (B) mice physiological hallmark to locate the submandibular vein, (C) the use of lancet to create small nick to the vein, and (D) blood collection process.

Result and Observation

Parameters	**Mice 1**	**Mice 2**
Body Weight		
OD at 1 minute		
OD at 15 minutes		
OD at 30 minutes		
Liver Weight		
Spleen Weight		
Carbon Clearance Rate		
Phagocytic Index		

Analysis and Discussion

1. What is the function of sodium carbonate in carbon clearance assay?

2. Summarize your findings below.

Chapter VI : Analysis of Human Endocrine System

Overview

The endocrine portion of the pancreas functions primarily in the regulation of glucose homeostasis. The

cells in the endocrine portion of the pancreas are located in the Islets of Langerhans, so named as they form islands of endocrine cells in a sea of exocrine glands. The beta and alpha cells located in the islets produce the counter regulatory hormones insulin and glucagon, respectively. The primary function of insulin is to lower blood glucose whereas glucagon acts to increase blood glucose levels. Together these hormones help maintain glucose homeostasis in the body. The goal of this lab is to perform glucose measurements following a fasting period and glucose consumption. At least one volunteer from each group will be chosen as subjects to undergo a full mock glucose tolerance test. Subject must be healthy individual and must sign and informed consent sheet.

Important Notes:

1. Students who fulfill the following inclusion criteria can participate:
    a. Weighing over 43 kg
    b. Generally in good health
2. The following students will be exempted from this experiment:

. Students with diabetes

a. Students undergoing physical stress (recent surgery, trauma, or severe infection) or extreme psychological stress

3. For one week before the session, do not smoke.
4. Subjects must fast at least 8 hours (but not more than 16 hours) before the test – no food, no sugary drinks, mineral water only).

Procedure

A. Initial Blood Draw

1. Prepare all of the materials and equipment on your work bench. Clean your work bench with tissue paper and 70% alcohol. Read carefully the instruction manual for the glucose meter.
2. Have your subject seated comfortably. Clean the area (the distal phalange of the second or third finger of the hand) to be pricked with an alcohol pad and dry completely. Set the depth of the blood lancet (usually 3-5, depending on subject's skin thickness). Then prick the finger with the lancet. Press gentle the finger until a small drop of blood appears. You may need to apply a little pressure by squeezing the fingertip. Collect the blood drop with the test strip.
3. Follow the instructions for inserting the glucose test strip and using the glucose meter. Read the reading provided by the glucose meter. Record your results.
4. Dispose the lancets in the sharp bin. Dispose alcohol pads, gauze pads, and band aid in the designated biohazard disposal container.
5. Clean lab bench thoroughly with 70% alcohol and tissue paper.

Mock Glucose Tolerance Test

1. Prepare a 75-g glucose solution by mixing 75 g of sugar homogenously in 400 cc of water in a beaker glass using a stirrer. Put the solution in plastic cup.
2. Have your subject drink the glucose solution all at once if possible. Glucose solution must be finished in no longer than 15 minutes.
3. Repeat the blood draw and glucose testing every 30 minutes following the glucose drink. Do this over the course of 120 minutes. Record your results.

Results and Observations

Time point	**Glucose reading (mg/dL)**
0 min (before glucose drink)	
30 min	
60 min	
90 min	
120 min	

Plot a graph showing the changes in blood glucose level over time

--- insert chart here ---

Did the subject consume caffeinated beverages before the experiment? YES/NO If YES:

How long before the experiment starts? _____

_____ What type

of caffeine? _____

Analysis and Discussion

1. Summarize the findings above.

2. Are your test subject's results considered normal? If not, describe what the expected normal response would be.

3. How would the results be for someone who has:
   a. Type 1 Diabetes Mellitus?

   b. Type 2 Diabetes Mellitus?

4. Explain how caffeine, cigarette smoking, and food consumption (no fasting) would affect the results of the experiment.

Chapter VII : Analysis of Human Cardiorespiratory System

Overview

The cardiovascular system and the respiratory system work together to provide nutrients and oxygen to fuel the workings of all parts of the body. Every living cell performs cellular respiration, a process that converts chemical energy from nutrients and oxygen to ATP, which can then be utilized to perform the cell's functions. Upon stressful conditions, such as during exercise, the demands for nutrients and especially oxygen would increase, inducing the cardiorespiratory system to work harder in coordination in order to sustain the increased needs of the tissues.

This experiment aims to demonstrate the effect of stress, in the form of physical exercise, on the cardiorespiratory system.

Procedure

1. Get the subject to sit in a comfortable position, and calculate their heart rate by placing your index and middle fingers on the inside of the wrist, below the thumb. Count the pulse for a full minute. Record this as the resting heart rate.
2. While still sitting in a relaxed manner, count the breathing over a full minute. Record this as the resting breathing rate.
3. Perform the exercise according to the table below:

1st Exercise	**2nd Exercise**	**3rd Exercise**
Jumping jacks for 30 seconds	Jumping jacks for 90 seconds	Jumping jacks for 3 minutes

4. Immediately after the 1st Exercise, count the heart rate and the breathing rate again. Record this as post-exercise heart rate and breathing rate. Calculate the percentage change in heart rate and breathing rate by comparing to the resting heart rate and resting breathing rate, respectively.

5. Before starting the next exercise, rest for 5-10 minutes, and check the heart rate and breathing rate again. Do not start the 2nd Exercise until the heart rate and breathing rate are both similar (within ± 5) to the rates before the 1st exercise.
6. Repeat the procedure for the 2nd and 3rd Exercises.
7. Record the findings in the Results table and in the Class Data spreadsheet.

Results and Observation

A. Group data

Name of Subject:_____

	30 sec exercise	**90 sec exercise**	**3 min exercise**
Resting heart rate			
Post-exercise heart rate			
% change in heart rate			
Resting breathing rate			
Post-exercise breathing rate			
% change in breathing rate			

B. Class data

	30 sec exercise	**90 sec exercise**	**3 min exercise**
Average resting heart rate			
Average post-exercise heart rate			
% change in heart rate			
Average resting breathing rate			
Average post-exercise breathing rate			
% change in breathing rate			

Analysis and Discussion

1. Summarize the above results and describe any trends that can be discerned

2. Explain why exercise would induce such changes in heart rate and breathing rate.

3. Identify possible factors that can influence heart rate and breathing rate in different individuals.

Experiment 2: Diving Response

Overviews

During a dive, the body activates the "diving response", a collection of responses aimed at conserving energy, which includes reduction of heart rate, reflex to hold breath, and peripheral vasoconstriction.

This experiment aims to demonstrate the dive response and to investigate the factors that triggers the activation of diving response.

Procedure

1. While the subject is sitting in a comfortable position, calculate their heart rate by placing your index and middle fingers on the inside of the wrist, below the thumb. Count the pulse for 15 seconds, multiply by 4 to get heart rate per minute. Record this as the resting heart rate.

2. The subject is to immerse their face up to the temples for 30 seconds in the slightly cold water. At the 15 second mark, start counting their pulse until the end of the 30 second immersion period. Record this heart rate and calculate the percentage change in heart rate.
3. Rest for several minutes and check their heart rate again. Proceed only when the heart rate has returned to the original resting heart rate.
4. Without immersing face in water, the subject is to hold their breath for 30 seconds. Record your 15-30 s heart rate and calculate the percentage change in heart rate.
5. Rest for several minutes and check the heart rate again. Proceed only when the heart rate has returned to the original resting heart rate.
6. Using the snorkel or bendable straw as a breathing apparatus, the subject is to immerse their face for 30 seconds in the slightly cold water without holding breath. Record the 15-30 s heart rate and calculate the percentage change in heart rate.
7. Rest for several minutes and check the heart rate again. Proceed only when the heart rate has returned to the original resting heart rate.
8. Change the cold water with room temperature water, and immerse face for 30 seconds while holding breath. Record the 15-30 s heart rate and calculate the percentage change in heart rate.

Results and Discussion

A. Group data

Name of subject: _____

	Resting heart rate	**Heart rate during 15s-30s**	**% change in heart rate**

Face immersed in cold water, apnea			
Face not immersed, apnea			
Face immersed in cold water, breathing			
Face immersed in room temp water, apnea			

Class data

	Average resting heart rate	**Average heart rate during 15s-30s**	**Average % change in heart rate**
Face immersed in cold water, apnea			
Face not immersed, apnea			
Face immersed in cold water, breathing			
Face immersed in room temp water, apnea			

Analysis and Discussion

1. Summarize the effect of simulated diving on heart rate. Identify which factor(s) is/are responsible to trigger the result: apnea (breath-holding), immersion of face in water, or cold temperature?

2. Explain why a simulated dive would induce such changes in heart rate. How would those changes benefit us when we are diving?

3. What suggestions would you make to improve the experiment or data analysis method?

Chapter VIII : Analysis of Human Digestive System

Overview

Different enzymes are used to digest different food substances. This session will look on how

digestive enzymes break down a) proteins, and b) polysaccharides

Proteins

The digestion of proteins is catalyzed by several proteolytic enzymes, such as pepsin of gastric origin, trypsin, chymotrypsin, carboxypeptidases, and elastase of pancreatic origin. Chemical digestion of protein begins in the stomach. The lining of the stomach produces gastric juice in response to neural and mechanical stimulation. Gastric juice contains a number of substances such as HCl and pepsin. The low pH (~2) of the gastric juice aids protein digestion in a couple of ways. First, the low pH denatures the tertiary structures of ingested protein, making them easier to digest enzymatically. Secondly, the low pH is required for the activation of pepsin. Pepsin is activated from pepsinogen and breaks peptide bonds between amino acids with hydrophobic side chains in the middle of polypeptides into shorter polypeptides.

Polysaccharides

Saliva is a mixture derived from the secretions of three pairs of glands; the parotid glands scattered diffusely over the mucosa of the mouth. The amount secreted in normal adult human is 1 to 1.5 litres in 24 hours. The composition of saliva is 99.5% water and 0.5% of inorganic solids. The amylase in saliva hydrolyses the α(1□4) linked D-glucose units in polysaccharides. At pH of about 6- 7 and in the presence of chloride ions, α-amylase catalyses the hydrolysis of starch to the disaccharide maltose with the intermediate formations of various dextrins. Starch and higher dextrins give a blue color with iodine, the intermediate dextrins give a reddish-brown color, while the lower dextrins and maltose give no reaction with iodine. Thus, the action of α-amylase can be

followed by observing the time taken to reach the point at which the reaction mixture no longer gives a color with the iodine solution, “the achromic point”. The maltose produced can be detected by Benedict’s test.

Procedure

A. Protein: Chemical Digestion of Protein by Pepsin

1. Do this part of experiment first! (it takes 90 minutes for the results to come out)
2. Set up all of your materials and equipment.
3. Set of these following tubes:

Tube contents	T1	T2	T3	T4	T5
Type 3 water	10 drops	-	-	5 mL	-
Pepsin solution	5 mL	5 mL	5 mL	-	5 mL
1 M HCl	-	10 drops	10 drops	10 drops	-
1 M NaOH	-	-	-	-	10 drops

4. Add a small sliver of hard-boiled egg white to each of the five tubes
5. Place the tubes T1, T2, T4, T5 in 37˚C water bath for equilibration for 90 minutes. Place T3 on ice bath.
6. Examine the egg white following incubation. Note any digestion that occurs to egg white and change of color of the solution.

B. Carbohydrate: Hydrolysis of Starch by Salivary Amylase

1. Prepare all of the materials and equipment.
2. Collect a sample (2-3 mL) of your own saliva using a plastic pipette into a small beaker. Salivation can be enhanced by taking deep sniffs of a lemon slice (or amyl alcohol, or peppermint aroma)
3. Take an aliquot (0.5mL) into a test-tube and serially dilute it 1:20 and 1:40 with 0.1 M sodium phosphate buffer pH 6.7. Place the saliva samples on the ice.
4. Set up the following tubes

Tube contents	C1	C2	T1	T2
0.5% starch solution	5 mL	-	5 mL	5 mL
0.1 M sodium phosphate pH 6.7	5 mL	5 mL	-	-
1% NaCl	1 mL	1 mL	1 mL	1 mL

5. Place the tubes in 37˚C water bath for equilibration for 5 minutes
6. Add 1 mL of the 0.1 M sodium phosphate buffer to each of the two control tubes (C1 and C2). Draw one drop of the mixture from both tubes and mix (using a glass rod) with a drop of the iodine solution on the white tile. Use the colors developed as visual indications of a positive (C1) and negative (C2) starch reaction.
7. At zero time, add 1 mL of the 1/20 diluted saliva to T1 and mix the tube contents thoroughly. At intervals of 1 min, draw one drop of the reaction mixture from the tube and mix with a drop of iodine on the tile. Run your stopwatch to measure time taken to reach the point at which the reaction mixture no longer gives a color (achromic point).
8. Repeat step 7 by adding 1mL of the 1/40 diluted saliva to T2

9) The time taken to when the tube content gives a negative starch reaction with the iodine. solution is the achromic point. A convenient value for the achromic point is 5-10 min. Adjust the dilutions of your saliva and repeat the experiment if necessary.

10) Add 0.5mL of your undiluted saliva to C1 and C2, incubate at 37 ˚C for 10 min. Then add 0.5 mL of the final reaction mixture to 5mL of Benedict's reagent. Mix and place the tubes in a boiling water bath for 3 minutes. Cool and note the results.

Results and Analysis

A. Protein: Chemical Digestion of Protein by Pepsin

1. Describe the changes in the egg white and the color changes in the solution.

2. Explain the action of pepsin.

3. Describe and explain the effects of temperature on the reaction.

4. What is the other function of pepsin? (hint: micronutrient absorption) and what is the effect of pepsin deficiency?

B. Carbohydrate: Hydrolysis of Starch by Salivary Amylase

1. How long does it take for T1 and T2 to reach a chromic point?

-

2. Describe and explain the result of mixing the final reaction mixture with Benedict's reagent.

3. Explain the role of NaCl solution and sodium phosphate in the reaction.

4. Apart from the α-amylase present in the human alimentary canal, there is β-amylase. What is the natural occurrence of β-amylase and how does this enzyme digest starch?

Chapter IX: Analysis of Human Urinary System

Overview

The urinary system functions to regulate water and electrolyte balance in the body. Urine is formed in

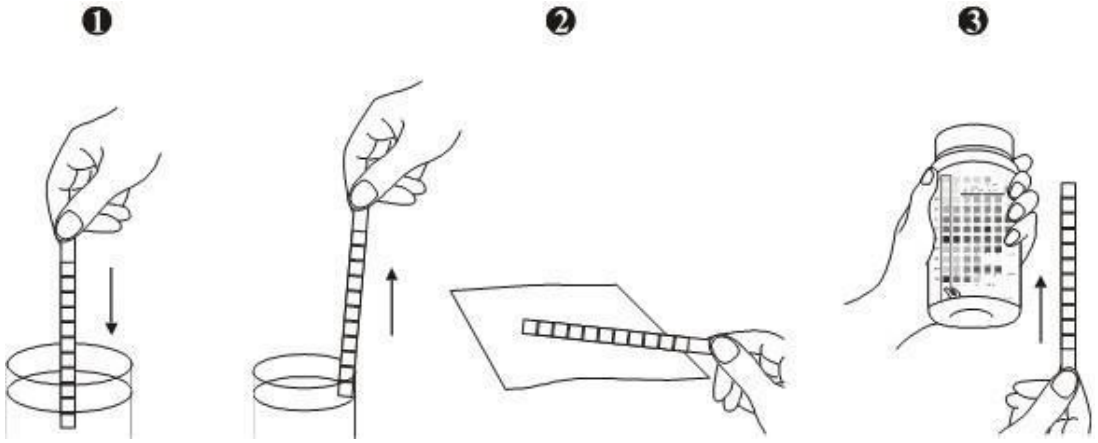
the glomeruli of the kidneys where water and solutes are filtered into the renal tubule of the Bowman's capsule, reabsorption of water, glucose amino acid and ions are reabsorbed, and hydrogen ions, potassium ions, creatinine and drugs are removed. The process is regulated by the Renin-Angiotensin- Aldosterone system (RAAS), which responds to changes in perfusion pressure due to blood volume changes, and changes to blood sodium content. With the RAAS activated, changes in urine volume and composition will be affected to maintain water and electrolyte homeostasis.

In this experiment we will look at the effects of water loading, salt loading, and consumption of caffeine on urine output.

Important Notes:

1. The following students will be exempted from this experiment:
   1. Females having their menstrual period
   2. Students with hypertension, diabetes, heart disease, kidney disease, or previous kidney injury
2. For one hour before the start of the session, do not eat anything. You may drink plain water but not excessively.
3. Only handle your own urine, minimize spillage and avoid contact of urine with open wound. Wash your hands with soap after handling urine.

Procedure



1. Empty your bladder at the start of the session. Collect the urine and perform the urine strip test:

Figure 5. Urine strip test

- Briefly dip the test strip into the urine sample, ensuring that all the pads are immersed.
- Gently run the back of the strip against the collection cup to drain off excess fluid.
- Place the strip in the plastic bag provided and wait 30 seconds, while keeping the strip horizontal to prevent mixing of adjacent pads.
- After 30 seconds, take picture of the urine strip result.
- Compare the result with the reference chart at on the test packaging.

2. You will be randomly assigned to treatments 1-5. Consume the following as fast as possible (within 5 minutes):

Treatment	**Consume**
1	500 ml of drinking water
2	1 liter of drinking water
3	500 mg of salt + non-excessive water
4	500-750 ml of coffee/tea
5	Normal water intake (drink non-excessively **when thirsty**)

3. Collect your urine every 30 minutes using the collection cup. Measure the volume at every collection, and additionally perform the urine strip test at 1-hour and 2-hour time points.
4. Urine volume measurement:
   a. Measure the volume using a measuring cup.
   b. Record the urine volume collected every 30 minutes in the Result section and compile the data for the whole class.
   c. Record your body weight and calculate the urine flow rate using the following formula: volume of urine collected / (body weight x time period in hour).
   Note that the normal urine flow rate is approximately 1 ml/kg/hr.
5. At 1-hr and 2-hr time points, perform the urine strip test as described in part (a). Compare the urine strip test results across the time points.

Results and Observation

A. Urine Strip Test

	Strip test result (insert picture)	**Trends observed**

Treatment	**0 min**	**60 min**	**120 min**	[identify which parameters have changed and describe the change (increase/decrease)]
1				
2				
3				
4				
5				

B. Urine volume

Treatment	**Average volume of urine collected at time points** (ml)					**Average total volume of urine** (ml)	**Average urine flow** (ml/kg/hr)
	30 min	**60 min**	**90 min**	**120 min**	**150 min**		
1							
2							
3							
4							
5							

Based on class data above, construct a multiple line chart illustrating the cumulative volume of urine collected over the five time points.

--- insert chart here ---

Analysis and Discussion

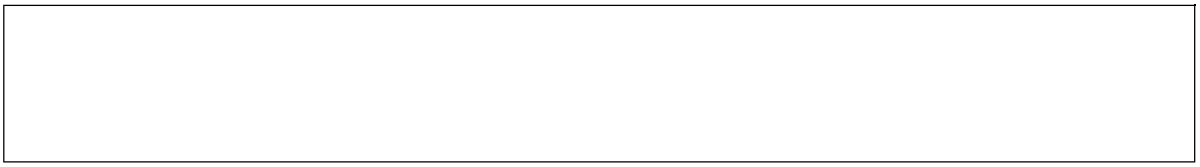
1. Summarize the findings above.

2. Explain why 500 ml and 1 liter water loading result in the findings above. Describe the homeostatic mechanism employed in maintaining water balance in the body.

3. Explain why salt loading (Treatment 3) results in the findings above. Describe the homeostatic mechanism employed in maintaining salt balance in the body.

4. Explain why coffee/tea consumption in Treatment 4 results in the findings above.

5. Explain the trends observed in the urine strip test across three time points. Which urine parameters were changed and by which treatments?



Chapter X : Analysis of Human Reproductive System

Overview

The reproductive system of a female shows regular cyclic changes that prepare the body for pregnancy
and fertilization. The average menstrual cycle lasts 28 days, starting from the first day of period and ending on the day before the next period starts.

This case study introduces the human menstrual cycle and its associated misconceptions, covering the phases of the menstrual cycle, the hormones involved, the negative feedback, and the prediction of ovulation.

Read the case study below and answer the accompanying question

What Happened to 28 Days? – A case study about the human menstrual cycle

*Adapted with modifications from case study by Tamar L. Goulet, University of Mississippi, 2011.

Ann is a newlywed college student. Ann worries that she is pregnant and confides in her friend, Karen, a biomedicine major.

"Karen, I'm already two days late on my period, do you think I could be pregnant? I'm really not ready to be a mom yet" lamented Ann.

"So, when was the 1st day of your last period?"
asked Karen. "February 2nd," replied Ann.

"How long is your menstrual cycle?" asked Karen, her pen
poised in the air. "28 days," Ann replied quickly.

"Is it always 28 days?" asked Karen.

"Isn't every woman's menstrual cycle 28 days?" asked Ann.

Q1: What is the length of a human menstrual cycle?

a. **20 days**
b. **24 days**
c. **28 days**
d. **32 days**
e. **Any of the above**

"Ok, let's step back a bit. Which organs are directly involved in the menstrual cycle?" asked Karen.

Q2: List the organs that are directly involved in the menstrual cycle.

"Are these organs physically connected?" asked Karen.

"Well, no" answered Ann incredulously. "After all, my hypothalamus is up here," said Ann, pointing to her head, "and my ovaries are down there," she continued as she pointed to her jeans, and then looked around self-consciously to see if she had been overheard.

"Good, good," nodded Karen. "So, how does the hypothalamus communicate with the ovaries?"

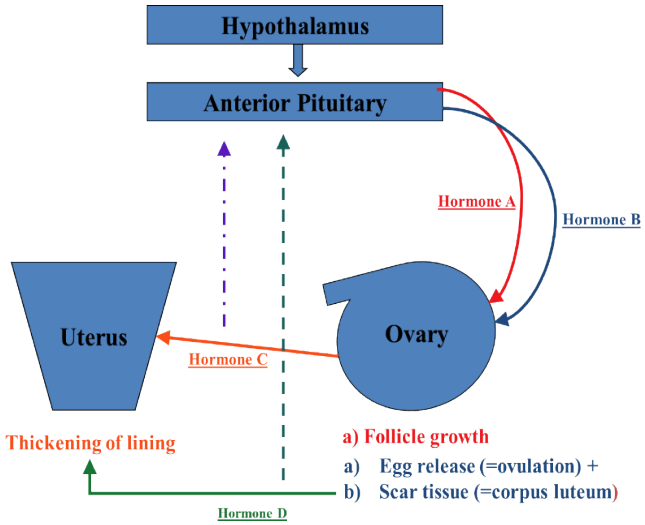
Q3: List the hormones that are involved in the menstrual cycle and specify where they are produced.

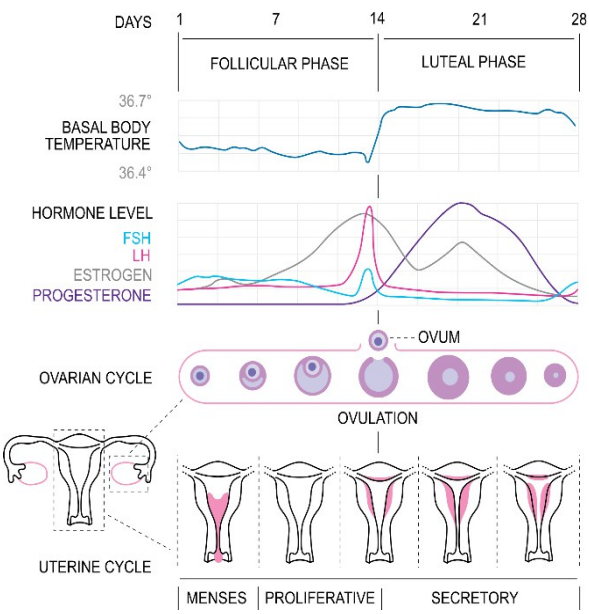
Q4: How do hormones get from the anterior pituitary to the ovaries?

a. **Gravity**
b. **Osmosis**
c. **In the bloodstream**
d. **In the interstitial fluid**
e. **Through the nerve impulses**

"Ann," Karen asked gingerly. "Do you know what happens during a menstrual cycle?" "Of course!" replied Ann adamantly.

Q5: Use the following diagram to briefly explain what happens during a menstrual cycle.


Hypothalamus
Anterior Pituitary
Hormone A
Hormone B
Uterus
Ovary
Hormone C
Thickening of lining
a) Follicle growth
a) Egg release (=ovulation) +
b) Scar tissue (=corpus luteum)
Hormone D



Q6: Unless a pregnancy occurs, the corpus luteum will degenerate after _days.

a. **6**

b. **10**

c. **12**

d. **14**

e. **18**

Q7: Without the corpus luteum and progesterone, the uterine lining will:

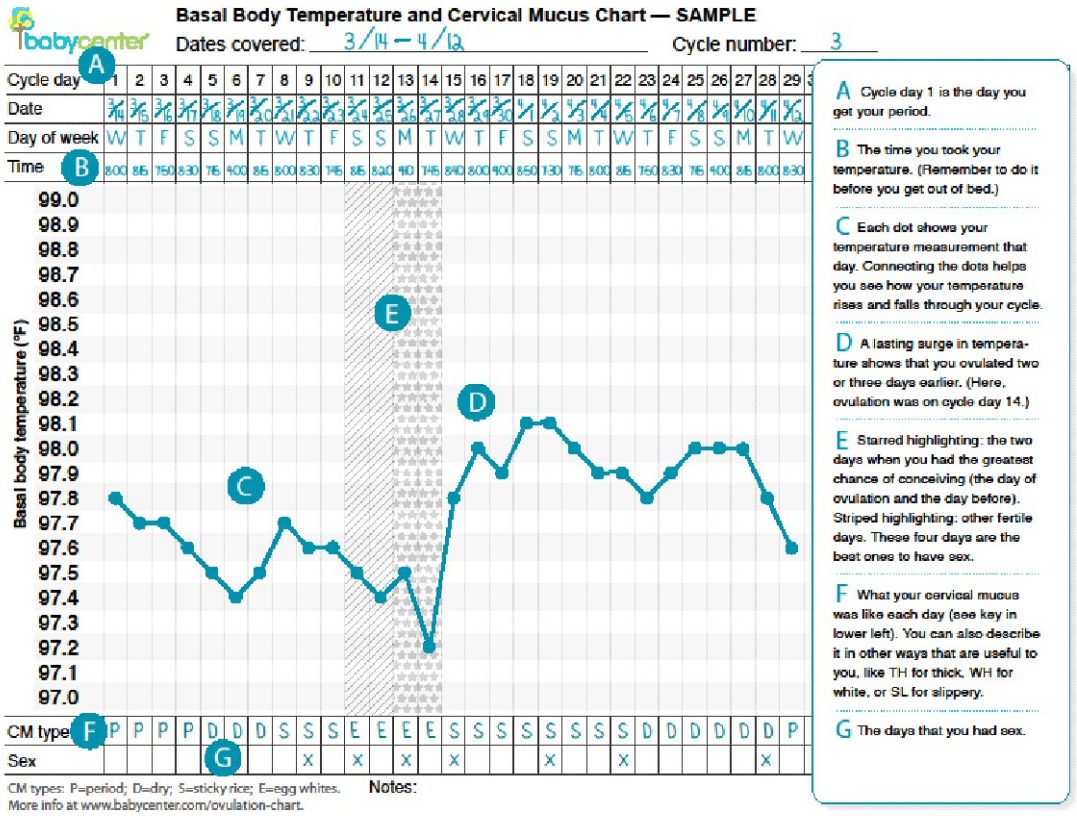
a. **Remain the same thickness**

b. **Increase in thickness**

c. **Decrease slightly in thickness**

d. **Shed (i.e. menstrual flow)**

"Ok, what was the first day of your last menstrual cycle?"
asked Karen. "February 2nd," repeated Ann.
"And what was the first day of the cycle before that?"
asked Karen. "Umm, January 1st," said Ann.
"So, how long is your personal menstrual cycle?" prodded Karen.
Ann paused to think. What was it…? "So your cycle is 32 days long,"
stated Karen. "Can you figure out when you ovulate?" she asked.
"I don't feel when I ovulate," said Ann.
"Most women do not," stated Karen. "But, there are signs."


babycenter
Basal Body Temperature and Cervical Mucus Chart — SAMPLE
Dates covered: 3/14 – 4/12
Cycle number: 3
Cycle day
Date
Day of week
Time
Basal body temperature (°F)
99.0
98.9
98.8
98.7
98.6
98.5
98.4
98.3
98.2
98.1
98.0
97.9
97.8
97.7
97.6
97.5
97.4
97.3
97.2
97.1
97.0
CM type
Sex
CM types: P=period; D=dry; S=sticky rice; E=egg whites.
More info at www.babycenter.com/ovulation-chart.
Notes:
A Cycle day 1 is the day you get your period.
B The time you took your temperature. (Remember to do it before you get out of bed.)
C Each dot shows your temperature measurement that day. Connecting the dots helps you see how your temperature rises and falls through your cycle.
D A lasting surge in temperature shows that you ovulated two or three days earlier. (Here, ovulation was on cycle day 14.)
E Starred highlighting: the two days when you had the greatest chance of conceiving (the day of ovulation and the day before). Striped highlighting: other fertile days. These four days are the best ones to have sex.
F What your cervical mucus was like each day (see key in lower left). You can also describe it in other ways that are useful to you, like TH for thick, WH for white, or SL for slippery.
G The days that you had sex.

Q8: After ovulation, for the remainder of the cycle, a woman's basal body temperature:

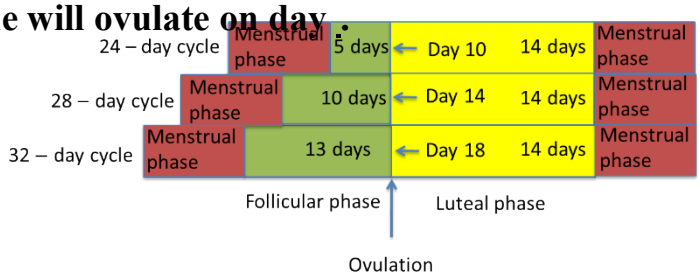
a. **Does not change**
b. **Oscillates, generating hot flashes**
c. **Goes up slightly and stays up**
d. **Goes down slightly and stays down**

"I didn't watch out for those signs!" wailed Ann.

"Even if you did not, we can still calculate when you ovulated," said Karen.

Q9: Ann has a 32-day cycle. She will ovulate on day

a. **6**
b. **10**
c. **12**
d. **14**
e. **18**



"Since we had sex on day 16, I have nothing to worry about," said Ann with a sigh.

"Not necessarily," said Karen. "A male's sperm can stay alive in a female body for up to 5 days."

"You have got to be kidding," whispered Ann in an appalled voice.

"But," Karen pointed out, "you assumed you had a 28 day cycle when in fact yours is 32. So guess what? You're not late," stated Karen.

A week later, Ann & Karen met for coffee again.

"I did not hear from you, so I assumed… Well…?" asked Karen picking

up their conversation from the previous week.

“I got my period,” said Ann. “I never counted how many days were between my periods,” she admitted sheepishly. John & I are checking our options for family planning. I’m not ready yet to be a mom.”

“At least you know your own body better now,” concluded Karen. “What kind of coffee do you want today? My treat.”

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This textbook is an essential resource for students and lecturers, providing a comprehensive yet accessible exploration of the human body's structure and function. It covers key biological systems through a combination of theoretical insights and practical applications. Anatomy and Dissection: Students gain hands-on experience in mammalian dissection, using rodent models to explore anatomical structures that are foundational to human biology. Histology: The textbook details the microscopic study of tissues, guiding students through tissue processing, sectioning, and staining techniques that reveal cellular structures. Integumentary and Skeletal Systems: It examines the skin's role in protection and temperature regulation, alongside the skeletal system's function in support and movement. Nervous System: This section explores the nervous system's response to stimuli, including reaction time and sensory perception exercises, highlighting the system's critical functions. Lymphatic and Immune Systems: The textbook introduces carbon clearance assays to study the immune response, focusing on how the body handles foreign substances. Endocrine System: Students learn about hormonal regulation, particularly glucose homeostasis, through practical exercises like glucose tolerance tests. Cardiorespiratory System: The textbook covers the interplay between the cardiovascular and respiratory systems, emphasizing their roles during physical exercise and stress. Digestive System: It provides insights into the chemical digestion of food, focusing on the enzymatic breakdown of proteins and carbohydrates. Urinary System: The textbook explores the mechanisms of water and electrolyte balance, detailing the kidney's role in urine formation and the effects of different substances on this process. Reproductive System: The final chapter discusses the female reproductive cycle, offering a case study to clarify common misconceptions and explain hormonal regulation. This textbook serves as a comprehensive guide, blending theoretical knowledge with practical skills, making it an invaluable tool for both students and instructors in the study of human biology

ABOUT THE AUTHOR

Dr. Wided Kouidhi was born in Tunisia and holds a Bachelor's degree in Biotechnology and a Master's degree in Human Physiology. She earned her Ph.D. from the University of Burgundy in France, where her research focused on the effects of endocrine disruptors—chemicals that interfere with the endocrine system—specifically on the oral cavity and salivary glands.

Following her Ph.D., Dr. Kouidhi worked as a postdoctoral researcher at both the University of Hong Kong and the University of Malaya in Malaysia. Her research expertise centers on toxicology, with a particular emphasis on the harmful effects of endocrine disruptors such as pesticides, plastic compounds, and phytoestrogens on human health. She has published numerous research articles in these fields.

In 2018, Dr. Kouidhi joined the Indonesia International Institute for Life Sciences (i3L) as a faculty member in Biomedicine. Since joining i3L, she has been actively involved in teaching various courses, including Human Structure and Function Laboratory, Tissue Biology, General Biology, Principles of Neurosciences, Toxicology, and Bioethics. Her current research focuses on the effects of Bisphenol S on neurodevelopment, exploring how this endocrine disruptor may impact brain development.In 2021, she furthered her expertise by obtaining a Certificate of Achievement in Pharmacology and Toxicology from Harvard Medical School.

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