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ABSTRACT

This laboratory manual, containing 44 exercises, is intended to be used as part of an audio-tutorial approach to laboratory work in a life-science course for student nurses. Exercises include basic techniques of miscroscopy, microbiology, electrophysiology, routine biochemical analyses of blood and urine, and microscopic examination of prepared histological sections. There is a small number of experiments involving frog embryology, enzymatic digestion and radiation effects, and dissections of the heart, kidney, and digestive and respiratory systems. An appendix lists supplies needed for permanently equipping each audio-tutorial booth, and additional needs for each exercise. A bibliography of readings assigned for each term and a list of reference books for each section of the course are included. (AL)

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Investigations Into LIFE SCIENCE

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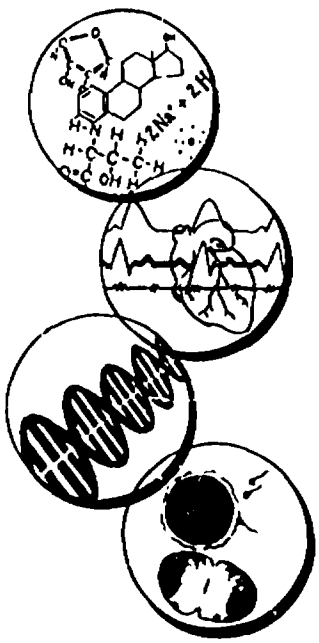

Investigations Into LIFE SCIENCE

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PREFACE

This manual was developed to provide the student nurse at the Washington Hospital School of Nursing with meaningful laboratory experiences. It contains 44 laboratory investigations which provide science background for the student nurse. Each week, the science investigations are correlated with the subject matter in the Medical-Surgical Nursing and Nutrition and Diet Therapy.

Some investigations are original; others have been adapted from various sources. The adapted exercises are common to many laboratory manuals; therefore, no attempt was made to credit any specific source. All of the investigations have been tried, used, and modified by the author in order to provide meaningful science investigations for the student nurse.

This manual is not a substitute for a textbook; it does not follow any one particular textbook. It is to be used in conjunction with various references and textbooks listed in the appendix.

Dean Samuel Mentzer

CONTENTS

INTRODUCTION

Investigations into Life Science is the guide the beginning student nurse will use in his learning of Life Science in the Audiotutorial Laboratory at the Washington Hospital School of Nursing.

The Audiotutorial Laboratory is unique in that most of the equipment to be used is placed in independent booths. Besides routine science laboratory supplies such as a microscope, slides, stains, etc., auditory and visual aids are available. These include a tape recorder, slide viewer, 8-mm. filmloop projector and film screen. All the laboratory work is accomplished by the audiotutorial approach, utilizing the various additional aids available each week of the course.

The Audiotutorial Laboratory is designed and equipped to accomplish the following objectives:

1. To create an environment in which the student is motivated to become involved in the learning process.
2. To place the responsibility for learning upon the individual student.
3. To develop in the student a self-directed approach to learning.
4. To provide the opportunity for the student to progress at his own rate in the learning process.

The Laboratory Investigations are preceeded by a list of specific objectives which the student must be able to do or accomplish at the completion of each exercise. The student's laboratory progress and accomplishment of the specific objectives will be evaluated weekly by one of the science instructors on the following basis:

Attitude	Performance	Results
Motivation	Ability to work independently	Productiveness
Professionalism	Responsibility	Completeness
Interest	Neatness	Accuracy
Acceptance of Criticism	Technique	
	Self-Direction	

At the completion of each exercise, the student will be given a numerical rating of 0-3 for attitude, performance and results.

3 = very satisfactory

2 = satisfactory

1 = poor

0 = unacceptable

LABORATORY INVESTIGATION 1A The Microscope and Cells

Objectives:

1. To identify and name the parts of the compound microscope.
2. To focus on objects under low power (4X), medium power (10X), high power (43X), and oil immersion (100X).
3. To clean the microscope properly and identify when it needs to be cleaned.
4. To prepare a wet mount on a slide.
5. To identify the parts of a cell.
6. To compare and contrast an animal cell and a plant cell.
7. To explain why oil is used with the oil immersion lens.

The Compound Microscope

Introduction: The Compound Microscope is one of the most important tools of the biologist and one you will be using a great deal in this course. Because it is a precision instrument, consisting of many parts all of which must work together perfectly, the microscope must be handled properly and must be taken care of properly.

You may have used microscopes before but it has been our experience that even if you have, the chances are very good that you can learn to use it much more effectively. Though you may have observed things with a microscope before, the likelihood is that much detail which would have been seen, escaped you. You may have learned something about reporting what you have seen but there is probably still more for you to learn about how to do this most effectively. Concentrate today on learning to improve your skills no matter what their starting level may be.

Parts of the Microscope

Procedure: Examine your microscope carefully and identify the parts listed below. Then label these parts on the prepared drawing provided by the instructor. Print these labels neatly.

1. Stage. This is the platform upon which will be placed microscope slides of the material to be examined. In the center of the stage is a hole or opening known as the stage aperature.

2. Mechanical Stage. Located on top of the stage. It secures the slide and is used to move the slide in any direction.

3. Iris Diaphragm. This is located under the stage and is a shutter-like mechanism which makes it possible to control the size of the opening through which light must pass to reach the microscope slide. Locate this and find out how to open and close the diaphragm.

4. Body Tube. This is the barrel-like portion of the microscope which has a lens system at each end.

5. Ocular. This is the eyepiece at the top of the body tube.

6. Revolving Nosepiece. This circular structure is located at the lower end of the body tube and holds the objectives. Grasp the objectives firmly and find out how you can shift their position by a circular movement of the nosepiece.

7. Objectives. These are the lenses attached to the nosepiece. The objective that is being used should be in portion directly below the body tube and above the microscope slide. This microscope contains low power (4X), medium power (10X), high power (43X), and oil immersion (100X) objectives. The number of the objectives is to indicate the magnification power.

8. Coarse Adjustment. This is the larger wheel closest to the body of the microscope. It is used to raise or lower the objectives in order to sharpen the focus. This knob should be used only when the low power objective is in the operating position.

9. Fine Adjustment. The wheel next to the coarse adjustment. This knob should be used to bring the subject under study into the sharpest possible focus.

Cleaning the Microscope

A microscope is only as good as the quality and condition of the lenses. Cleaning the quality lenses with a cloth or paper towel tends to scratch them. The only acceptable way to clean a lens is with lens paper, which is both lint-free and smooth enough to avoid scratching. The ocular and objective lenses should be cleaned before and after each laboratory exercise with lens paper only.

Learning to Use the Microscope

Procedure: Using a strand of hair as the material for observation, prepare a slide and practice getting it into focus according to the following instructions. See Fig. 1, page 7.

1. Make sure that the LOW POWER OBJECTIVE (4X) is in the operating position.

2. Place the slide you have just prepared on the stage of the microscope, with the object which you want to examine lying directly above the opening in the stage.

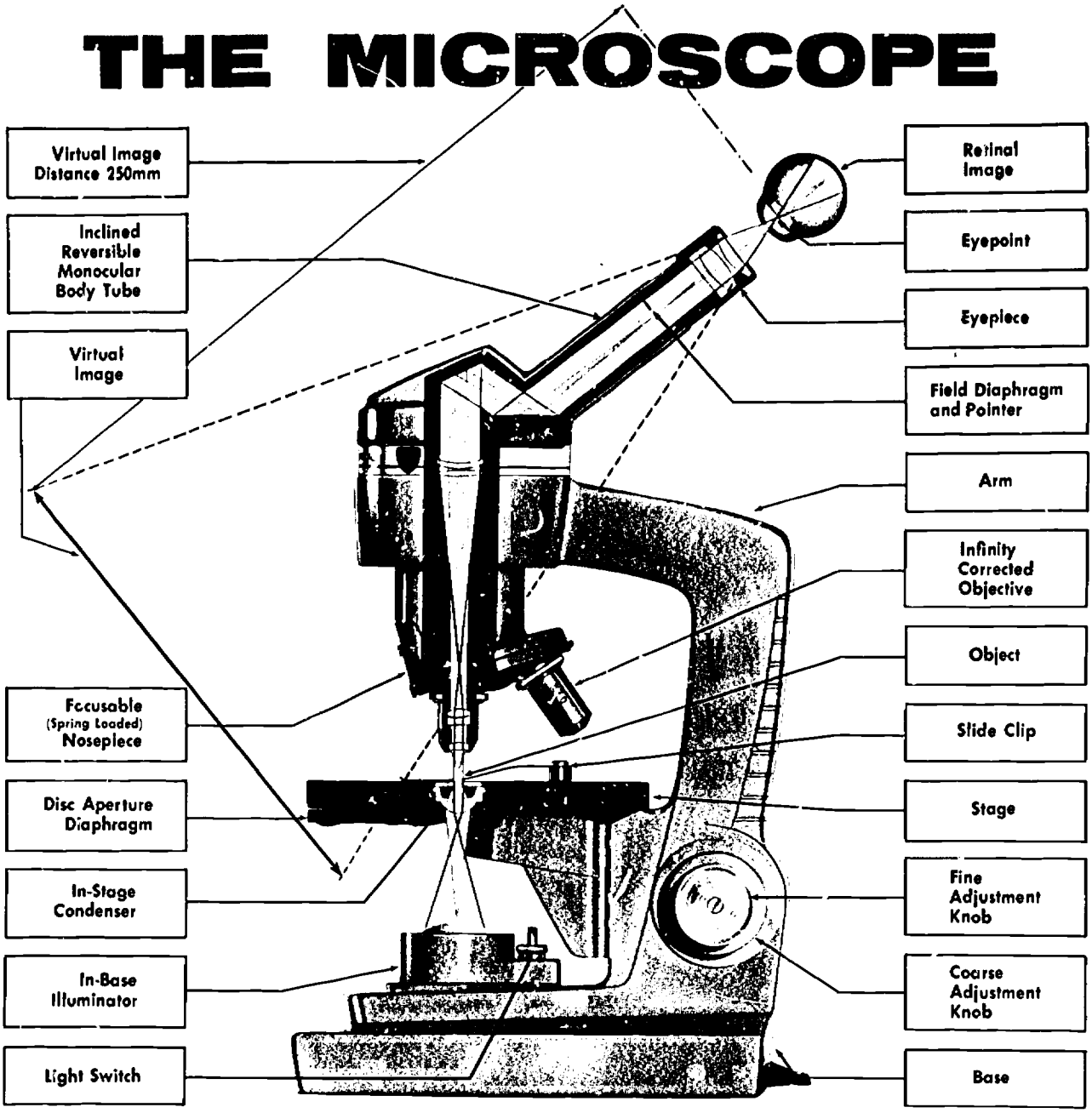
3. While watching from the slide, lower the body tube by turning the coarse adjustment knob until the tip of the low power objective is about ¼ inch above the slide, or until it is automatically stopped and cannot be lowered further.

4. Now place your eye over the ocular and slowly raise the body tube and the low power objective by turning the coarse adjustment knob. If this is done slowly and carefully the object will then come into focus. A few turns of the fine adjustment knob forward or backward will help you to find the sharpest and clearest focus possible for your eye.

5. To get the object in focus under the high power objective after they are in focus under low power, carefully turn the revolving nosepiece until the high power lens comes into position over the slide, watching carefully from the side. Then use only the fine adjustment knob to bring the object into the sharpest possible focus under high power. Also, adjust the light by means of the iris diaphragm each time that you change to a different magnification.

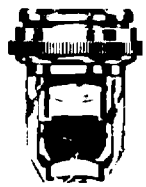
6. Move the slide from left to right and forward and backward to see all parts. Make sure that the slide is held securely by the mechanical stage. Turn the knobs on the mechanical stage to observe various areas of the slide.

THE MICROSCOPE

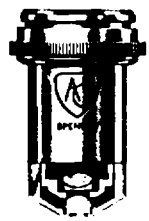


Optical and Mechanical Features of Series SIXTY Microscope

Cross section of scanning objective, 4X.



Cross section of low power objective, 10X.



Cross section of "high dry" objective, 43X.

Cross section of oil immersion objective, 97X.

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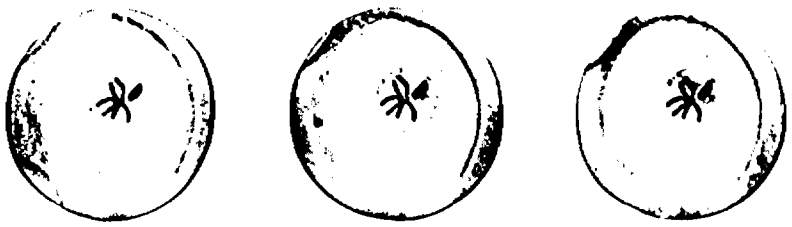


The three photomicrographs show the relationship of numerical aperture to resolving power, and the failure of magnification to provide increased detail. All three specimens are magnified 650X. The one at the left was taken with a 10X, N.A. 0.25 objective and enlarged photographically. The center picture was taken with a 43X, N.A. 0.66 objective and also enlarged photographically. The one at the right was taken with a 90X, N.A. 1.30 objective. Note the superiority of contrast and sharpness of image in the right hand picture.

Illumination: The full capabilities of a microscope cannot be realized unless the illuminator is efficient. Microscopes may be illuminated in several ways. Daylight, but not direct sunlight, can be used with a mirror tilted and adjusted to reflect the light uniformly into the condenser and through the specimen. Daylight is of variable quality and not always available. Consequently, artificial light is more reliable than daylight.

The In-base illuminator is ideally suited for good results because it is an integral part of the instrument . . . assures correct alignment and fully illuminates the field of view . . . also satisfies the numerical aperture requirements of all available objectives 4X, 10X, 43X and 97X. If multiple electrical outlets are not conveniently available, a table globe type microscope illuminator is also suitable and, if centrally located, can be used for four microscopes simultaneously.

Virtual Image: The apparent size and position of the object specimen. See chart on opposite page. This image (not a real or retinal image) seen through the microscope appears to be about 10" away from the eye . . . approximately the same distance at which average print is read.

Magnification: The ratio of the apparent size of an object as seen through the microscope (virtual image) to the size of the same object as it appears to the unaided eye at a distance of 10". The ratio is linearly expressed in terms of "diameters," "power," "X," or "times."

The total resultant magnification of an eyepiece-objective combination equals the product of the initial magnifications of the two. For example—the 10X eyepiece used in combination with a 10X objective produces a linear resultant magnification of 100X.

Magnification alone is not the aim of the finest microscope. The amplified or enlarged image isn't helpful unless more detail . . . resolution . . . becomes apparent. Therefore, always use the lowest practical power objective which effectively reveals the detail in which you are interested.

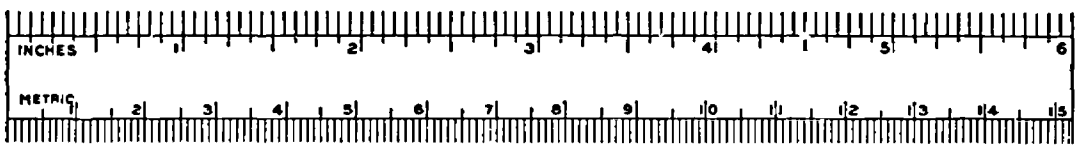
Numerical Aperture (N. A.): A designation, usually engraved on objectives and condensers, expressing mathematically the solid cone of light delivered to the specimen by the condenser and gathered by the objective. It is a criterion of resolving power. The higher the numerical aperture of an objective, the greater its resolving power, provided the N.A. of the condenser is equal to or greater than the N.A. of the objective. For example, a stained preparation of bacteria can be most effectively resolved if viewed with a 97X N.A. 1.25 oil immersion objective used in combination with an Abbe condenser having a corresponding N.A. of 1.25.

Resolving Power (Resolution): The ability of a microscope to reveal fine detail. It is stated as the least distance between two points or lines at which they are seen as two, rather than as a single blurred object. Resolving power is a function of numerical aperture and serves as an indication of which objective should be used to depict any degree of detail.

Definition: The faithfulness with which the instrument magnifies and reproduces specimen detail. The brilliance, clarity, distinctness and sharpness of the microscope image.

Working Distance: The distance between the front mount of the objective, when the microscope is focused on a thin specimen preparation, and the top of the cover glass. The greater the initial magnification of the objective . . . the shorter the working distance. Most objectives are corrected for use with a cover glass thickness of 0.18mm. For this reason, as well as to prevent specimen liquids from touching the objective, such cover glasses should always be applied to the specimen preparation.

Depth of Focus: The thickness of the specimen which may be seen in focus at one time. The lower powered objectives, because of their longer focal lengths and greater depth of focus, are usually more suitable for the study of the general arrangement of the specimen . . . also, the field of view is larger and the image brighter.



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Preparing Wet Mounts

Procedure: Prepare a wet mount of each item listed. Remember always to start with low power then change to a higher power - adjusting only the light and fine adjustment. Make a large drawing of each object observed.

1. Hair
2. Cotton Strands
3. Shreds of lens paper
4. A letter of a word from typed newsprint (prepared slide)

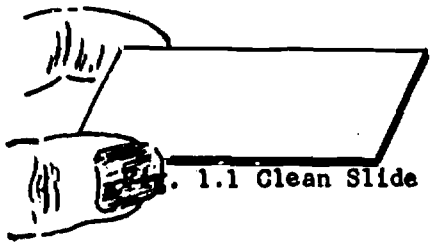


Fig. 1.1 Clean Slide

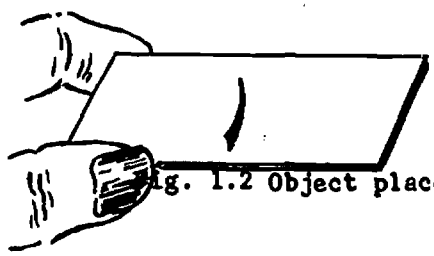


Fig. 1.2 Object placed on slide

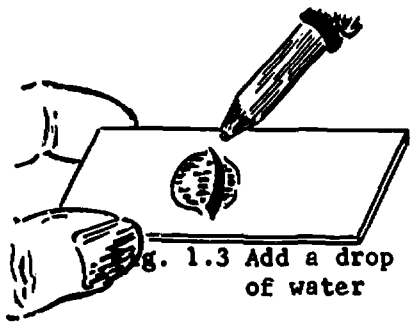


Fig. 1.3 Add a drop of water

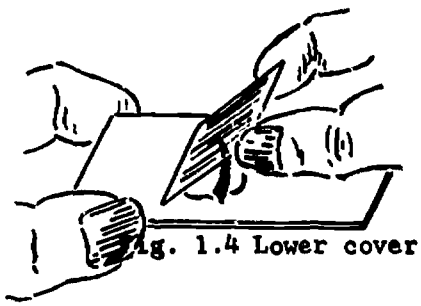


Fig. 1.4 Lower cover Slip

Fig. 1.5 Completed Wet Mount

Use of the Oil Immersion Lens (100X)

Procedure:

1. Use only prepared slides under the oil immersion lens. Never put a wet mount under oil immersion.

2. For this exercise start with a prepared slide of frog's blood.

3. Start with low power and get a sharp focus. Then switch to the next higher power - adjust only the light and fine adjustment. The coarse adjustment does not need to be adjusted.

4. Get a sharp focus under the 43X objective. There should not be any oil on the slide at this time.

5. Revolve the nosepiece only part-way to allow enough space to place one drop of oil on the slide. Now turn the oil immersion lens (red rings) into place. The object should be in focus. You may possibly need more light if it is too dark and then adjust with only the fine adjustment.

6. Check with the instructor to be sure that you are operating the oil immersion lens correctly.

7. Make a large drawing of one red blood cell.

Study of an Animal Cell

Procedure:

1. Place a small drop of water in the center of a clean slide.
2. With the blunt end of a toothpick, lightly scrape the inner surface of your cheek.
3. Place this end of the toothpick into the drop of water on the slide and stir gently.
4. Add a drop of dilute methylene blue and then cover with a cover glass.
5. Observe under the microscope. Make a drawing of one of the cells and label the parts.

Study of a Plant Cell

Procedure:

1. Peel back a leaf or section of the onion bulb.
2. With a pair of forceps, carefully remove a piece of epidermis from the outside shiny surface of a leaf, and prepare a wet mount. The onion skin must be thin enough to see through.
3. Add a drop of dilute iodine solution and cover with a cover slip.
4. Observe under the microscope. Make a drawing of one of the cells and label the parts.

LABORATORY INVESTIGATION 2A Bacteria

Objectives:

1. To locate on a prepared slide and identify the following types of bacteria: coccus, bacillus, spirillum, streptococcus, staphylococcus, diplococcus, and streptobacillus.

2. To prepare a simple stain of bacteria using each of the following stains: crystal violet, methylene blue, carbol fuchsin.

3. To locate and identify the type of bacteria on each of the three (3) simple stains.

4. To prepare a gram stain and to differentiate between gram negative and gram positive organisms.

BACTERIA - Morphology and Staining

Introduction: Some of the major characteristics of bacteria are morphology, size, shape, arrangement, and structure. These characteristics can be determined by examination of prepared specimens. Being that most bacteria are nearly transparent and difficult to see, it is desirable that the cells be stained to make them more readily visible.

Staining of a bacterial film or smear may be performed simply to reveal the shape, size, and arrangement of the cells. The cells are stained by the application of a single staining solution. However, it is possible to acquire additional information about the morphology of bacteria through the use of differential staining techniques. Differential staining procedures usually involve treatment of the smear with a series of reagents. The appearance of the cells following this treatment may permit one to distinguish between two different bacteria on the basis of the color they retain; e.g. one type might appear blue, another red. One may also distinguish between structural entitles within the cell proper or exterior to the cell wall.

Careful examination of appropriately stained bacteria provides invalueable information for the morphological characterization and identification of the specimen.

Procedure:

1. Examine prepared slides of the different types of bacteria.

2. Observe bacteria under the oil immersion lens.

3. Observe the following prepared slides to identify the various kinds of bacteria.

   a. *E. coli*
   b. *Sarcina lutea*
   c. Mixed bacteria
   d. *Bacillus subtilis*

4. Make drawings after identifying the following kinds:

   a. spirillum
   b. streptococcus
   c. staphylococcus
   d. streptobacillus
   e. diplococcus

The Simple Stain

Procedure:

1. Wash and dry several microscope slides. (Use Comet or a strong detergent).

2. Place a small drop of water on the slide by use of a dropper bottle. Remove a very small portion of a bacteria colony with the transfer loop. There should only be a small amount of bacteria on the edge of the loop. If the loop is completely filled with bacteria, you have entirely too much.

3. Emulsify this growth in the drop of water on the slide. Make a smear by spreading out the material over the center of the slide. (See Fig. 2). The smear should be a thin film on the slide.

4. Always flame the needle before and after transferring bacteria.

5. Allow the smear to air-dry then "fix" the smear by passing the slide through the flame two or three times. If the bacteria are heated too much, they will become dehydrated which will distort the shape.

6. Select one of the following stains for each of the different growths of bacteria and cover the smear for the designated times:

gentian violet	30 to 60 seconds
methylene blue	1 to 2 minutes
carbol fuchsin	15 to 30 seconds

7. After the smear has been exposed to the stain for the required time period, remove excess stain by washing with a gentle stream of water, using the plastic washing bottle, then blot dry with a paper towel.

8. Examine the stained preparation under the microscope, using the oil-immersion objective. Do not put a cover slip over the stained bacteria smear.

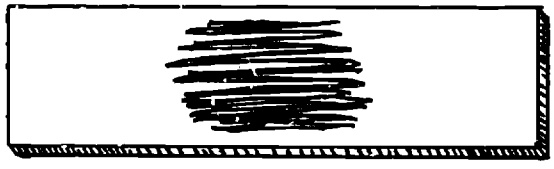


Fig. 2 Bacteria Smear

The Gram Stain

Introduction: One of the most important and widely used procedures for characterizing bacteria is the Gram stain. In general terms, bacteria are divided into two groups, based on whether they retain or lose the primary stain (gentian violet). Those organisms which retain the gentian violet (appear dark blue or violet) are designated gram-positive; those which lose the gentian violet and are susequently stained by the safranin (appear red) are designated gram-negative.

The Gram stain is an important diagnostic test, but in recent years it has been demonstrated that this reaction is quite variable and so its use must be attended with care as the age of the organism tested, the kind of culture medium used, and other environmental factors that might be responsible for variation must be considered.

Procedure:

1. Prepare smears of bacteria and "fix" the same as for a simple stain.

2. Stain the smear with gentian violet solution for one minute; then wash with water for a few seconds. Drain off excess water.

3. Apply Gram's iodine solution for one minute; then wash with water for a few seconds. Drain off excess water.

4. Decolorize with alcohol (95%) until free color has been washed off (approximately 30 seconds). Wash slide with water and drain.

5. Counterstain smear for 30 seconds with safranin; wash, and blot dry.

6. Make microscopic examination under the oil immersion lens for each stained preparation. Make a large diagram and label correctly.

Results: Record Data

Organism	Morphology	Color	Gram Reaction (+) or (-)
E. coli			
B. subtilis			
S. lutea			
R. rubrum			

LABORATORY INVESTIGATION 3A The Digestive System

Objectives:

1. To locate and identify the parts of the gastro-intestinal tract of a living animal (Rat).

2. To locate and identify the diaphragm and to distinguish between the thoracic and abdominal cavities.

3. To differentiate between the types of peritoneum and mesenteries.

4. To locate and identify the pancreas, liver, and spleen.

5. To locate and identify the following layers of the gastro-intestinal tract:
   a. serosa
   b. muscle layer
   c. submucosa
   d. mucosa

6. To compare and contrast the layers of the esophagus, stomach, small intestine, and large intestine.

7. To locate and identify the villi of the small intestine.

Digestive System

Introduction: The digestive tract is a long tube which begins with the mouth and ends with the anus. On its way through the G. I. tract, the food undergoes complex mechanical and chemical changes. It is forwarded through the tube by the contraction of its muscular walls, and is digested by the secretions of the various parts of the alimentary system and its accessory glands. A part of the digested food is absorbed through the walls of the intestine and passes into the circulation, the residue is eliminated as feces.

Opening the Body Cavity

Expose the organs in the thoracic and abdominal cavities by making an incision through the body wall. Cut all the way through the body wall with a pair of scissors, but lift the body wall toward you to prevent cutting any internal organs. Continue the incision from the neck region to the groin.

Make lateral incisions through the body wall just posterior to the attachment of the diaphragm. Also make lateral incisions just anterior to the hind legs, now you can bend back the flaps of the body wall.

Digestive Organs and Accessory Glands and Tissues

1. Liver: The most conspicuous organ in the abdominal cavity is the liver. It fits under the dome of the diaphragm. It is the largest gland in the body.

2. Esophagus: The muscular tube from pharynx to stomach. Notice its close association with the dorsal surface of the trachea. Turn to the abdominal cavity and observe the penetration of the diaphragm by the esophagus before the latter enters the cardiac end of the stomach.

3. Peritoneum: This is the membrane lining the abdominal cavity an enclosing the viscera.

4. Stomach: Identify the fundus, the anterior blind pouch, the greater curvature, the portion of the stomach to which the spleen is attached; lesser curvature; cardiac end, the part of the stomach nearest the heart; pyloric end, the part of the stomach which becomes continuous with the duodenum.

5. Duodenum: The first portion of the small intestine leading away from the pyloric end of the stomach. It is usually identifiable as the part of the small intestine which includes the pancreas between itself and the stomach.

6. Jejunum and Ileum: These two regions of the small intestine comprise the rest of the alimentary tract as far as the large intestine and cannot be differentiated from one another. Between the coils of small intestine is located the mesentery. Take the probe and loosen up some of these coils.

7. Mesenteries: Each mesentery consists of two layers of peritoneum between which are connective tissue and blood vessels and nerves passing through.

8. Colon: This is the first part of the large intestine. The ileum opens into the colon at an oblique angle and in such a manner as to form a blind pouch, the caecum, at the beginning of the colon; it can be seen by pushing the coiled mass to one side.

9. Rectum: The posterior part of the large intestine, continuing from the colon as a dark straight tube, and terminating in the anal opening.

10. Diaphragm: A dome shaped muscle that separates the abdominal and thoracic cavity.

11. Pancreas: An elongated granular mass lying in the angle between the curve of the stomach and the duodenum. Use the probe to expose it and loosen it from the stomach and small intestines.

12. Spleen: Long reddish-brown structure along left side.

Miscroscopic Anatomy of the Digestive Tract

Introduction: The walls of the gastro-intestinal tract consists of four (4) layers. Beginning with the outside and progressing toward the lumen the layers are (1) serosa, (2) muscle layer, (3) submucosa, and (4) mucosa.

Serosa: This is a layer of epithelium (visceral peritoneum) supported by connective tissue and containing a few blood vessels.

Muscle Layer: There are two muscle layers; the longitudinal muscle layer and the circular muscle layer. These two layers are antagonistic to each other, and they are responsible for the peristaltic movements that propel food along the G. I. tract.

Submucosa: This layer consists of loose fibrous connective tissue containing many blood vessels.

Mucosa: This is the inner most layer, adjacent to the lumen, which contains the glands that secrete intestinal enzymes.

Procedure:

1. Examine prepared slides of different regions of the digestive tract: Esophagus, stomach, small intestine, large intestine.

2. Observe and identify the four different layers in cross section. Observe under low and medium power, do not use oil immersion.

3. Make a large diagram of the layers of the (1) esophagus, (2) stomach, (3) small intestine, and (4) large intestine. Label the layers.

LABORATORY INVESTIGATION 4A Enteric Bacteria - Antiseptics

Objectives:

1. To identify which antiseptics are effective against bacteria.
2. To determine and list which antiseptics are best as bacterial growth inhibitors.
3. To determine and list which antiseptics do not inhibit bacterial growth.
4. To determine the Gram stain (reaction) of Proteus vulgaris.
5. To identify and determine the morphology of pathogenic enteric bacteria.
6. To explain the difference between pathogenic and nonpathogenic bacteria and be able to give some examples of each.
7. To differentiate between motile and non-motile bacteria.
8. To locate and identify bacteria flagellum.

Inhibition of Bacterial Growth by Antiseptics

Introduction: This exercise presents a simple method for determining bacterial growth inhibition by household and hospital antiseptics. Some of the antiseptics are good and others are not too effective.

Procedure:

1. Cover the surface of an agar plate with a thin film of a bacteria-broth suspension. Use a disposable pipete to apply a few drops of bacteria culture to the plate.

2. Use the inoculating loop to spread the suspension over the agar surface.

3. Be sure to flame the inoculating loop before and after using it.

4. Using sterile forceps, place the filter paper discs, saturated with an antiseptic, on the agar surface.

5. Invert the agar plate and incubate for 24-96 hours. Be sure to label the petri dish with your name and date.

6. Make a sketch of each zone of inhibition.

Results: Make measurements of the zones of growth inhibition around the filter paper discs. (Measure the radius of the zone in mm.)

ANTISEPTIC	SHAPE OF FILTER DISC	ZONE OF INHIBITION (size in mm.)
Cepacol		
Lavoris		
Tincture of Zephrian		
Cidex		
Phisohex		
2% Lysol		
70% Alcohol		
Zephrian Chloride		

Conclusion: Evaluate on the basis of your results.

BEST Antiseptics	NOT Affective as an Antiseptic
1.	1.
2.	2.
3.	3.

Enteric Bacteria

Introduction: The enteric bacteria refers to the bacteria found in the intestinal tract. These include both the nonpathogenic bacteria that normally inhibit the intestinal tract and the highly pathogenic bacteria that invade and injure it.

The normal inhabitants which are nonpathogenic include species in the genera Escherichia, Aerobacter, and Proteus. In contrast, the pathogenic ones include species of the Salmonella and Shigella. The genus Salmonella comprises the causative organisms of the salmonellosis. It includes some 250 species of motile enteric bacilli which are found almost everywhere.

Dysentery due to certain members of the Shigella genus of bacteria is known as bacillary dysentery. Dysentery due to Shigella dysenteriae is much more severe than is that due to the other organisms because this bacillus produces a powerful toxin.

Procedure:

1. Make a gram stain of E. coli and Proteus vulgaris. Observe under the oil immersion lens and make a detailed drawing of each.

2. Observe and identify under oil immersion the Salmonella group of bacteria. Make drawings of each species available.

3. Observe and identify under oil immersion the Shigella group of bacteria. Make drawings of each species available.

4. Observe a prepared slide of Proteus vulgaris. Locate and identify the flagellae. Make a diagram of one bacterium and label the flagellum.

LABORATORY INVESTIGATION 5A Carbohydrates, Fats, and Proteins

Objectives:

1. To determine what substances contain reducing sugars by employing the Benedict Test.
2. To differentiate between a monosaccharide and disaccharide.
3. To list examples of monosaccharides and disaccharides and to determine the approximate percent of reducing sugar.
4. To identify substances containing starch using the iodine test.
5. To differentiate between a polysaccharide (starch) and a disaccharide (sugar) by writing a chemical formula.
6. To list examples of foods containing starch.
7. To write a chemical reaction that occurs when adding iodine to a unsaturated fat.
8. To write the chemical structure of a saturated fat.
9. To determine the relative amount of unsaturation of a fat by the addition of 2% iodine in CCl_4.
10. To list examples of oils that are saturated, slightly unsaturated, and poly-unsaturated.
11. To identify foods containing protein by the Biuret method.
12. To list examples of foods containing protein.
13. To write the structural formula of two amino acids showing the peptide linkage.

Benedict's Test for Sugar

Introduction: Benedict's test is for a particular group of carbohydrates, called reducing sugars. The reducing ability of a sugar is involved in its ability to reduce Cu^{++} to Cu^{+}, thereby changing the color of the copper solution. This reduction of copper ions is brought about by the aldehyde or ketone group on a long-chain carbon molecule. All aldehydes and ketones contain the necessary aldehyde or ketone group for reaction with Benedict's reagent. Benedict's test also distinguishes between monosaccharides and disaccharides. Although all monosaccharides give a color change with Benedict's reagent, only some disaccharides do.

The Benedict test is used to determine if reducing sugars are present in the solution being tested in clinical medicine. This test is most often done to find out if the patient has sugar (glucose) in the urine and to approximate the quantity of sugar present. The color of the precipitate gives an indication of the percent of reducing sugar.

Color	Percent of Reducing Sugar
blue	0
blue-green	trace
yellow	1%
orange	2%
brick red	over 2%

Procedure:

1. Prepare a water bath (See Page 26).
2. Place about 1/8 teaspoon of each substance to be tested in separate test tubes. It should be enough to fill the rounded ends of the tubes.

3. Label each tube correctly (indicating what it contains).

4. Fill each tube ⅓ full with Benedict's solution (3-5 ml.).

5. Shake each tube to mix the material. (You may shake by holding your finger over the open end or stir with a glass stirring rod.)

6. Place test tube in water bath and boil for five minutes.

7. Remove the tube and let it cool. After cooling, shake to mix.

8. Record and interpret results.

Results: Benedict's Test

Carbohydrate	Color	Remarks (Explain the Color Change
glucose (dextrose)		
fructose		
sucrose		
lactose		
powdered milk		
maltose		
unknown		

Conclusion: Indicate which were good reducing sugars and which ones were not.

1. Write the structural formula for a disaccharide.

2. Write the structural formula for a polysaccharide.

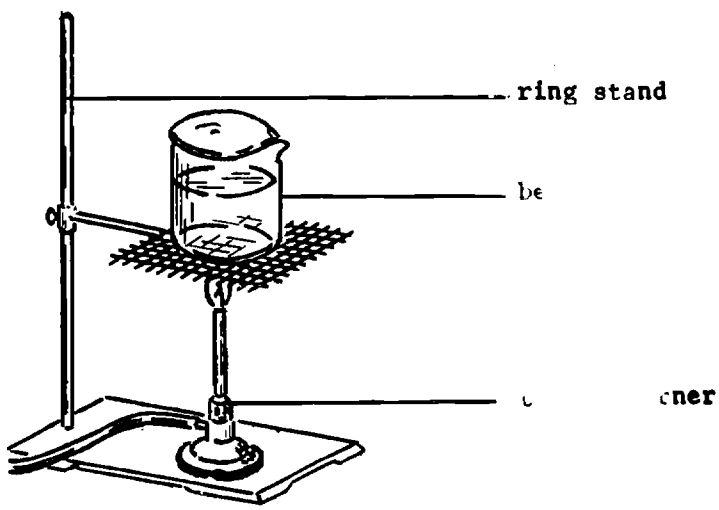
Preparation of Water Bath

Introduction: It is best to heat solutions in a test tube in a water bath. Before you get started with this exercise set up a water bath. (See diagram below)

Procedure:

1. Put wire mesh over ring stand.
2. Fill large beaker (500 ml.) 1/2 - 3/4 full of water and set on wire mesh on the ring stand.
3. Light the Bunsen Burner and boil the water.
4. The water bath will be needed throughout the laboratory period. After the water starts to boil turn the flame down and boil with a low flame.

When heating beakers or flasks, place them on an asbestos screen in order to distribute the heat evenly. Be sure that the iron ring that supports the container is tightly secured to the ring stand.



Iodine Test for Starch

Introduction: The iodine test is for the detection of starch. In the presence of plant starch, the color will change to blue-black.

Procedure:

1. Place a very small amount of material to be tested in a test tube. (Only enough to fill the rounded bottom part of the tube.)
2. Label each tube indicating what it contains.
3. Fill the tube 1/3 with water.
4. Add 1-3 drops of Iodine to each tube. Use the Gram Iodine (Bacteria Stain).
5. Stir with a stirring rod and record the color.
6. If the material to be tested is a solid, it must first be ground up into a pulp with a mortar and pestle.

Results: Iodine Test

Substance	Color	Remarks (Explain the Color Change
glucose (dextrose)		
starch		
powdered milk		
cracker		
potato		
onion		
Unknown		

Conclusion: List foods containing starch.

1.
2.
3.
4.
5.
6.

FATS - Amount of Unsaturation

Introduction: Fatty acids may be subdivided into two principal groups. These are called the saturated fatty acids and the unsaturated fatty acids. The saturated fatty acids predominate in solid fats whereas unsaturated fatty acids are found in large quantities in oils.

Unsaturated fats are characterized by the presence of one or more double bonds in the molecule. These unsaturated fats are able to add hydrogen to produce saturated compounds. The process of adding hydrogen to unsaturated linkages is called hydrogenation. Hydrogenation is of particular value in the preparation of shortenings, for a vegetable oil (liquid due to its unsaturated fatty acid content) is hydrogenated until the proper consistency and melting point is obtained.

The unsaturated fatty acid portion of the molecule will add halogen atoms (iodine, bromine, or flourine) in a similar manner to the addition of hydrogen. Practical use is made of the addition of halogens in determining the amount of unsaturation in any fat or oil. In this exercise you will add iodine to the unsaturated fats. In this way, you can determine the amount of unsaturation by the amount of iodine added to the double bonds.

Procedure:

1. Fill a separate test tube 1/3 full with each oil to be tested.
2. Label each tube indicating what it contains.
3. Add 3-5 drops of 2% iodine dissolved in carbon tetrachloride. Mix by shaking and allow to stand for 15 minutes. This is not the same as the Iodine used to test for starch.
4. Watch each carefully for color changes. The iodine combines with unsaturated fatty acid radicals within the fats to make saturated iodized compounds. Any reaction, therefore, is indicated by a decrease of the iodine color in the solution, and the comparative extent of the reactions in these instances is suggestive of the degree of unsaturation.

Results:

Fat or Oil	Original Color	Degree of Saturation
coconut oil		
olive oil		
corn oil		
poly-unsaturated		
unknown		

Conclusion:

Saturated	Slightly Unsaturated	Poly-unsaturated

Write the structural formula for s saturated fat.

Write the structural formula for an unsaturated fat.

Color Test for Proteins

Introduction: The color tests for proteins depends upon certain groupings present in the amino acids of the proteins. The peptide linkage involved in joining two aminio acids together is responsible for the Biuret Test. Protein gives a purple color when treated with the Biuret Reagent.

Procedure: Biuret Test

1. Place a small portion of the substance to be tested in a test tube. If the substance is a solid, make it into a solution and fill each tube 1/3 full.
2. Label each test tube indicating what it contains.
3. Add 3-5 drops of Biuret Reagent, stir with a glass stirring rod.
4. Record the color change. Record the original color of the Biuret Reagent.
5. Write the Structural formula for a peptide bone.

Results: Biuret Test for Protein

Substance	Original Color	Remarks (Color Change)
egg albumin		
gelatin		
casein		
starch		
powdered milk		
onion		
potato		
unknown		

LABORATORY INVESTIGATION 6A Osmosis and Diffusion

Objectives:

1. To compare the rate that molecules of different sizes pass through a selectively permeable membrane.

2. To determine if starch molecules pass through a membrane by testing with iodine.

3. To determine if sodium chloride can pass through a membrane by testing with silver nitrate.

4. To determine if glucose gets through a membrane by testing with Benedict's solution.

5. To write a chemical equation to demonstrate the result of combining sodium chloride with silver nitrate.

6. To observe intracellular movement of protoplasm.

7. To determine the direction of osmosis and the effects upon living cells that are placed in the following environments:
   a. isotonic solution
   b. hypertonic solution
   c. hypotonic solution

8. To compare and contrast the composition of the following solutions:
   a. isotonic
   b. hypertonic
   c. hypotonic

Molecular Diffusion

Introduction: Biological membranes are differentially permeable. They allow certain substances to pass through the membrane while blocking the passage of other materials. It is generally true, for water-soluble materials, that this choice depends mainly on molecular size. The membrane acts as though it possesses pores of certain size, which permit small molecules to pass while blocking the passage of larger molecules. Cell membranes tend to permit the passage of fat-soluble molecules, almost regardless of size. Many cell membranes tend to pass uncharged molecules much more readily than charged molecules and many pass negative ions more readily than positive ions. In any event, the passage of materials from one side of a membrane to the other occurs by diffusion and will take place when there is a difference in the concentration of the solute particle on both sides of the membrane. The net diffusion is always from the more concentrated side to the side of lower concentration, and the rate of diffusion is directly proportional to the difference in concentration on the two sides of the membranes.

The procedure of separating colloidal particles from particles of molecular dimension is known as dialysis. Dialysis can occur through a membrane such as a cellophane bag containing both dipersed colloidal particles and the solute dissolved in water. The bag is suspended in a beaker filled with water. Depending upon the concentration and molecular size, certain particles will diffuse through into the container of water.

Procedure:

1. Prepare a starch solution by mixing approximately one teaspoon of cornstarch in a little cold water and add this to 100 ml. of boiling water, stirring as you add the water.

2. Boil for 2 minutes, cool. This enables the starch to become dissolved and go into solution.

3. Add approximately one tablespoon of sodium chloride to the starch solution and stir.

4. Add approximately one tablespoon of glucose to the starch and salt solution. Now you have a solution containing starch, salt, and glucose.

5. Pour the starch-salt-glucose solution into a dialyzing bag and tie both ends securely. Wash the outside of the bag with water to remove any solution that might have spilled over the outside.

6. Suspend in a beaker of water as shown below. Let stand for at least one hour.

7. After one hour, fill three test tubes 1/3 - 1/2 full of the water from the beaker.

8. Test one tube for starch - 1-2 drops of iodine.

9. Test the second tube for glucose: add 2-5 ml. of Benedict's solution. Heat in a water bath.

10. Test the third tube for sodium chloride. Add 2-5 drops of silver nitrate. Formation of a precipitate indicates the presence of sodium chloride. If the solution becomes cloudy, this indicates a slight precipitation.

11. Write a chemical equation for the reaction of sodium chloride plus silver nitrate.

Caution: $AgNO_3$ stains

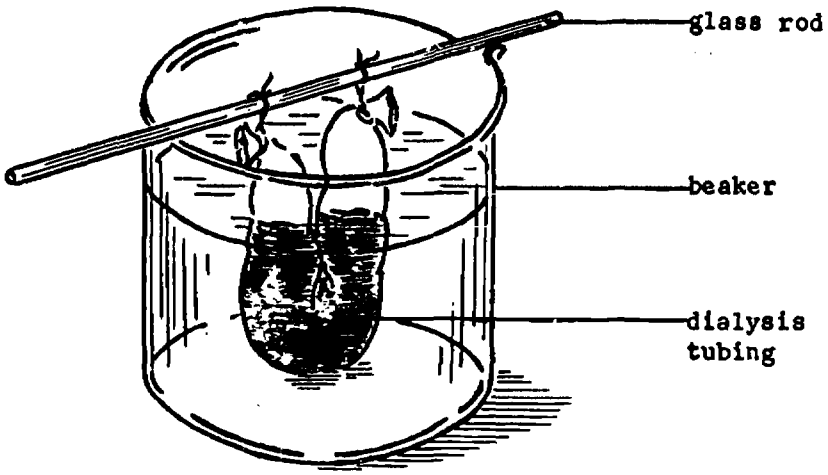


Fig. 6 Diffusion Set-Up

Results:

Tube Number	Test	Color	Remarks
1 add Iodine	Starch		
2 add Benedict's	Sugar		
3 add $AgNO_3$	Salt		

Conclusion: What molecules diffused through and why? Which did not and why?

Protoplasmic Streaming

Introduction: Water and dissolved materials are constantly moving into and out of a cell. Within the cell there is also a constant movement. This movement within a cell is called streaming or rotation in the cytoplasm.

One of the best kinds of cells to show protoplasmic streaming are cells in the leaf of Elodea, also called Anacharis. This plant may be found almost anywhere in the U.S. in streams, ponds, and lakes. The leaf is small, narrow, and pointed. Choose one leaf from near the apex of a stem. It is only two cells in thickness for the most part, one cell thick at the margin. In the cytoplasm are numerous bright green oval bodies, the chloroplasts.

After the leaf recovers from the shock of being removed and mounted, a regular streaming or rotation will be observed in the cytoplasm carrying the chloroplasts along. If no movement is seen, warm the slide a trifle, or mount the leaf in a drop of water in which several pennies have been kept for a few days.

Procedure:

1. Make a wet mount of one leaf. Observe the cells at the margin of the leaf.
2. Determine the direction and speed of the streaming.
3. Make a large drawing of one cell. Label the parts.

Osmosis

Introduction: Osmosis is the diffusion of water through a membrane, not just any membrane but rather a membrane which will differentiate between materials. This type of membrane is said to be semi-permeable or differentially permeable. Some molecules pass through with no restriction, some with moderate restriction and for some there is absolute restriction. The plasma membrane of living cells works in this manner.

If a plant cell is placed in a solution with an osmotic concentration greater than the osmotic concentration of the cell, water will move out of the cell and the solution is said to be hypertonic to the cell. As water leaves the cell, the protoplasm contracts away from the cell wall. The cell is now said to be plasmolyzed. Plasmolyzed cells often recover if they are returned to pure water. They would now be in a hypotonic solution and water would move into the cell. If the cell was placed in an isotonic solution, there would be no net movement of water because the osmotic pressure would be the same inside the cell as outside.

Procedure:

1. Prepare a wet mount of plant cells in an isotonic media. An Elodea leaf or another aquatic plant are the best to use.
2. Examine under low and high power.
3. Prepare a wet mount of similar cells in a hypotonic media. You may use distilled water.
4. Examine under high and low power. Wait a few minutes for the exchange of water.
5. Prepare a third wet mount using hypertonic solution (10% NaCl).
6. Examine under high and low power. Wait a few minutes for the exchange of water.
7. After getting plasmolysis, try to reverse this by placing the cells in a hypotonic solution.
8. Make a drawing of a plant cell under each of the above conditions.

Results:

Concentration	Cell Environment	Direction of Water Movement
.9% NaCl	Isotonic	
distilled H_2O	Hypotonic	
10% NaCl	Hypertonic	

Conclusion:

1. What is plasmolysis?

2. Is the plasmolyzed condition reversible?

3. What would happen to Red Blood Cells under the above conditions?

LABORATORY INVESTIGATION 7A Acids - Bases - Salts

Objectives:

1. To differentiate between an acid and a base.
2. To determine the specific pH of a solution using various indicators.
3. To determine the strength of an acid and a base.
4. To neutralize an acid by titrating with a base.
5. To form a salt from an acid and a base.
6. To write a chemical equation showing the reaction of an acid and a base.
7. To calculate the volume of base required to neutralize a known volume of acid.
8. To explain how to prepare a normal solution.

Acids - Bases

Introduction: The acidity or alkalinity of a liquid environment is one of the factors that dictate which organism will maintain an active existence. All cells have an optimal pH, that is, one in which the efficiency of the cell is maximum. Different areas of the body will require a different optimal pH. For example, the blood requires a pH of 7.3 - 7.4; whereas the HCl in the stomach must have a pH of 2 in order for the enzyme pepsin to work at its optimum.

The strength of an acid or base is determined by the degree to which the compound dissociates in water. This is done by measuring the amount of free H^+ or OH^- ions present in the solution.

$$pH = \log \frac{1}{H^+}$$

$$H_2O \rightleftharpoons (H)^+ + (OH)^-$$

H_2O = .0000001 gram of H^+

$$\text{pH of } H_2O = \log \frac{1}{.0000001} = 7$$

Procedure:

1. Check the available solutions with the acid and base indicators.
2. Using a glass stirring rod, apply a drop of solution to be checked to the indicator paper. Use a clean stirring rod for each solution.
3. In order to test with the liquid indicators, fill a test tube 1/3 full of acid or base and add only 1-2 drops of liquid indicator.

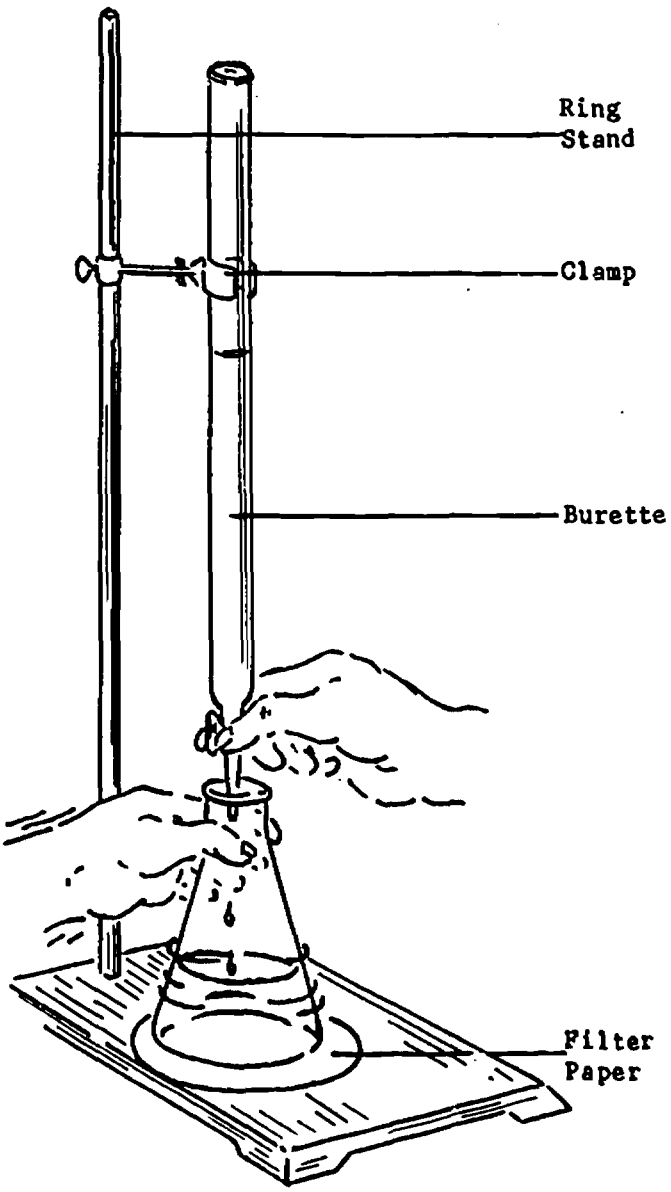
Results: Data Sheet: Record the color change

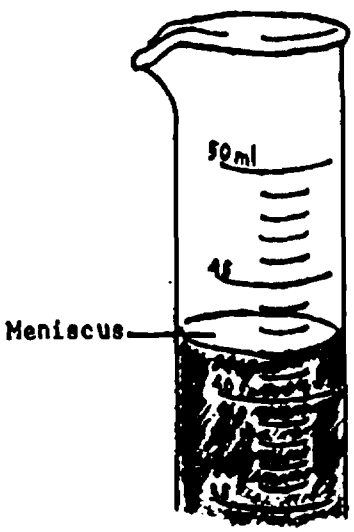
Solution	Red Litmus Paper	Blue Litmus Paper	pH Indicator Paper	Phenolphthalein	Bromothymol Blue	Litmus Solution
hydrochloric acid						
acetic acid						
sodium bicarbonate						
vinegar						
saliva						
distilled water						
soap solution						

TITRATION SET-UP

IMPORTANT POINTS

1. Place sheet of filter paper on ring stand to aid in end-point determination.
2. Add solution drop-by-drop.
3. Rotate flask as adding solution.





READING OF LIQUID VOLUME:

Eye level must be parallel with liquid level.

Accurate reading is <u>bottom</u> of meniscus.

In example, the liquid volume is 42 ml.

Titration

Introduction: It is possible to neutralize an acid with a base and form a salt. The amount required depends upon the concentration (normally) and volume of both the acid and the base. If one has a basic solution of known normality and an acid solution of unknown normality, it is possible to calculate the normality of the acid solution. This can be measured experimentally by determining the volume of the alkaline solution that is required to exactly neutralize a given volume of the acid solution. Phenolphthalein is used as an indicator to determine when the solution becomes exactly neutralized. The first indication of a slight pink color that remains for 30 seconds, indicates the solution is neutral.

Normality X volume (acid) = normality X volume (base)

CAUTION: Extreme care should be taken when working with an acid or base. If it is spilled on your skin, flush immediately with water.

Procedure:

1. Fasten a burette to a ring stand and fill it with 0.15 N NaOH.

2. Add 10 ml. of 0.10 N HCl to a flask. Add 2-3 drops of phenolphthalein. Titrate with 0.15 N NaOH very slowly while mixing the acid. Place a sheet of white filter paper under the flask so the first indication of a pink color can be detected. Determine the volume of base required to neutralize the HCl.

3. Titrate with .15 N NaOH (in burette). Add exactly 20 ml. of 0.15 N HCl and 2-3 drops of phenolphthalein to a flask. Determine the normality of the HCl.

4. Determine the normality of an unknown acid. Add 10 ml. of the acid to a flask, add 2-3 drops of phenolphthalein and titrate with 0.15 N NaOH.

5. Write a chemical equation for the chemical reaction in each titration.

Results:

#1).

Base: NaOH	**Acid:** HCl
normality = _____	normality = _____
volume (measured) = _____	volume = _____
volume (calculated) = _____	

#2).

Base	**Acid**
normality = _____	normality (calculated) = _____
volume = _____	volume = _____

Conclusion:

1. What was the normality of the unknown _____.
2. What volume of a 0.33 N solution of an acid is required to neutralize 27 ml. of a 0.44 N solution of a base?
3. If it required 40 ml. of an acid to neutralize 30 ml. of a 0.3 N solution of a base. Calculate the normality of the acid.
4. How was the end point determined in each titration?
5. Write a chemical equation to illustrate each titration.

LABORATORY INVESTIGATION 8A Digestive Glands and Enzymes

Objectives:

1. To observe the chemical breakdown of protein (digestion of egg white) with the enzyme pepsin.
2. To write the structural formula for protein digestion.
3. To observe the effects of Rennin on milk.
4. To identify the curds and whey separation in milk.
5. To determine the digesting effect of Pancreatin solution upon fats.
6. To write the chemical reaction that occurs in fat metabolism.
7. To determine the effect of HCl upon starch hydrolysis.
8. To write a chemical formula illustrating what occurs during hydrolysis of starch.
9. To observe a section of pancreas and to determine where the enzymes are produced.
10. To differentiate between the endocrine and exocrine portion of the pancreas.
11. To observe a section of liver to determine how bile is formed and collects from a network in the liver.
12. To locate and identify a lobule of the liver and to observe the blood supply to the lobules.

Enzymes

Introduction: Digestion takes place in several regions of the digestive tract. Digestion is the chemical breakdown of food with the aid of enzymes. Enzymes are organic catalysts which speed up chemical reactions in the body. Enzymes are protein in nature and some are specific in that they can only act upon one substrate. Hydrolytic enzymes are those involved in digestion; their action produces hydrolysis of the substance.

Protein Digestion

Procedure I. Egg White

1. Place a very small piece of hard-boiled egg white in each of two test tubes.
2. Add 5 ml. of pepsin solution (5%) to one test tube. Label the tube.
3. Add 5 ml. of distilled water to the second tube. Label the tube.
4. Incubate at 37° C. Observe for changes during an hour.

Results:

Test	Enzyme	Observable Results
Exp.		
Control		

Procedure II. Curd Formation in Milk

1. Add a Rennet tablet to 20-30 ml. of fresh skim milk.
2. Warm the milk to 37°C - 40°C.
3. Observe for curd formation.
4. Separate the curds and whey by filtering through a funnel lined with filter paper.

Results: What happened to the milk protein?

Fat Digestion

Procedure:

1. Fill two (2) test tubes each 1/3 full of fresh cream.
2. Add 2-3 ml. of Pancreatin solution (5%) to one tube and label.
3. Add 2-3 ml. of distilled water to the second tube and label.
4. Stir each tube with a glass stirring rod and incubate in a water bath at 37°C.
5. After 30 minutes add a few drops of blue litmus solution (enough to give the solution a pale blue color).
6. When fat is digested, fatty acids are released which will make the solution acidic and give a pink color.

Results: Fat Digestion

Test Tube	Color Change	End Product
1		
2		

Show by a chemical equation the digestion of a fat.

Carbohydrate Hydrolysis

Procedure:

1. Polysaccharides can be partially hydrolyzed in the stomach by the hydrochloric acid (HCl).
2. Fill two test tubes 1/3 - 1/2 full of 5% starch solution.
3. Add 2 ml. of HCl (pH 2) to one tube and 2 ml. of distilled water to the other tube. Label each tube.
4. Boil both tubes in a water bath for 5 minutes.
5. Let the tubes cool slightly and add 2-3 ml. of Benedict's Solution.

Results:

Test Tube	Color Change	End Products
1		
2		

Show by a chemical formula what happens during hydrolysis.

Digestive Glands

Liver: The liver is the largest gland in the body. It receives blood from the intestinal tract. This blood contains all the material absorbed from the alimentary canal. Among many other functions, the liver also produces bile.

The lobule is the basic unit of the liver. In cross section, lobules appear as hexagons with a central vein in the center. In the corners of the hexagons are portal canals, consisting of the portal vein, hepatic artery, and bile duct surrounded by connective tissue. In most mammals there is no boundary between the lobules. In the pig and a few other mammals there is a well-defined connective boundary which clearly separates the lobules.

Procedure:

1. Observe a section of the liver under the microscope.
2. Locate and identify a lobule, central vein, portal vein, hepatic artery, and bile duct.
3. Make a large diagram of several lobules and label the parts.

Pancreas: It consists of an exocrine portion which secretes digestive juices and an endocrine portion which is responsible for the production of insulin. The pancreas is a compound gland whose lobules are bound together by loose connective tissue through which run blood vessels, nerves, lymphatics, and excretory ducts. The acini which form the exocrine secretion vary from rounded structures to short tubules. The acinar cells show rather striking differences in the various stages of secretion.

In addition to the external-secreting portions of the gland, the pancreas also contains islets of Langerhans. These are irregular masses of cells which are separated from the acini by a thin layer of reticulum and are provided with an extensive blood supply.

Procedure:

1. Observe a section of pancreas under the microscope.
2. Locate and identify the acini cells and the islets of Langerhans.
3. Make a large diagram and label the islets of Langerhans and the acinar cells.

Salivary Glands: There are three pair of salivary glands, the parotid, submaxillary and the sublingual. These glands secrete saliva which helps to prepare food for digestion. Saliva is a mixture of secretions containing mostly water, mucin, and the enzyme salivary amylase. This enzyme starts the digestion of starch in the oral cavity.

Procedure:

1. Observe a secretion of a salivary gland under the microscope.
2. Locate and identify the secretory cells and the salivary duct.
3. Make a large diagram and label the parts.

LABORATORY INVESTIGATION 9A Parasites

Objectives:

1. To locate and identify living parasites of the liver, lungs, and gastro-intestinal tract in the frog.

2. To locate, identify, and diagram the life cycle of the following worm parasites: (a) pinworm
   (b) tapeworm
   (c) porkworm
   (d) hookworm
   (e) liver fluke

3. To observe under the microscope and identify the protozoa which causes African Sleeping Sickness.

4. To locate and identify the protozoa which causes malaria.

5. To diagram and explain the life cycle of Plasmodium vivax.

6. To identify and diagram the life cycle of Entamoeba histolyticia.

Parasites

Introduction: Parasitology is the study of parasites and their relationship to their hosts. Not all parasites are capable of destroying the host's tissues, and even among those that do, there is a large variation in the degree of damage. Some parasites injure the host's tissues when they enter the host, while others cause tissue damage after they have entered.

The human intestinal tract is infected by many parasitic organisms. Most of these parasites gain entrance into the gut by fecal contamination of food and water. Some, however, first enter the body by penetration of the skin. They penetrate in the larval stage and migrate through various tissues on the way to the intestine, where they mature.

Some parasites live their entire life on one host while others live different stages of their life on different hosts. A major feature which distinguishes life cycles of worm parasites from unicellular Protozoan parasites is the lack of multiplication of worm parasites within a host body. For most worms, the eggs or larvae produced within a host must leave that host before they can infect the same or another host.

Parasites Infecting Internal Organs

Procedure:

1. Open the thoracic and abdominal cavity of a frog.
2. Remove the lungs and place in a finger bowl with a small amount of water.
3. Remove the liver and place in a separate finger bowl with a small amount of water.
4. Remove as much of the gastro-intestinal tract as possible and place in a third finger bowl with a small amount of water.
5. Using your dissecting needles tease through the lungs and liver to free any available parasites from the tissue.

6. Using the scissors cut open the stomach and intestines. Let the digested food float in the bowl.

7. Using forceps, remove the tissues from the finger bowl.

8. Observe under the dissecting microscope for worm parasites.

9. Identify as to the number in each tissue and determine whether they are round, flat, or segmented worms.

Results:

Body Region	Number of Parasites	Types of Parasite
Lungs		
Liver		
G. I. Tract		

Worm Parasites

Procedure: Observe under low and medium power and make drawings of each organism. Determine the size and structure of each. Make a diagram of the life cycle of each organism.

1. Enterobius vermicularis: The pinworm is located in the caecum and appendix region of the intestines.

2. Taenia saginata: The beef tapeworm is located in the intestines. The mature worm may be 4-10 meters long and composed of 1,000-2,000 proglottids. Draw the head and one large proglottid.

3. Trichinella spiralis: The larva form is encysted in muscle fibers.

4. Necator Americanus: The hookworm enters the blood stream in the larvae form which travels to the lungs. The adult form is found in the small intestines.

5. Trematode: The human liver fluke is a large oval shaped worm.

Protozoan Parasites

Procedure: Observe prepared slides under oil immersion and make drawings of each organism studied. Determine the size, structure, and life cycle of each. Also, make a diagram of the life cycle of each organism.

1. Trypanosomes: They are flagellated protozoa found in the blood and cause African Sleeping Sickness.

2. Plasmodium vivax: Lives an asexual stage in the blood of man and a sexual stage in the mosquito. This protozoa causes malaria.

3. Entamoeba histolytic: It is found in lesions of the host's large intestine. Infections cause diarrhea and dysentery.

LABORATORY INVESTIGATION 10A Bacteria in Food, Air, and Soil

Objectives:

1. To isolate pure cultures of bacteria from a mixture of cultures.

2. To identify pure cultures of bacteria as to the following: color and types of colony; gram stain; morphology.

3. To identify the kinds and amount of bacteria that are found in fresh pasturized milk.

4. To locate and identify potential pathogenic bacteria in milk.

5. To observe and identify the following pathogenic soil bacteria:
   a. Cl. botulinum
   b. Cl. tetani
   c. Cl. perfringens
   d. B. anthracis

6. To differentiate between a spore forming and a non-spore forming bacteria.

7. To observe and identify a pathogenic bacterium transmitted in contaminated milk and water; Salmonella typhosa.

Bacteriologic Examination of Milk

Introduction: Most bacteria grow well in milk. As sold on the market, milk usually contains thousands of harmless bacteria per ml. Mere number of bacteria, however, are not alarming; it is the kind that is important. Freshly drawn milk may contain as few as 100 bacteria per ml. By the time milk reaches the dairy (24-48 hours later), the number of bacteria ranges from 3,000 per ml. in the very best milk, to millions per ml. in poor milk.

Pasteurization helps to reduce the number of bacteria and thus helps to delay the souring of milk. Also, another major reason for pasteurizing milk is to kill potential pathogens. One human pathogen that is transmitted in milk and water is the typhoid bacillus, Salmonella typhosa. This organism gets into milk and water that has been contaminated with fecal material.

Procedure:

1. The bacteria in milk are readily made visible and may be counted.

2. Make a smear of milk. Use one drop of milk and spread a thin film over the slide. Using the innoculating loop to spread the milk. Flame the loop before and after using it. Allow the milk smear to air dry, then heat "fix".

3. Stain with methylene blue, and examine under oil immersion.

4. The smear will show whether the predominating species are streptococci (potential pathogens).

5. Make a drawing of each type of bacteria and determine the type: streptococcus, staphlococcus, or bacillus.

6. To separate into pure cultures, streak an agar plate with a loop full of milk. Incubate the plate for 24-48 hours. Observe plate and record your data.

7. Observe a prepared slide of Salmonella typhosa under the oil immersion lens. Make a large diagram of the organism.

Isolation of Pure Cultures

Introduction: Microorganisms contaminating food are either saprophytic and may cause chemical changes that render the food unfit for human consumption; or pathogenic and may cause serious infections or poisoning when eaten. Microbiological examination of foods may provide information concerning the quality of the raw food and the sanitary conditions under which the food was processed as well as the effectiveness of the method of preservation.

In order to identify bacteria from a mixture, they must first be isolated into pure cultures. A pure culture is one containing only one kind of microorganisms. In preparing a pure culture of bacteria, it is very convenient to have a solid surface on which to spread out the mixture. If a mixture of bacteria is spread over the surface of a sterile agar plate (petri dish), separate colonies will grow on the surface.

Bacteria colonies vary greatly in appearance. Some look like dew drops, others are white and glistening like the head of a pin; while others have various colors. These peculiarities are used in identification.

Procedure:

1. Using an inoculating needle streak a plate with a loop full of ground meat suspension. Heat flame the loop before and after using it. Label the agar plate: name, date, source, of bacteria.

2. Incubate the plate for 48-96 hours. Invert the petri dish during incubation to prevent condensation on the growing surface. Study the types of colonies and record your results on the data sheet.

3. Make a gram stain of each type of colony. Make a drawing of each organism.

Results: Description of Bacteria Colonies

Source	Ground Meat	Milk
Color		
Amount of Growth (number of colonies)		
Shape of Colony (even circle or irregular)		
Surface Consistence (flat or elevated)		
Gram Stain		
Morphology		

Bacterial Pathogens of the Soil

Introduction: Many species of pathogenic microorganisms occur in the soil. The bacterial pathogens of man that are indigenous to fertile soil are the following: Clostridium botulinum causes a form of food poisoning. Clostridium prefringens causes gas gangrene. Bacillus anthracis causes anthrax.

Procedure:

1. Observe under the oil immersion lens and make drawings of prepared slides of the following:

   Cl. botulinum
   Cl. tetani
   Cl. perfringens
   B. anthracis

Results:

1. Which ones are spore formers?

LABORATORY INVESTIGATION 11A Antibiotics

Objectives:

1. To differentiate between mold and bacteria.
2. To observe and identify molds from which antibiotics are derived.
3. To identify and distinguish common bread mold from other molds.
4. To determine the sensitivity of bacteria to antibiotics by the "disc method".
5. To compare and contrast the growth inhibiting effects of antibiotics on *Aerobacter aerogens* and *Staphyloccus aureus*.

Antibiotics

Introduction: An antibiotic is a chemical substance which is produced by microorganisms and prevents the growth or destroys other microorganisms.

(1). Penicillin is an antibiotic derived from two stains of mold: Penicillium notatum and Penicillium chrysogenum. These molds are commonly seen growing on bread or fruit. Penicillin, the first clinically effective antibiotic, was discovered by Sir Alexander Fleming in 1929.

(2). Streptomycin is an antibiotic obtained from certain strains of Streptomyces griseus.

(3). Chloramphenicol (chloromycetin) is an antibiotic originally derived from Streptomyces venezuelae, but it is now produced synthetically.

(4). Actinomycin D is an antibiotic obtained from Streptomyces antibiolicus. Actinomycin is used against certain types of cancer.

(5) Tetracyclines: The tetracycline group of antibiotics includes Achromycin, Terramycin, and Aureomycin. Although they are all derived from a different species of Streptomyces, they all have similar, but not identical, antibiotic properties.

Mold Cultures

Procedure:

1. Observe under the dissecting microscope and make a large diagram of the following mold cultures:
    a. Penicillium notatum
    b. Streptomyces antibiolicus
    c. Streptomyces venezuelae
    d. Streptomyces griseus

2. Observe the mold culture, Rhizopus nigricans growing on bread. This is the common bread mold. There are no antibiotics obtained from this mold. However, this mold is so common and wide spread, it should be observed so that it is not mistaken for another mold. This bread mold will grow as a contaminent on other cultures of mold.

Sensitivity of Bacteria to Antibiotics

Introduction: The susceptibility of bacteria to antibiotics can be determined by the disc - plate method This consists of inoculating the organisms on an agar plate, placing discs containing antibiotics on the inoculated surface, and after incubation, observing zones of inhibition. The size of the zone of inhibition is influenced by many factors such as the rate of diffusion of the drug through agar, the size of the inoculum, the rate of growth of the organisms, and its susceptibility to the antibiotic.

Procedure:

1. Inoculate the surface of one trypticase - soy-agar plate with a broth culture of bacteria of Aerobacter aerogenes and another plate with Staphyloccus aureus.

2. Use a disposable pipet or dropper to apply several drops of broth culture so that the culture covers the plate completely.

3. Use the innoculating loop to spread the broth culture over the agar surface. Flame the loop before and after using it.

4. Place six discs (one of each antibiotic), evenly spaced, over the inoculating surface. Use sterile forceps to place discs.

5. Invert the plate and incubate at 37°C for 24-48 hours.

Results: Data Sheet: Measure the zone of inhibition in millimeters. Measure from the center to the outer edge (radius).

Antibiotic	Size of the zone of inhibition	
	Aerobacter aerogens	Straphyloccus aureus
1. Penicillin		
2. Streptomycin		
3. Aureomycin		
4. Chloramphenicol		
5. Terramycin		

Conclusion: Rate antibiotic in order of their ability to inhibit bacterial growth.

LABORATORY INVESTIGATION 1B Blood Chemistry

Objectives:

1. To determine the amount of hemoglobin in blood.
2. To compare and contrast the normal amount of hemoglobin in males and females.
3. To type ones own blood and an unknown sample as to the ABO system and Rh factor.
4. To identify and determine the conditions necessary for RBC's to agglutinate.
5. To determine the clotting time of blood.
6. To identify and determine the mechanism of phagocytosis by observing amoeba engulfing food particles.
7. To determine the amount of sugar in the blood.

Hemoglobin Determination

Introduction: Hemoglobin is a conjugated protein made up of a protein part, globin, and an iron - containing part, heme. Heme contains four pyrrole groups joined together with an iron ion in the center. Four heme molecules combine to form one molecule of hemoglobin. Hemoglobin carries oxygen to all cells of the body. When oxygen is taken into the lungs, it combines with the hemoglobin of the blood to form oxyhemoglobin. Each hemoglobin molecules contain four iron ions which enables it to carry four molecules of oxygen.

The normal amount of hemoglobin varies with age and sex, but it is usually between 12 and 16 grams per 100 ml. of blood. If the hemoglobin content of the blood falls below normal, the condition is called anemia.

Procedure:

1. Swab the end of your finger with 70% alcohol.
2. Using a clean, sterile lancet, puncture the skin.
3. Place one drop of fresh blood on a piece of test paper.
4. Match the blood spot with the scale provided by comparing colors.
5. Follow the same procedure with an unknown sample of blood.
6. From the chart, determine the normal for your age and sex.

Results:

Blood Sample	Percent Hemoglobin
Student Sample	
Unknown Sample	
Expected Amount (normal)	

Blood Typing

Introduction: The blood of human beings can be grouped into four types on the basis of the antigens present in the blood and associated with the red blood cells. The four types are A, B, AB, and O. These groups are named according to two antigens (agglutinogens) which may or may not appear in the red blood cells. Group A contains agglutinogen A; Group B contains agglutinogen B; Group AB contains both agglutinogens, and Group O contains neither agglutinogen.

Blood Group	Antigen in RBC	Antibody in Plasma
A	A	anti-B
B	B	anti-A
AB	A and B	none
O	none	anti-A and anti-B

The Rh factor refers to the presence or absence of another protein in the red blood cell. About 85 per cent of the population are Rh^+ (positive) and about 15 per cent are Rh^- (negative).

Procedure:

1. You will need three (3) depression slides for your blood typing.
2. Label each slide as follows: (1) "anti-A", (2) "anti-B", (3) Rh^+.
3. Place a drop of blood on each of the slides.
4. Add a drop of anti-A test serum to the slide marked anti-A.
   Add a drop of anti-B serum to the slide marked anti-B.
   Add a drop of anti-Rh^+ serum to the slide marked Rh^+.
5. Mix each with a separate clean toothpick. After mixing, observe and determine presence or absence of agglutination.
6. Follow the above procedure with an unknown sample of blood.

Results: Indicate presence or absence of agglutination

AGGLUTINATION RESULTS

Blood Sample	Anti-A	Anti-B	Anti-Rh^+
Student Sample			
Unknown Sample			

Conclusion: On the basis of your results, what are the blood types?

Sample	Type	Rh Factor
Student Sample		
Unknown Sample		

Blood Sugar Determination

Procedure: Determine the amount of sugar in the blood using a Dextrostix. Follow the directions on the bottle and compare the color to the color chart.

Results:

Sample	Color	mg.%

Clotting Time

Introduction: Whenever cells are damaged and small blood vessels are broken, the body has a mechanism to stop bleeding. Substances within the blood react with substances released from the injured cells to form a network of fibers to trap the blood cells and stop the flow of blood.

Procedure:

1. Obtain a capillary tube.
2. Puncture the end of one of your fingers. First swab with 70% alcohol.
3. Allow about two drops to appear on the end of the finger, then hold the capillary tube horizontal and place one end into the blood.
4. Note the time the drop of blood appeared.
5. Allow the blood to fill the tube.
6. After two minutes, break off a small piece of the tube and see if a thread of fiber connects the broken ends. If so, this should be recorded as your clotting time.
7. If no fibers appear, repeat every 30 seconds until your clotting has been determined.

Results: Record the length of time required for clot formation.

Phagocytosis

Introduction: The white blood cells or leukocytes are large and less numerous than the red cells. The white cells have a very important function as scavengers in the blood. Any foreign particles, such as dead tissue cells or bacteria, finding their way into the blood or tissues are promptly engulfed by the white cells.

Certain of the leukocytes move and respond similar to certain protozoa called amebae. They both have no fixed shape but move about by sending out temporary finger like cytoplasmic extentions called pseudopodia. Some leukocytes have this power of ameboid movement, they can extend pseudopodia around particles, draw them in and engulf them.

Procedure:

1. Make a wet mount of living amebae and study and observe them with the microscope under low and high power. Place a drop or two of fluid (amoeba are along the bottom of the container) on a depression slide. Use a cover glass.

2. Observe the ameboid movement and how the ameba engulfs foreign particles. Make a drawing of an amoeba.

LABORATORY INVESTIBATION 2B Blood Cells

Objectives:

1. To determine the hematocrit of blood.

2. To compare and contrast ones own hematocrit with an unknown sample.

3. To make and stain a blood smear.

4. To locate and identify the different types of blood cells on both a freshly prepared blood smear and a commercially prepared slide.

5. To differentiate between the following types of white blood cells:
   a. lymphocyte
   b. monocyte
   c. neutrophil
   d. eosinophil
   e. basophil

Hematocrit

Introduction: When blood is centrifuged, it separates into two distinct fractions. The upper fraction is a clear straw colored fluid called plasma and the lower fraction is packed with blood cells. The percent of cells by volume is referred to as the hematocrit. In the normal male the hematocrit is approximately 45% of the total volume; in the female it is approximately 42%.

$$\text{Hematocrit} = \frac{\text{mm packed cells}}{\text{mm total sample}} \times 100 = \quad \%$$

Procedure:

1. Clean the end of your finger with 70% alcohol.
2. Prick your finger with a sterile lancet. Allow a drop or two of blood to form.
3. Quickly fill a heprinized capillary tube with blood. The blood will flow up the tube dua to capillary action. It will flow easier if the tube is held in a horizontal position.
4. Cap the bottom of the tube and centrifuge for 15-20 minutes. Check with the instructor when using the centrifuge. Place the capillary tuba into a centrifuge tube for centrifuging.
5. Determine your own hematocrit. Measure the height of blood in the capillary tube, then measure the height of compact blood cells. Make all measurements in millimeters.
6. Determine the hematocrit on an unknown sample of blood.

Results:

Sample	Height of Blood (millimeters)	Height of RBC (millimeters)	Hematocrit (percent)
Student's Blood			
Unknown			

Making and Staining Blood Smears

Procedure:

1. The first essential is to have an absolutely clean slide. It should be cleaned with a cleaning powder such as Comet.

2. Place a drop of blood near one end of the slide. Take a cover slip, tipped at an angle of 45 degrees to the slide, lower it until its edge comes in contact with the drop of blood. The blood will spread out along the edge of contact. Now pull the tipped cover slip ahead with its edge pressed against the slide. A film of blood will be left behind.

3. Allow the blood smear to air dry.

4. Add a few drops of Wright's blood stain to the blood smear, enough to cover the film.

5. Allow to stand for one minute; then add about the same amount of distilled water. The water will cause a thin metallic film to rise from the stain. It is only after adding the water that the staining process actually begins.

6. Now stain for 3 to 5 minutes, then rinse off the solution with water. Use the washing bottle to flood the stain off with water.

7. Blot dry with a paper towel.

PREPARATION OF A BLOOD SMEAR

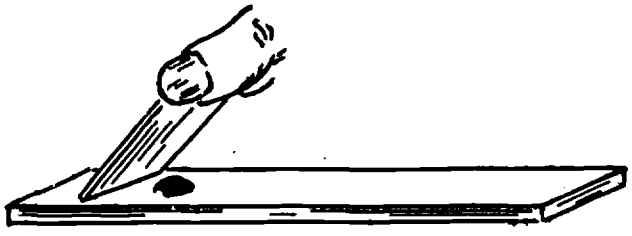


Fig. 10a. Cover Slip Angled and Contacting Slide

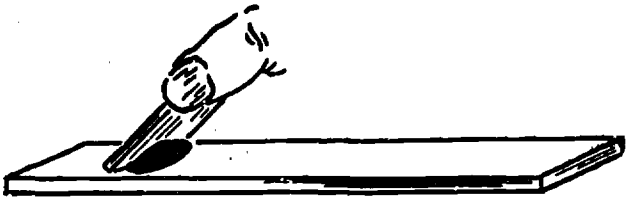


Fig. 10b. Cover Slip in Contact with Drop of Blood

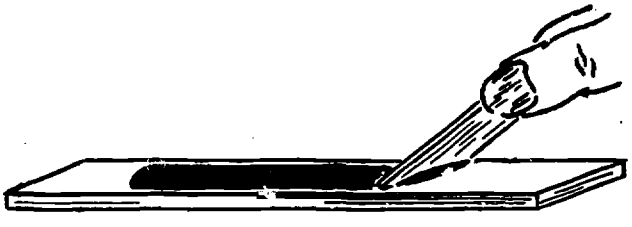


Fig. 10c. Cover Slip Pulled Across Slide, Leaving Thin Film of Blood

Types of Blood Cells

Introduction: The blood contains several different types of cells. The red blood cells are disc-like cells without a nucleus. It normally appears red because of the oxygenated hemoglobin.

The white blood cells are defensive agents of the body. There are different types of leucocytes or white blood cells. The lymphocyte is the smallest of the white blood cells, only slightly larger than the RBC. It has a distinct nucleus that fills almost the entire cell and stains dark blue with Wright's stain.

The monocytes are the largest of the white cells, ranging from two to three times the size of red blood cells. The nucleus stains dark purple. There is a large amount of non-granular cytoplasm surrounding the nucleus.

Neutrophils, eosinophils, and basophils all have nuclei divided into lobes which stain dark blue-purple. The cytoplasm is granular. The granules stain red in the eosinophils, purple in the neutrophils, and blue in the basophils.

Procedure:

1. Make and stain a blood smear of your own blood. Locate and identify the different types of blood cells.

2. Examine prepared slides of human blood. Locate, identify, and make a drawing of each type of blood cell. Label each drawing, as to the type of blood cell.

LABORATORY INVESTIGATION 3B Dissection of the Heart

Objectives:

1. To locate and identify the four chambers of the heart.
2. To differentiate between the ventral and dorsal surface of the heart.
3. To differentiate between auricles and atria.
4. To locate and identify the four different valves in the heart.
5. To compare and contrast the structure of the various heart valves.
6. To locate and identify the blood vessels that carry blood to the heart.
7. To locate and identify the blood vessels carrying blood away from the heart.

Dissection of the Heart

Introduction: For proper dissection of the heart, certain outer markings must be identified to differentiate the right from the left and the front from the back. The tip of the heart, or apex should be at your right as you are looking at the heart. The outstanding characteristic to distinguish the front or ventral surface is the coronary sulcus. The coronary sulcus is a groove that contains the larger vessels of the heart. This groove also separates or divides the ventricles into a right and left chamber.

Procedure:

A. Open the left side of the heart. Using the scalpel, start cutting into the left auricle through the left atrium and make the incision down toward the apex of the heart. After opening the left side, take a blunt end of the probe and run it into the chamber and out through the blood vessels to trace the flow of the blood. Locate and identify each of the following structures:

1. Left auricle. Ear-like flap over the left atrium.
2. Left atrium. Chamber receiving blood from the lungs.
3. Left ventricle. Chamber that forces blood out of the aorta.
4. Aortic semilunar valve. Located at the entrance of the aorta.
5. Mitral valve. Located between the left atrium and left ventricle.
6. Chordae tendineae. Chord like structures attached to the flaps of the mitral valve.
7. Papillary muscles. Muscles which anchor the chordae tendineae to the myocardium.
8. Aorta. Carries blood from the left ventricle.
9. Pulmonary veins. Four veins which enter the left atrium. They carry oxygenated blood from the lungs.

B. Open the right side of the heart. Follow the same technique used to open the left side. Locate and identify each of the following structures:

10. Right atrium. Chamber receiving venous blood from the body.
11. Right auricle. Ear-like flap over right atrium.
12. Right ventricle. Chamber that forces blood to the lungs to pick up oxygen.
13. Tricuspid valve. A valve with three flaps located between the chambers. The Chordae tendineae and papillary muscles are also attached as on the left side.
14. Inferior vena cava. Large vein entering right atrium. It drains blood from the lower parts of the body.
15. Superior vena cava. Large vein entering the right atrium. It drains blood from the head and upper thoracic region of the body.

LABORATORY INVESTIGATION 4B Blood Vessels and Blood Pressure

Objectives:

1. To observe the flow of blood through capillaries.
2. To measure the systolic and diastolic pressure with the sphygmomanometer.
3. To determine the pulse rate during various activities.
4. To locate and identify the three layers, (1) tunica intima, (2) tunica media, (3) tunica externa; in an artery and vein.
5. To compare the thickness of the layers of an artery to that of a vein.
6. To determine the thickness of the capillary walls.
7. To observe and identify the major arteries and veins.

Capillary Circulation

Introduction: To appreciate fully the intricacies of a closed circulatory system, you should observe the capillaries in living tissues.

The diameter of the capillaries varies considerable depending upon the amount of blood flow. The thin walls of the capillaries which are elastic, but not muscular, do not actually control the flow of blood. This control is largely exerted by the small arteries leading into the capillaries.

Procedure:

1. Anesthetize a frog injecting one (1) ml. of 10% urethan into the spinal cord. Wrap the frog in a wet towel and place it on a frog board.
2. Pin the web of one back leg over the hole in the board.
3. Observe under low power with the compound microscope.
4. Identify a capillary by its small size compared with the other vessels.
5. Change to high power and note the comparison between the diameter of red cells and that of the capillary lumen.

Blood Pressure and Pulse Rate

Introduction: The highest pressure obtained during each cardiac cycle is called the systolic pressure and the lowest pressure is called the diastolic pressure. The difference between the two is termed pulse pressure.

The blood pressure is generally recorded in the arm with the patient in a sitting position. The arm should be slightly flexed and supported by a smooth, firm surface. The artery over which the blood pressure is to be recorded should be at a level with the heart. The instrument used to determine blood pressure is called a sphygmomanometer.

The compression cuff is applied evenly and snugly, but without constriction around the arm. The brachial artery should be palpated to determine where to place the stethoscope. The compression cuff is then inflated rapidly to about 30 mm. Hg above the pressure at which the radial pulse disappears. The cuff is then deflated at a rate of 2 to 3 mm. Hg per heartbeat. While watching the meniscus of the mercury column, the pressure at which characteristic changes in the Korotkoff sounds occur is noted. From the changes in these sounds, the systolic and diastolic blood pressures are determined.

The systolic pressure is determined at the moment that you first begin to hear a faint, clear rhythmic tapping or thumping sound that gradually increases in intensity. The pressure at which the sound disappears is the diastolic pressure.

Procedure:

1. Steps in taking blood pressure.
    a. snug application of compression cuff.
    b. inflation of compression cuff above systolic pressure.
    c. careful placement of stethoscope.
    d. deflation of the cuff at a rate of 2 to 3 mm. Hg per heartbeat to determine systolic and diastolic blood pressure.

2. Take the blood pressure three (3) times for each activity, then record the average of the three in your results.

3. Take the pulse on the radial artery prior to taking the blood pressure.

Results: Record Data

Activity	Systolic Pressure	Diastolic Pressure	Pulse Pressure	Pulse Rate
Sitting				
Lying down				
Vigorous exercise				

Structure of Blood Vessels

Introduction: Arteries and veins are each composed of three layers: tunica intima is the inner layer; tunica media is composed of smooth muscle tissue; tunica externa (adventitia) is composed of elastic connective tissue. Arteries are thicker and more elastic than veins; this is to enable the arteries to withstand the higher pressure. The thinnest vessels are the capillaries which are only one cell layer thick.

Procedure:

1. Observe prepared slides of cross-sectional views of arteries, capillaries, and veins.
2. Make a cross-sectional drawing of each. Label the drawings.
3. Compare the thickness of the layers.

LABORATORY INVESTIGATION 5B Chemical and Thermal Stimulation to the Heart

Objectives:

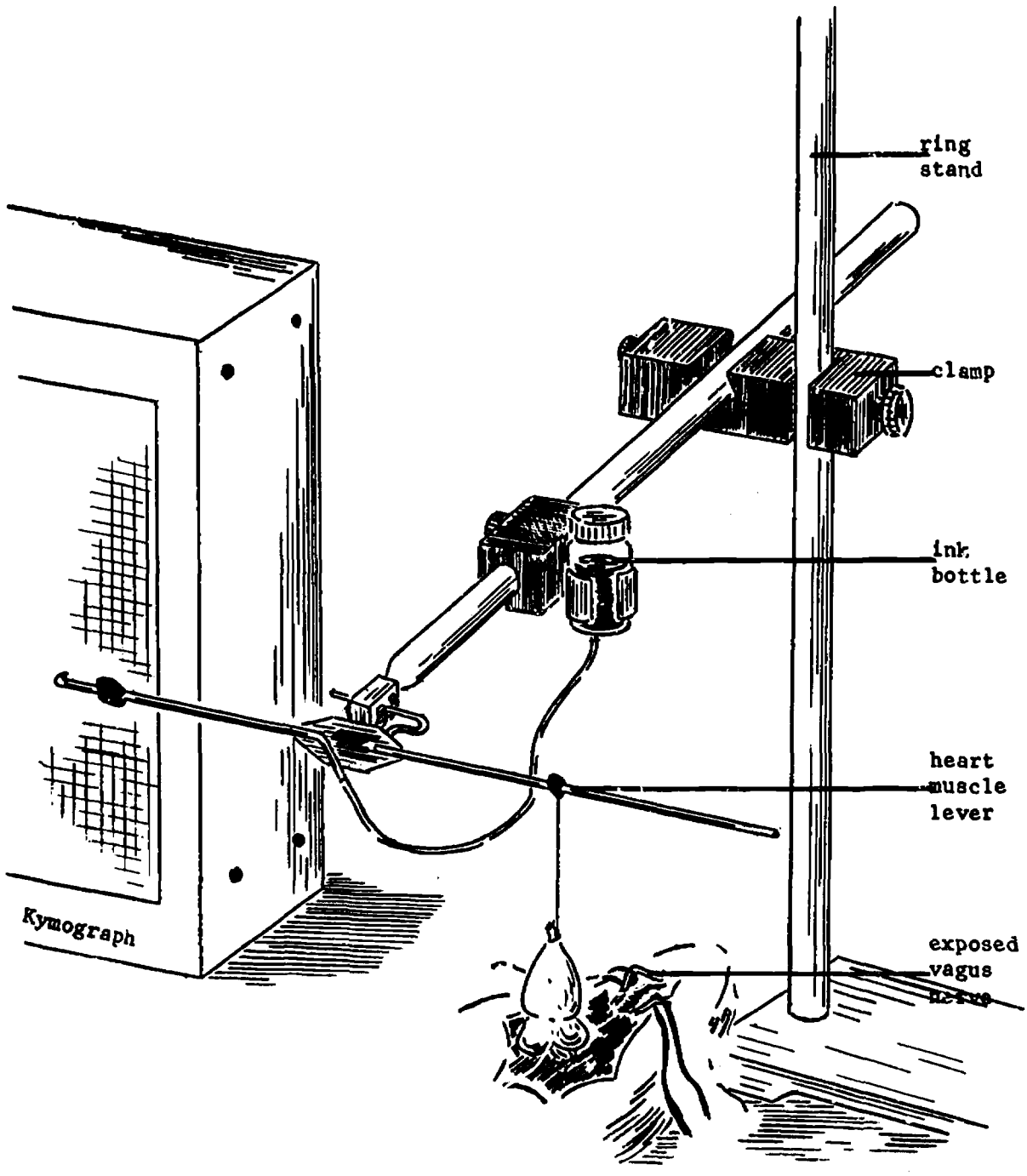
1. To obtain tracings on the kymograph of frog heart contractions.

2. To determine normal heart rate and vigor of contractions from the heart tracings.

3. To determine the effects of temperature change on normal heart contractions.

4. To determine the variation in heart contractions after the administration of the following chemicals:
   a. adrenaline
   b. acetylcholine
   c. digitalis
   d. atropine

Kymograph Set-Up for Heart Tracings



Introduction: The kymograph is an instrument for recording movement or in this experiment you will be recording tracings of heart contractions. The heart is connected to an ink-writing lever. Each time the heart contracts the lever moves up and down. The more forceful the heart contracts the higher the lever moves. From these tracings you can determine rate and vigor.

Depending upon the type and amount of stimulation you will be able to observe variations in both rate and vigor.

Procedure:

1. Anesthetize a frog by injecting 1.0 ml. of 10% urethane into the spinal cord.

2. Open the thoracic cavity and expose the heart. Keep the heart moist with frog ringer's solution.

3. Attach a fish hook or bent pin into the apex of the ventricle.

4. Use a light weight string to attach the hook to the ink-writing lever. (Use a piece of clay or putty to attach the string to the lever.)

Procedure: Thermal Stimulation

1. Record the normal tracing for at least one minute. The rate of contractions can be determined by counting the contractions from the kymograph tracing for a given period of time.

2. The vigor can be determined by measuring the height of the tracing. A decrease in height from normal indicates a decrease in vigor and conversely, an increase in height is an increase in vigor.

3. Apply chilled ringer's solution (0°C) with an eye dropper over the heart. Keep applying the chilled ringer's while you are recording the contractions.

4. Determine the changes in rate and vigor and record on the data chart in your results.

Procedure: For Chemical Stimulation

1. Record the normal tracing for at least one minute. The rate of the contractions can be determined by counting the contractions from the kymograph tracing for a given period of time.

2. Apply 0.5 ml. of adrenaline directly onto the heart. Record the tracing for several minutes. Be sure to label and indicate on the tracing the time of injection; 1 minute, 2 minutes, 3 minutes, etc. after the injection.

3. Measure height of contraction and determine the rate.

4. Allow at least 10 minutes between administration of different drugs. (Allow the heart to become normal.)

5. Apply 0.5 ml. of acetylcholine directly onto the heart. Record the tracing for several minutes. Record the rate and vigor.

6. Again allow the heart to return to normal, then apply 0.5 ml. of digitalis and record for several minutes.

7. Repeat the above procedure with 0.5 ml. of atropine.

Results:

Stimulus	Rate of Contraction	Percent Increase or Decrease	Height of Contraction	Percent Increase or Decrease
Normal				
Adrenaline				
Acetylcholine				
Digitalis				
Atropine				
Temperature				

LABORATORY INVESTIGATION 6B Electrocardiogram

Objectives:

1. To observe and identify the parts of an EKG tracing on the oscilloscope.

2. To label an electrocardiogram as to the P, QRS, and T wave.

3. To observe the variations in a normal EKG tracing during various activities.

4. To connect the three electrodes to the skin and operate the ImpScope in order to obtain a EKG tracing.

The Human Electrocardiogram

Introduction: The ImpScope is a five inch oscilloscope and stimulator. It is applicable to the study of electrophysiological phenomena. Muscular contractions may be converted to electrical signals for display on the oscilloscope.

Electrical changes are always associated with and are all thought to cause muscular contraction, although the chemical link between the electrical changes and the resulting mechanical effects is not understood. The electrical phenomena related to muscle contraction are due to the ionically charged nature of the muscle cell membrane. The resting muscle cell possesses a polarized membrane. The outside surface is positive, the inside negative. Any stimulus which disturbs this polarized state sufficiently to depolarize the entire membrane will produce a contraction of the muscle fiber. The rate at which the cell repolarizes determines how soon the cell will again be responsive.

The heart at rest can be visualized as polsrized, covered outside with a layer of positive charges. When the S-A node initiates depolarization, a wave of negativity spreads over the surfaces of the heart in a fairly well understood sequence. A typical electrocardiogram or EKG is shown in Figure 6. The letters P, Q, R, S, and T correspond to definite electrical events.

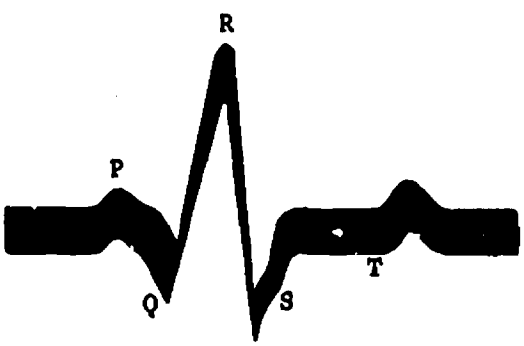


Figure 6. An Electrocardiogram. The different waves are known simply by the letters P,Q,R,S, and T. The wave P is caused by the contraction of the atria; Q,R, and S by the contraction of the ventricles; and T by the relaxation of the ventricles.

A. Procedure: To operate the ImpScope.

1. Turn the on-off switch to the first click. Wait several minutes (2-3) for the instrument to warm-up.

2. The horizontal and vertical switches (large knobs) should be set with the marker at "12:00 o'clock". These two knobs will center the sweep. The electron beam should now be moving from left to right.

3. Initially set the sensitivity switch to 5 MV/cm.

4. If the sweep is not moving the entire distance across the screen, adjust with the small dial on the vertical control. This will regulate the height of the tracing.

B. Procedure: Connecting ImpScope leads.

1. Cleanse the area of skin with alcohol:
   a. Upper right arm
   b. Upper left arm
   c. Left wrist

2. Apply EKG paste to Telectrodes.

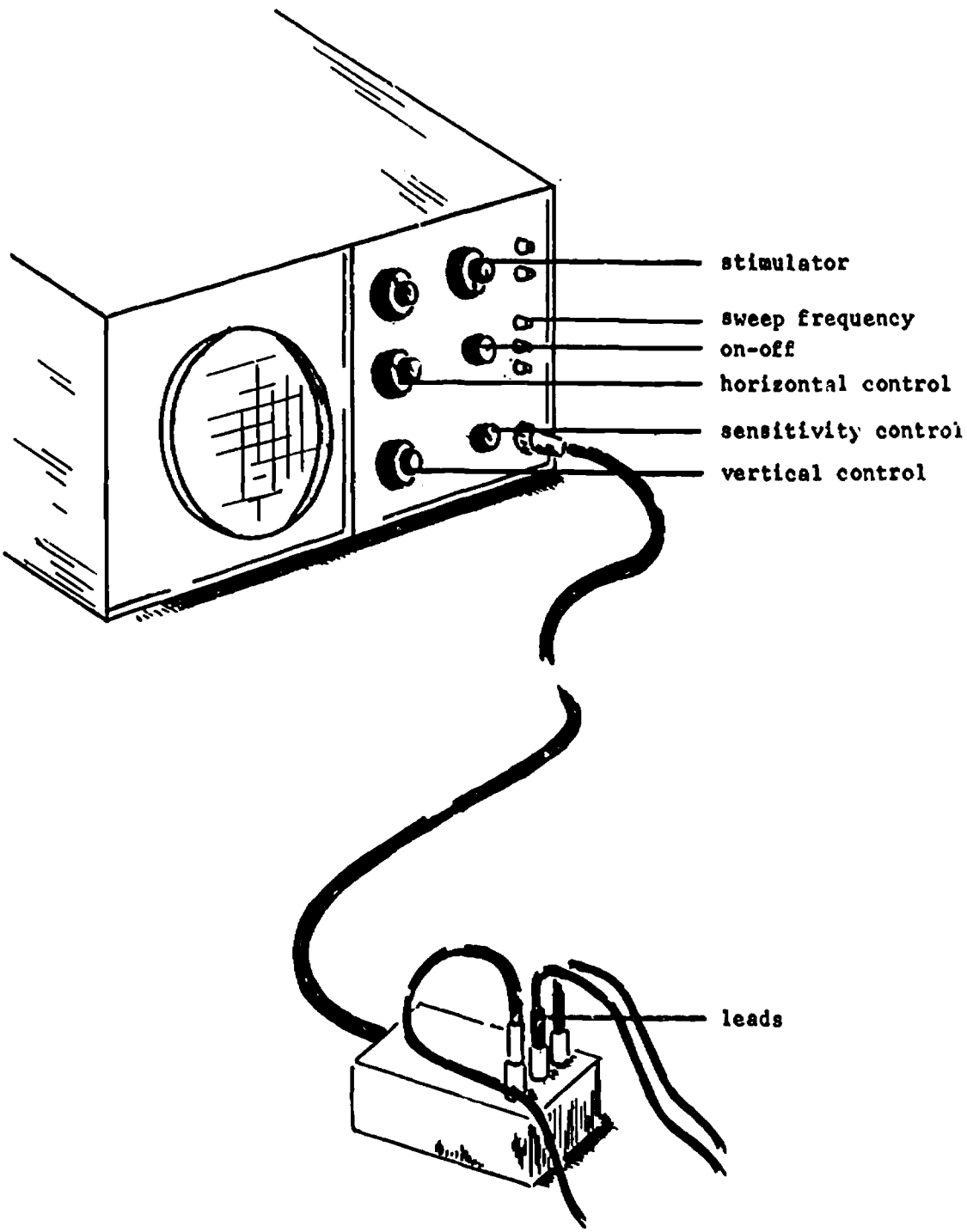
3. Apply Telectrodes to skin and connect leads.

4. Connect the "white" lead to the upper left arm.

5. Connect the "red" lead to the upper right arm.

6. The "black" lead is the ground, connect it to the left wrist.

IMPSCOPE


stimulator
sweep frequency
on-off
horizontal control
sensitivity control
vertical control
leads

C. Procedure: For EKG observation.

1. Connect the input box to the ImpScope and connect the leads from the patient to the input box.

2. Wait a minute for the sweep to re-adjust and have the patient lying very still and quiet.

3. Change the sensitivity switch to 1 MV/cm and adjust the vertical height tracing.

4. Depending upon the electrical conductivity of the skin, it may be necessary to set the sensitivity switch to 2000 μV/cm.

5. For better observation of the EKG, it may be necessary to vary the speed of the tracing. The slower the sweep, the more compact is the EKG wave; and conversely, the faster the wave the more spread out the wave becomes.

D. Procedure: Making EKG tracings.

1. Obtain a good tracing on the oscilloscope with the patient in a sitting position. Sketch the tracing on graph paper and label the wave.

2. Remove the input plug to the oscilloscope, hand it to the patient and have him hop up and down on each foot 20-40 times. Have him immediately recline so that you can replace the input plug and observe the EKG immediately after exercise. Sketch the tracing and label.

3. Observe the EKG during the administration of pressure or pain. Observe for variations and make a sketch.

4. Observe the EKG while exposing the patient to anxiety or emotional stress. Sketch the EKG wave.

LABORATORY INVESTIGATION 7B Nerve and Electrical Stimulation to the Heart

Objectives:

1. To observe and determine the effects of an electrical shock during ventricular contraction.

2. To observe and determine the effects of an electrical shock during ventricular relaxation.

3. To observe and determine the effects of a continuous electrical stimulation to the heart.

4. To observe and determine the effects of electrical stimulation to the vagus nerve.

5. To observe and determine what effects cutting the vagus nerve has on the rate of heart contractions.

Introduction: Both heart rate and vigor can be observed and determined from kymograph tracings of a contracting turtle heart. The height of the tracing is an indication of the force of vigor. A normal tracing must first be observed and recorded in order that variation or changes can be determined. If the height of the tracing increases, it is an indication of an increase in force. Conversely, a decrease in height is indicative of a decrease in force.

The distance between contractions is an indication of the rate. If the tracings are spaced apart, the heart is slowing down; and if the tracings become closer together the heart rate is increasing.

Procedure:

1. Anesthetize a turtle with an injection of 1.0 ml. urethane directly into the spinal cord.

2. The instructor will then sacrifice the turtle with a hard blow to the head.

3. Remove the ventral shell by cutting along the sides with a cast-cutter. Then use the scissors to cut through the attached skin and connective tissue.

4. The heart can be observed beating under the pericardium. Very carefully remove the pericardium. Notice the frenulum, connective tissue at the apex of the ventricle. Tie a string around the frenulum as close as possible to the heart. After the string is tied, cut the frenulum free of the body so that the heart is suspended by the string. The string will later be attached to the heart lever.

5. Examine the vessels leaving the aorta. Two of these may be traced upward along either side of the neck toward the head. These are the carotid arteries. Careful observation and dissection in the vicinity of these arteries low in the neck will reveal near each a white, thread-like structure. These are the right and left vagus nerves. With the probe to loosen the vago-sympathetic trunks insert a string under the nerves. This is to enable one to lift up and readily expose the vagus. The vagus will be stimulated later in the experiment.

Electrical Stimulation

Procedure:

1. Observe and record the normal tracing for about one (1) minute with the chart moving slowly.

2. Be sure to label all tracings as to what was done under each stimulation.

3. Stimulate the heart directly with a single shock. (Regulate the voltage to get a response.)
   (a) set the voltage to administer 20 volts.
   (b) set the frequency to 1/sec.
   (c) put the switch on multiple and touch the heart with electrodes.

4. Stimulate with a single shock when the ventricle is relaxed (bottom of the tracing).

5. Stimulate with a single shock when the verticle is contracted (top of tracing).

6. Repeat procedure 4 and 5 three times each.

7. Using a multiple stimulation, stimulate the heart uninterrupted for 5 seconds, 10 seconds, 15 seconds. (Repeat three times each) Gradually increase the frequency from 1-100 while you are stimulating the heart.

8. Measure the height of the tracing in mm. under each of the above conditions.

Results:

Stimulation	Height of Tracing	Percent Increase or Decrease
Normal		
Single Shock		
Shock During Contraction		
Shock During Relaxation		
Continuous Shock 5 seconds		
10 seconds		
15 seconds		

Nerve Stimulation

Procedure:

1. Record the normal tracing for at least one minute.
2. Stimulate the left vagus with a multiple stimulus for 5 seconds, 10 seconds, 15 seconds. Then repeat with the right vagus. Gradually increasing the frequency from 1-100.
3. Cut the left vagus and record for several minutes. Cut the right vagus and record.

Results:

Stimulus	Expected Results
Stimulate left vagus	
Stimulate right vagus	
Cut left vagus	
Cut right vagus	

LABORATORY INVESTIGATION 8B Dissection of the Respiratory System

Objectives:

1. To examine and make gross observation of the respiratory system of the rat.
2. To identify the parts of the respiratory system.
3. To observe the pleura membranes and to determine how they function in breathing.
4. To observe the cartilage rings in the trachea.
5. To locate and trace the bronchi and the branches throughout the lungs.
6. To observe ciliary action of the frog tongue and paramecium.
7. To observe a prepared slide of the alveoli under the microscope.

The Respiratory System

Introduction: The respiratory system consists of those organs that make it possible for blood to exchange gases with air. These include the nose, pharynx, larynx, trachea, bronchi, and lungs.

The lungs are cone-shaped organs, large enough to fill the pleural portion of the thoracic cavity completely. They extend from the diaphragm to a point slightly below the clavicles and lie against the ribs. The medial surface of each lung is roughly concave to allow room for the mediastinal structures and for the heart.

The left lung is divided by fissures into two lobes and the right lung into three lobes. Internally, each lung consists of millions of microscopic alveoli with their bronchioles and bronchi.

Procedure: Gross Observation

1. Examine the trachea.
   a. Observe its size and shape.
   b. Try to compress the trachea.

2. Examine the pleura membranes.

3. Examine the lungs.
   a. Note divisions of the lungs into lobes and lobes into lobules.

4. Cut off part of the trachea and examin the cartilage rings.

5. Trace the bronchus through one of t bes to the smallest bronchioles.

Procedure: Microscopic Examination

1. Examine a prepared slide of lung tissue under the microscope.

2. Observe and determine how many cell layers make up the structure of an alveoli.

The Physiology of Ciliary Action

Introduction: The tracheobronchial tree is a system of open tubes designed for the transmission of air into the lungs. Air contains foreign material and pollutants that gather on the walls of these tubes and must be removed.

The Cleansing mechanism consists of a special type of epithelial lining, the surface of which is covered with tiny hairlike processes, called cilia. All these cilia stroke in a marvellously coordinated manner, directing the stroke upward toward the larynx. Each cilium beats about 1,000 times a minute. This ciliary action is similar to the ciliary action in unicellular organisms.

Procedure: Ciliary action may be observed on the tongue of a frog and as a means of locomotion in the Paramecium.

1. Anesthesize a frog and take a very thin sliver off the surface of its tongue. Make a wet mount.

2. Place one drop of 14% polyvinyl alcohol on a depression slide. Add a drop of liquid containing Paramecium. The polyvinyl alcohol is a quieting agent to slow down the organisms.

LABORATORY INVESTIGATION 9B Disease Organisms of the Respiratory Tract

Objectives:

1. To make a culture of the bacteria from the throat.
2. To observe and identify the various types of hemolysis.
3. To observe and identify various bacterial organisms that cause diseases of the respiratory system.
4. To compare and contrast virus with bacteria.

Throat Culture

Introduction: Under normal conditions, the mouth and throat may contain enormous numbers of bacteria Most of these are harmless parasites; some are potential pathogens; sometimes virulent organisms may be present which do not produce disease in the individual harboring them but may cause infections in others.

Procedure:

1. Depress the tongue with a sterile tongue depressor; then pass a sterile cotton swab into the mouth and rub gently but firmly against tonsils and/or back of the mouth and throat region.

2. Streak the swab back and forth over the surface of a blood agar plate. Be sure to label the plate correctly: Name, Time, Date.

3. Invert the plate and incubate at 37°C for 48-96 hours.

4. Observe for the presence of hemolytic areas surrounding the colonies. Make a drawing of each type of colony.

5. Describe the type, color, and number of colonies.

   Alpha - Hemolysis manifests itself by the presence of a greenish coloration of the agar and partial hemolysis in the immediate vicinity of the colonies.

   Beta - Hemolysis is indicated by the presence of completely hemolyzed, clear, colorless zones around the colonies.

   Gamma - No visible change of blood cells occurs.

Results: Hemolysis usually indicates the presence of certain streptococci.

Type	Color	Number
Alpha		
Beta		
Gamma		

CAUTION: Do not open the petri dishes with cultures. Many of the bacteria may be pathogenic.

Respiratory Infections

Introduction: Infections of the respiratory tract range from the "common cold", the most common of all infections, to tuberculosis, the most deadly infectious disease in North America and second only to malaria in the world. In the present exercise, we shall examine the morphological features of the principal bacterial pathogens that attack this vital passageway.

Procedure:

1. Examine under oil immersion the prepared slides that are available and make a large drawing of each.

2. Make a large drawing of each organism.

Prepared Slides:

Diplococcus pneumoniae
Klebsiella pneumoniae
Mycobacterium tuberculosis
Corynebacterium diptheriae
Staphylococcus aureus
Streptococcus pyogenes
Bacillus antharacis

Results: Summarize your observations

Organism	Morphology	Habitat	Disease

Procedure:

1. List the virus that cause the following diseases. Indicate the size of the virus and the location of infection.

Results:

Disease	Virus	Size	Area of Respiratory Affected
Common Cold			
Influenza			
Pneumonia			

LABORATORY INVESTIGATION 10B Vital Capacity and Pulmonary Pressure

Objectives:

1. To measure the amount of tidal air in the lungs with the Spirometer.
2. To measure the amount of supplemental air in the lungs with the Spirometer.
3. To measure the vital capacity of the lungs.
4. To determine the normal breathing rate.
5. To measure and determine variations in breathing rate after various activities.
6. To form carbonic acid from exhaled air.
7. To form a precipitate of calcium carbonate by the addition of CO_2 to lime water.
8. To measure and record the pressure changes of the thoracic cavity during imspiration and expiration.

Vital Capacity

Introduction: An apparatus called a SPIROMETER is used to measure the amount of air exchanged in breathing. The amount of air exhaled normally after a normal inspiration is termed tidal air. A forcible expiration after a normal inspiration represents the Supplemental Air. Complemental Air is the amount that can be forcibly inspired over and above a normal inspiration. No matter how forcefully an individual exhales, he cannot squeeze all the air out of his lungs. Some of it remains trapped in the alveoli. This amount of air that cannot be forcibly expired is known as residual air.

Procedure:

1. Tidal Air. While at rest, apply the noseclip and exhale through your mouth into the mouthpiece. Repeat this three times and record the average. How many cc. of air is expired with each normal breath? The Spirometer records in liters; convert the readings to cc.
   Trial: 1._____ 2._____ 3._____

2. Supplemental Air. After a normal expiration, exhale as deeply as possible into the mouthpiece. Repeat three times.
   Trial: 1._____ 2._____ 3._____

3. Vital Capacity. Take as deep a breath as possible and exhale as deeply as possible into the mouthpiece. Repeat three times.
   Trial: 1._____ 2._____ 3._____

4. Express the volumes as <u>percent of predicted</u>. In order to calculate this, divide the actual volume by the expected normal.

<u>Example</u>: The predicted normal for a 32 year old female is 2975 cc. The actual vital capacity was 3270 cc.

therefore: $\frac{3270}{2975} \times 100 = 109\%$

<u>Results</u>: Record Data

Volume	Spirometer Reading	Predicted Amount	Percent of Predicted
Tidal Air			
Supplemental Air			
Vital Capacity			

Breathing Rate

Introduction: In each of the following exercises, one partner should serve as test subject, the other should record the results. Count one inhalation and one exhalation both together as one breath. Allow at least one minute rest between tests.

Procedure:

1. Count the number of breaths per minute during normal breathing while sitting at rest.

2. Hyperventilate by breathing deeply and rapidly for two minutes. Immediately afterward, count respirations and note their depth for one minute.

3. Hold a paper bag tightly over your nose and mouth so that you are forced to breathe the same air over and over again for three (3) minutes. Immediately afterward, count respirations and note the depth for one minute.

4. Run in place or up and down some stairs for two (2) minutes. Immediately after this vigorous exercise, count the number of breaths per minute.

5. Take one deep breathe and hold your breath as long as possible. Record length of time. Immediately after record rate and depth.

Results:

Activity	Rate/Min.	Depth
1. Normal		
2. Hyperventilate		
3. Breath into Paper Bag		
4. After Exercise		
5. Holding your Breath Time:_____		

Elimination of Carbon Dioxide

Introduction: Respiration may be defined as the sum total of all processes involved in the exchange of gases between cells and their environment. Oxygen required for the release of energy from foods and carbon dioxide is formed as a waste product. This is eliminated by exhaling.

Carbon dioxide combines with water to form carbonic acid. This is a relatively weak acid.

$$CO_2 + H_2O \longrightarrow H_2CO_3$$

Procedure: Formation of Carbonic Acid.

1. To 100 ml. of water, add a few drops of litmus solution. Litmus is blue when basic and pink when acidic.

2. Using a straw or 1 ml. pipete, bubble air (from lungs upon exhaling) into the solution until the solution turns pink.

Results:

1. Record the time required for solution to turn pink.

2. Prove that the solution is acidic.

Procedure: Formation of Calcium Precipitate

1. Using a straw of 1 ml. pipete, bubble air into a 100 ml. of lime water solution.

2. Carbon dioxide from your exhaled air will combine with the calcium to form a precipitate. Write a chemical equation to illustrate this reaction.

Pulmonary Pressures

Introduction: During inspiration the thoracic cavity enlarges. When there is a increase in volume there is an accompanying decrease in pressure. It is this decrease in pressure or negative pressure which pulls air into the lunga.

During expiration the exact opposite effects occur. There is an increase in alveolar pressure which pushes air out of the alveoli into the atmoaphere.

Procedure:

1. Attach pneumograph around the chest. The pneumograph is a rubber bellows which when fastened securely around the chest, will produce preasure changes which are created by the breathing pattern.

2. The pneumograph will be attached to a mercury manometer by a plastic or rubber tubing.

3. In order to trap the proper amount of air in the pneumograph:
   a. Open the exhaust valve on the manometer.
   b. Have the student take ½ of his normal inspiration and hold his breath.
   c. Close the exhaust valve on the manometer. Now the student may breath normally.

4. Obtain a base line on the ink-writing Kymograph. To do this stop breathing (hold breath) in a semi-relaxed state.

5. Mark the base line as 0 degrees or 760 mm. Hg.

6. Any decrease in the graph is illustrating a decrease in pressure and when the graph goes higher illustrates an increase in pressure.

7. Write the type of activity and record the exact pressure on the kymograph recording.

8. Note: Each division on the manometer scale is mm. Hg. and the difference between the numbers is 10 mm. Hg. or 1 centimeter of Hg. Be careful to make all recordings in mm. Hg.

Results: Record all data in millimeters of mercury (mm.Hg.)

ACTIVITY		Trial 1	Trial 2	Trial 3	Average
Normal Breathing	Base Line Recording				
	Inspiration				
	Expiration				
Deep Breathing	Forced Inspiration				
	Forced Expiration				
Vigorous Exercise	Lowest Inspiration Pressure				
	Greatest Inspiration Pressure				

LABORATORY INVESTIGATION 11B Chemical Respiration

Objectives:

1. To determine if the presence of sugar in the mouth increases the amount of bacteria.
2. To determine how bacteria obtains energy from carbohydrates.
3. To ferment lactose sugar with an anaerobic bacteria.
4. To determine the end products of lactose fermentation.
5. To produce alcohol by fermentation of grape juice.
6. To determine the amount of CO_2 that is produced during fermentation.
7. To determine the end product of amino acid metabolism.
8. To observe under the microscope and identify yeast cells and Streptococcus lactis.

Bacteria in the Mouth

Introduction: Many bacteria utilize sugar as their energy source. For this reason it is believed that eating sweets and desserts may cause an increase of bacteria in the mouth. With an increase in food supply bacteria should increase in numbers.

Procedure:

1. Eat a candy bar. Chew the candy well and allow it to remain in the mouth for 2-3 minutes before swallowing. Do not drink any water after eating the candy.
2. Swab the mouth with a sterile cotton swab, especially around the teeth and streak on an agar plate one hour after eating.
3. Another student will gargle immediately after eating and then swab teeth and streak a plate after one hour.
4. Incubate the plates for 24-48 hours at 37°C. Be sure that each plate is labeled correctly.
5. Make a gram stain of each type of colony. Make a diagram of the bacteria.

Results:

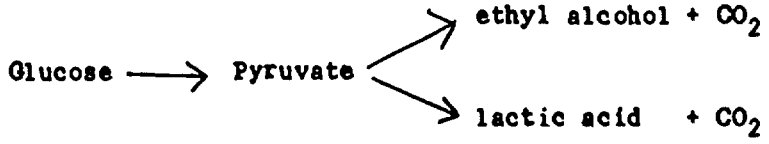
Plate	No. of Colonies	Appearance of Colonies	Gram Reaction	Bacteria Morphology
Gargle				
No Gargle				

Anaerobic Respiration (Fermentation)

Introduction: Respiration is the process by which carbohydrates are broken down for the release of energy. The primary gain to the cell from respiration is the energy provided by the breakage of the bonds in organic molecules. This energy is needed for cell maintenance and growth. The chemical products resulting from the breakdown of organic materials are actually waste products and must be eliminated from the cell.

Glycolysis involves the conversion of simple sugars to two molecules of pyruvate, which is a three-carbon molecule. The process does not require oxygen, and is referred to as anaerobic respiration. The metabolic pathways that follow the formation of pyruvate depend upon the enzymes present in the organism.

The formation of ethyl alcohol or lactic acid indicates the termination of the breakdown of the carbohydrate. Those organisms in which respiration is terminated with the formation of these waste products directly from pyruvate are called anaerobic because they grow in the absence of oxygen.



Procedure: Acid + CO_2 production

1. Fill a Smith fermentation tube with milk.
2. Inoculate with a drop of a 24-hour broth culture of Streptococcus lactis, mix gently.
3. Stopper the fermentation tube with a cotton plug.
4. Incubate for 48-96 hours at 37°C.
5. Test for acid with litmus and measure amount of gas production.

Procedure: Alcohol and CO_2 production

1. Fill a Smith fermentation tube with grape juice or a sugar solution.

2. Inoculate with a drop of yeast suspension (Saccharomyces cerevisiae). One package of Bakers yeast dissolved in 200 ml. of water.

3. Stopper the fermentation tube with a cotton plug. Mix gently.

4. Incubate for 48-96 hours at 37°C.

5. Measure the amount of gas production.

6. How can you determine if alcohol is formed?

Results:

Sugar	Organism	Amount of CO_2 Produced	Acid or Alcohol

Procedure: For microscopic examination of organisms causing fermentation.

1. Stain the bacteria causing lactose fermentation with a single stain. Examine under oil immersion and make a drawing of the organism.

2. With the yeast suspension make a wet mount and examine under high power. Make a diagram of a yeast cell.

Production of Ammonia

Introduction: The accumulation of ammonia in cultures of heterotrophic bacteria results from the deaminization of amino acids. Amino acids must first be deaminized prior to utilization for energy.

In the absence of a protein sparing action, amino acids are utilized for both structure and energy. Since more carbon is required for energy than nitrogen for structure, ammonia will accumulate in the medium. In the presence of a fermentable carbohydrate, a protein - sparing action may occur in which case the organisms utilize the amino acids for structure but not for energy.

Procedure:

1. Inoculate a tube of beef extract broth with one loopful of a 24 hour broth culture of E. coli or B. subtilis.
2. Incubate the broth at 37°C for 48 hours.
3. Remove the stopper from the incubated culture and heat in boiling H_2O for 5 minutes.
4. Hold a strip of litmus paper against the inside of the tube while heating the culture. Do not allow the broth to touch the litmus paper.
5. Write a chemical equation to illustrate the formation of ammonia from protein.

Results:

1. What effect would the presence of glucose have on ammonia production?
2. In the presence of a carbohydrate, under what conditions would you get (A) fermentation, (B) putrefaction?

LABORATORY INVESTIGATION 1C Skin and Mucous Membrane

Objectives:

1. To locate and identify the epidermis, the dermis, and the subcutaneous layers of the skin.

2. To differentiate between the stratum corneum and the stratum germinativium of the epidermis as to structure, function, and location.

3. To differentiate between the papillary and reticular layers of the dermis as to structure, function, and location.

4. To locate and identify accessory organs of the skin.

5. To compare and contrast the skin with the mucous membrane.

6. To list no less than six functions of the skin and explain each function.

Skin and Mucous Membranes

Introduction: Mucous membrane lines the cavities of the body which open to the exterior, such as the lining of the mouth and entire digestive tract, the respiratory passages, and the genitourinary tract. It consists of a surface layer of epithelial tissue over a deeper layer of connective tissue.

The skin or cutaneous covers the surface of the body and consists of two main layers: the surface epithelium, or epidermis, and the lower connective tissue layer, the dermis. Beneath the dermis is a looser connective tissue layer, the superficial fascia. The accessory organs of the skin consists of hair, nails, and sweat glands.

Procedure:

1. Examine under the microscope, a slide containing the cross-section of skin. Make a diagram showing the layers. Label the three layers: epidermis, dermis, subcutaneous.

2. The epidermis is composed of four layers; stratum corneum, stratum lucidum, stratum granulosum, and stratum germinativum. Label these layers.

3. The surface of the dermis is fused with the epidermis. This layer is called the papillary layer. The deeper layer is called the reticular layer. Label these layers.

4. Make a large drawing of each of the following accessory organs of the skin: Label the main structures.
   a. Hair Follicle
   b. Sweat Glands and Sebaceous Glands
   c. Nerve Endings

5. Observe the inner most lining of a section of the esophagus and identify the mucous membrane.

Results: List at least six functions of the skin

List Major Functions	Explain How They Are Accomplished
1.	
2.	
3.	
4.	
5.	
6.	

LABORATORY INVESTIGATION 2C Kidney Dissection and Urine Formation

Objectives:

1. To locate and identify the internal structures of the kidney.
2. To locate and identify the major blood vessels comprising the vascular system of the kidney.
3. To trace the pathway from urine formation to urine excretion by the kidney.
4. To identify and explain the function of each structure of the nephron.

Dissection of the Kidney

Introduction: The kidneys are located in the posterior part of the abdominal cavity, one on either side of the vertebral column, behind the peritoneum. The kidney presents a bean-shaped appearance. The lateral border is convex and the medial is concave. In the central portion of the concave border there is a deep longitudinal fissure where the vessels and nerves enter and leave and from which the ureter decends toward the urinary bladder.

Procedure:

1. Expose the interior of a preserved kidney by making a longitudinal incision through it.

2. Make a diagram of the longitudinal section of a kidney illustrating the following: Label all structures.
    a. Renal Cortex
    b. Renal Medulla
    c. Renal Pelvis
    d. Ureter
    e. Calyx
    f. Pyramid

3. Make another large diagram of the longitudinal section of a kidney. Diagram the renal vascular system. Label the following blood vessels:
    1. Renal Artery
    2. Interlobar Artery
    3. Arcuate Artery
    4. Renal Vein
    5. Interlobar Vein
    6. Arcuate Vein
    7. Glomerulus

Nephron

Introduction: Urine is formed from the blood plasma by the nephrons. They remove material from the blood plasma that are present in excess, while conserving those substances that are present in normal or subnormal quantities. There are over one million nephrons in each kidney.

Procedure:

1. Examine a section of kidney tissue under the microscope. Locate and identify the glomerulus. Make a large drawing of a single glomerulus.

2. Identify the major parts of the nephron:
   a. Glomerulus
   b. Bowman's Capsule
   c. Tubles

3. Make a large diagram of a nephron and label the following parts listed below.
   a. afferent arteriole
   b. efferent arteriole
   c. glomerulus
   d. proximal convoluted tubule
   e. loop of Henle
   f. distal convoluted tubule
   g. collecting tubule

Results:

Structure	Function
Afferent Arteriole	
Efferent Arteriole	
Glomerulus	
Brush Border	
Bowman's Capsule	
Proximal Convoluted Tubule	
Distal Convoluted Tubule	
Collecting Tubule	

LABORATORY INVESTIGATION 3C Urinary Sediment and Excretory Passages

Objectives:

1. To identify the excretory passages which carry the urine.

2. To compare and contrast the structure of the ureters with the urethra.

3. To separate the solid particles of the urine from the liquid part.

4. To locate and identify solid material in the urine.

5. To differentiate among the various solid particles of the urine.

Excretory Passages

Introduction: The excretory passages carry the urine from the kidney to the outside of the body. The walls of the excretory passages are composed of a outer layer of smooth muscle. The contractions of this muscle move the urine forward. The inner surface is lined with a mucous membrane.

Procedure:

1. Observe under the microscope and make a large diagram of the following:
    a. Ureter (cross-section)
    b. Urethra (cross-section)
    c. Urinary Bladder (section)

2. Label the following layers where appropriate on the above drawings.
    a. Musosa
    b. Logitudinal muscle
    c. Circular muscle
    d. Adventitia

Microscopic Examination of Urine Sediment

Introduction: The sediment of urine should be examined under a microscope in a complete urinalysis. In this examination such solids as white blood cells, red blood cells, crystals, casts (protein formations), bacteria, and epithelial cells can be found.

Procedure:

1. Fill a test tube approximately ½ full of urine.
2. Centrifuge for 5 minutes. (Caution: Be sure the centrifuge is balanced before operating it.)
3. Using a disposable pipete, remove the clear urine on top. Resuspend the sediment on the bottom by shaking the tube. Add 1-2 drops of stain (Sedi-stain) to the sediment in the tube.
4. Place a drop of the stained urine sediment on a microscopic slide; cover with a cover slip.
5. Examine microscopically using medium and high power.
6. Make a large drawing of each type of solid observed.
7. Record the kind and amount of solid particles.

Results:

Type of Sediment	Amount Present (per drop of urine)	Description (color and shape)
White Blood Cells		
Red Blood Cells		
Epithelial Cells		
Casts		
Bacteria		
Trichomonas vaginalis		

STAINED URINARY SEDIMENT

ELEMENTS IN URINARY SEDIMENT	USUAL DISTINGUISHING COLOR OF STAINED ELEMENTS	
1. Red Blood Cells	Pink to Purple	
2. White Blood Cells	Nucleii Purple Colorless or light blue	Cytoplasma Purple granules or Pale Blue
3. Renal Tubular	Dark Shade of blue-purple	Light purple or blue-purple
4. Bladder Tubular Epithelial Cells	Blue-purple	Light purple
5. Squamous Epithelial Cells	Dark shade of orange-purple	Light purple or blue
6. Hyaline Casts	Pale pink or pale purple	
7. Coarse Granular Inclusion Casts	Dark purple granules in purple matrix	
8. Fat Inclusion Casts	Fat globules unstained in pink matrix	
9. Blood (Hemoglobin) Casts	Orange-red	
10. Bacteria	Motile: Don't stain Non-Motile: Stain purple	
11. Trichomonas vaginalis	Light blue-green	
12. Mucous	Pale pink or pale blue	

LABORATORY INVESTIGATION 4C Urinalysis

Objectives:

1. To perform and interpret the results of a screening test on urine using combistix and Ketostix color indicators.
2. To determine the presence or absence of protein in the urine.
3. To explain possible reasons for protein in the urine.
4. To determine the amount of glucose in the urine.
5. To determine the amount of bilirubin in the urine.
6. To measure the specific gravity of urine using a urinometer.
7. To explain the reasons for variation in the specific gravity of urine.

Urinalysis

Introduction: Most labs will routinely screen for the normal urine contents by using Combistix strips (screening indicators available at any drugstore). These strips are ultrasensitive. If the test is negative, no further test is necessary. If, however, the test proves positive for either sugar or albumin, further tests are required. Run all test regardless of screening test results.

Procedure: A. Screening Test

1. Collect a specimen of urine in a wide-mouthed bottle.
2. Fill a test tube 1/2--2/3 full of urine.
3. Dip a Combistix strip completely into the specimen.
4. Remove strip and let stand for one minute.
5. Compare against color chart and record results.
6. Use a Ketostix to test for ketones.

Results: Combistix and Ketostix

Test	Color Change	Amount Present
pH		
Glucose		
Protein		
Ketones		

B. Urine Albumen Test

Introduction: When kidney tissue has been damaged, it becomes permeable to colloidal solutions and less permeable to many of the substances that it normally excretes. Albumen will then appear in the urine. The presence of albumen in the urine, however, does not always indicate the destruction of kidney cells.

Procedure: Protein Test

1. Fill a test tube 1/3--1/2 full of urine.
2. Add 5 drops of dilute acetic acid, then heat the urine in a water bath.
3. Protein will precipitate out upon heating.

C. Urine Sugar Test

Introduction: A positive result on a urine sugar test could be an indication of diabetes. Benedict's solution can be used to determine the amount of sugar in the urine.

Color of Solution	Amount of Sugar
Blue	Negative
Greenish-yellow precipitate	.5 - 1%
Yellow precipitate	1 - 2%
Orange-red precipitate	more than 2%

Procedure: Glucose Test

1. Fill a test tube ¾ full of urine.
2. Add 5 ml. of Benedict's solution. Mix well using a clean glass stirring rod.
3. Place in a boiling water bath for 5 minutes or until a color change is observed.
4. Record results.

Urine Bilirubin Test

Introduction: The Franklin Bilirubin test is a simple practical test for urinary bilirubin. It can be used to help diagnose acute infectious hepatitis before clinical jaundice is manifested.

Procedure:

1. Add 10 drops of urine onto a test tablet.
2. Add 2 drops of Fouchet's reagent.
3. Match with color chart and read results directly.

E. Specific Gravity of Urine

Introduction: The specific gravity of normal urine varies ordinarily between 1.015 - 1.025. The specific gravity gives an indication of the amount of solids in the urine.

For determining specific gravity, the urinometer is used. This is sufficiently accurate for clinical purposes. The urinometer is always calibrated for use at a certain temperature.

Procedure:

1. Fill the urinometer jar 2/3 full of urine.
2. Carefully lower the hydrometer float into the urine.
3. Specific gravity can be read directly. Note: Be sure to take the reading at eye level.

Results: Record the expected values for normal urine in the third column.

	Student's Urine	Unknown #1	(Normal Urine) Expected Results
Volume			
pH			
Specific Gravity			
Sugar			
Protein			
Bilirubin			
Ketone Bodies			
Solids			

LABORATORY INVESTIGATION 5G Disease Organisms of the Genitourinary Tract

Objectives:

1. To observe and identify the organism which causes gonorrhea.
2. To locate and identify Neisseria gonorrhoeae on a stained smear of urethral exudate.
3. To identify the organism which causes syphilis.
4. To list mode of infection, diagnosis, stages of infection, and treatment for venereal diseases.
5. To observe and identify organisms which are common agents of urinary tract infection.
6. To identify and diagram the life cycle of the body louse.

Venereal Disease Organisms

Introduction: Neisseria gonorrhoeae is the organism causing gonorrhea. It is a diplococcus that is gram-negative, non-motile and non-spore - forming. In smears, the opposing sides of the two cocci are flattened. The cocci are observed with their flat sides together. Each pair is surrounded by a capsule. Gonorrhea is a disease accompanied by a discharge from the genital tract. During the early stages the gonococci are found free in the serous exudate or attached to epithelial cells, but when the exudate becomes purulent, phagocytosis takes place and the gonococci are found within the cytoplasm of the pus cells (leukocytes). A single white blood cell may contain from 20 to 100 microorganisms. These gonococci are not dead and are infectious.

N. gonorrhoeae is a strictly human pathogen. It causes not only gonorrhea, but also it is the principla cause of acute infectious conjunctivitis in newborn infants.

Procedure:

1. Examine a prepared slide of gonococcus. Make a large diagram of the organism.

2. Examine a stained smear of gonorrhea exudate. Make a large diagram of the phagocytic cells containing gonorrhea. Label the structures.

Introduction: Syphilis is an infectious disease caused by Treponema pallidum. It is a spirochete bacterium which is a motile, slender, corkscrew-like organism. It can be found in practically every syphilitic lesion. The spirochete has six to fourteen spirals which may bend back upon itself.

The laboratory has at its disposal two important procedures applicable to the diagnosis of syphilis: (1). Demonstration of the organisms in the lesions and (2). Serological tests such as the complement fixation test (Wassermann Test).

Procedure:

1. Examine a prepared slide of the Treponema pallidum.
2. Make a large diagram of the disease organism that causes syphilis.

Results: Data Sheet

Disease	Gonorrhea	Syphilis
Organism		
Modes of Infection		
Stages of Infection		
Diagnosis		
Treatment		
Immunity		

Urinary Tract Infections

Introduction: Urinary tract infections frequently occur as a result of urologic procedures in the hospital. These include urethral and ureteral catheterizations and the use of catheters for continuous bladder drainage.

In many cases, infection is introduced by faculty technique, by inadequately sterilized or disinfected equipment, or by unwashed hands.

Procedure: Most common agents of urinary tract infection:

E. coli
Staphylococcus aureus
Streptococcus faecalis
Pseudomonas aeruginosa
Streptococcus viridans
Trichomonas vaginalis

1. Observe prepared slides of the above organisms and make a large diagram of each.

2. List the morphology and normal habitat of each organism.

Body Lice

Introduction: Phthirus pubis is a body louse which frequents the pubic hairs and perianal regions of man (and women). The adult louse is unable to survive for longer than a day when removed from its host. This louse causes more annoyance than actual physical harm. Spread of the organism is mostly through close body contact.

Procedure:

1. Observe the organism under the low power and make a diagram.

2. Where does the louse deposite the eggs?

LABORATORY INVESTIGATION 6C Male Reproductive System

Objectives:

1. To identify the internal structure of the testis.
2. To locate and identify the sperm producing cells of the testis.
3. To compare and contrast the structure and function of the seminiferous tubule with the epididymis.
4. To locate and identify sperm from a semen smear.
5. To identify and indicate where the chromosome, mitochondria, and cytoplasm are located in a sperm cell.
6. To compare and contrast the vas deferens with the epididymis.
7. To discuss the structure and function of the prostate gland and seminal vesicles.
8. To identify the internal structure of the penis.
9. To compare and contrast the various types of tissue which compose the structure of the penis.

Male Reproductive System

Introduction: The reproductive system is composed of structures whose function is to produce a new individual. The male provides the sperm and the female the egg. Combination of a single sperm and a single ovum forms a fertilized egg (zygote) which will develop into an embryo. The male reproductive system is composed of glands, ducts, and supporting structures.

Procedure:

1. Testis: The testis is composed of lobules. Each lobule is composed of seminiferous tubules separated by the interstitial cells of Leydig. Diagram the internal structure of a testis. Label the following parts:
   a. lobule
   b. seminiferous tubule
   c. cells of Leydig
   d. epididymis

2. Seminiferous tubule: Sperm is produced in the seminiferous tubules of the testis. Observe under the microscope a cross-sectional view of a seminiferous tubule. Make a diagram and label the following structures:
   a. spermatogonia
   b. primary spermatocyte
   c. secondary spermatocyte
   d. spermatid

3. Sperm: Observe a prepared semen smear under the high power and oil immersion objective. Make a large diagram of one sperm and label the head and tail.

4. Epididymis: The seminiferous tubules unite at the upper end of the testis in a series of ducts which pass through the testis and form a convoluted mass of coiled ducts called the epididymis. It is attached to the posterior surface of each testis and is greatly convoluted.

Observe under the microscope the cross-section of the epididymis. Make a diagram of the duct. Label the parts. How does the structure compare to the seminiferous tubules?

5. Vas deferens: This is a single duct which leads from the epididymis to the urethra. Observe under the microscope and make a diagram of the cross-sectional view of the vas deferens. Label the structure. How does it compare to the structure of the epididymis?

6. Accessory Glands: Observe under the microscope a section of each gland. Make a diagram of each and label the structures.
   a. Prostate
   b. Seminal Vesicle

7. Penis: The penis is formed by three cylindrical bodies of erectile tissue: two corpora cavernosa and the corpus cavernosum urethrae which surrounds the urethra.

   Each of the cavernous bodies is surrounded by a fibrous membrane, the tunica albuginea. The substance of the cavernous bodies is a network of large venous sinuses.

   Diagram the cross-sectional view of the penis and label the following structures:
   a. corpora cavernosa
   b. corpus cavernosum urethrae
   c. urethra
   d. tunica albuginea
   e. smooth muscle fibers

LABORATORY INVESTIGATION 7C Female Reproductive System

Objectives:

1. To locate and identify each of the following structures on the ovary: primary follicle, developing follicle, mature follicle with ovum, corpus luteum, and corpus albicans.

2. To differentiate among the three layers of the fallopian tube.

3. To describe how the mature ovum is transported from the ovary to the uterus.

4. To identify the layers of the uterus.

5. To list the stages of endometrium during the menstrual cycle.

6. To locate and identify changes in the endometrium during the various stages.

7. To calculate the thickness of the endometrium during each phase.

8. To list the hormones released during the menstrual cycle.

Female Reproductive System

Introduction: The human female reproductive system is composed of two ovaries, two fallopian tubes, a uterus, and a vagina. The associated structures include the external genitalia and mammary glands.

Procedure:

A. Ovary: The ovaries are responsible for the production of ova and the secretion of hormones. The surface of the ovaries consists of a single layer of epithelial cells. The interior is composed of connective tissue in which are embedded thousands of follicles. After puberty, the follicles increase in size and become Graafian follicles. After reaching a certain size, the Graafian follicle ruptures and the ovum is released. The cells of the ruptured follicle give rise to a yellow mass, the corpus luteum.

    1. Locate, on prepared slides of sections of ovaries, and diagram each of the following structures:
        a. primary follicle
        b. developing follicle
        c. mature follicle
        d. corpus luteum
        e. corpus albicans

B. Fallopian Tubes: The fallopian tube (oviduct) is composed of three layers: (1) mucosa which is ciliated, (2) smooth muscle, and (3) serosa. At the upper end, the fallopian tube expands into a funnel-like portion known as the infundibulum. This enables the ovum to enter the tube since the ducts are not actually connected to the ovaries.

    1. Diagram and label the layers of the fallopian tube (cross-sectional view).

C. Uterus: The uterus is a hollow, muscular organ which resembles an inverted pear. The upper portion of the uterus is called the body, and the lower constructed portion is called the cervix.

The wall of the uterus is composed of three layers: (1) the peritoneum, or serosal covering, (2) myometrium, consists of smooth muscle, and (3) the endometrium.

1. Locate on prepared slides and diagram the endometrium at various stages of the menstrual cycle.
    a. menstrual phase
    b. proliferative phase
    c. time of ovulation
    d. secretory phase
    e. premenstrual phase

D. Menstruation and the Endometrial Cycle: The endometrium undergoes cycles structural changes in which the uterus is being prepared for fertilization of the ovum and pregnancy.

1. Summarize the information in the chart.

2. Identify the pases or stages during the various days of the cycle.

3. Indicate the average thickness (in millimeter) of the endometrium during each phase.

4. List the functional activity of the endometrium during each phase (purpose).

5. List the hormones that are being released into the blood stream during each phase of the cycle.

Data: Summary of changes in the Endometrium during a 28 day menstrual cycle.

Day	Phase (stages)	Endometrium Thickness (mm.)	Function Activity	Hormones Released
1-5				
6-13				
13-15				
15-18				
19-25				
26-28				

LABORATORY INVESTIGATION 8C Cell Division

Objectives:

1. To locate and identify cells during each stage of mitosis.
2. To explain the location and function of the chromosomes during each stage of mitosis.
3. To tabulate the number of cells undergoing each stage of mitosis.
4. To calculate the amount of time required for a cell to complete each stage of mitosis.

PART I - Mitotic Cycle of Plant Cells

Introduction: Cells increase in number by cell division in which one cell divides and gives rise to two new cells. Nuclear division is known as mitosis and division of the cytoplasm is known as cytokinesis. In unicellular organisms, each cell is capable of division and is therefore able to give rise to two daughter cells, resulting in an increase in the size of the population. In multicellular organisms, special embryonic cells are capable of division and produce a number of different kinds of cells.

The mitotic cycle is made up of a series of stages that are arbitrarily established for convenience of observation. Since the genetic factors are contained on the chromosomes, the stages are based on chromosomal behavior.

Interphase: The chromosomes are preparing for division. The cell is growing and undergoing metabolic processes.

Prophase: The chromosomes condense and become visible. The disappearance of the nucleolus and distegration of a nuclear membrane mark the end of prophase.

Metaphase: The chromosomes line up along the central portion of the cell. Spindle fibers stretch from the centromere of the chromosome to one of the poles.

Anaphase: The chromosome separate and each newly formed daughter chromosomes migrate toward opposite poles.

Telophase: Each of the daughter chromosomes reach its respective pole and the new chromosomal complement become tightly packed together. A new nuclear membrane is formed around each group of chromosomes. The chromosome number remains constant for each organism.

Procedure:

1. Examine a prepared slide of allium (onion) root tip under low power (longitudinal view). Note: More cells undergoing division will be visible in the longitudinal view than in a cross-sectional view.

2. Locate the region of cell division. This region is located near the tip of the root just above the root cap region.

3. Locate and identify a cell in each stage of mitosis. Make a large diagram of each stage while observing with the oil immersion lens. Label each stage and label the parts of the cell.

II. Length of Time Required for each Stage of Mitosis.

1. The relative length of time required for the completion of each stage is directly correlated with the number of cells observed in the various stages. Given the additional information of how long the cycle takes, the time sequence for each of these stages can be calculated.

2. The mitotic cycle generally takes about one (1) hour or 60 minutes. This is just an approximation. On the basis of an hour cycle, work out the approximate time in minutes that is spent in each stage.

   Example: There were 40 cells observed in prophase out of 200 cells counted.

   $$\frac{\text{Cells counted per stage}}{\text{total cells counted}} \times 100 = \% \times \text{time}$$

   $$\frac{40}{200} \times 100 = 20\% \times 60 \text{ min.} = 12 \text{ minutes}$$

3. Choose a single field in the zone which seems to have been actively dividing at the time the slide was prepared.

4. Observe this field under high power (43X) and count the number of cells in each stage. You should count a total of several hundred cells. You may have to move the slide around in order to view several hundred cells. Observe several different viewing fields under the microscope.

5. Record your individual results. Compare them to the total class results.

Results:

DATA SHEET

Individual Data	No. Cells	Percent Cells	Time (min.)
Interphase			
Prophase			
Metaphase			
Amaphase			
Telophase			
Total			

Class Data	No. Cells	Percent Cells	Time (min.)
Interphase			
Prophase			
Metaphase			
Amaphase			
Telophase			
Total			

LABORATORY INVESTIGATION 9C Effects of Radiation Upon Bacteria

Objectives:

1. To determine the effects of UV-irradiation upon bacteria.

2. To locate and identify gross, microscopic, and chemical changes which occur to bacteria after UV-irradiation.

3. To measure the frequency of mutation.

4. To explain variations in color of bacteria colonies.

5. To explain variations in colony color of E. coli growing of EMB agar.

Effects of Irradiation

Introduction: Living cells are very sensitive to damage by radiation. Such damage may find expression in gross harm and death to the organism exposed or it may be transmitted as a hereditary change to the offspring (Mutation).

Ionizing radiation can disrupt any chemical bond in any molecule of any cell that happens to absorb radiant energy. Depending upon which molecules are disrupted will determine the specific effects upon the cell.

Procedure: Pigment Synthesis in Serratia marcesans and Sarcina lutea.

1. Using an inoculating loop, streak an agar plate from a broth culture of Serratia marcesans and a second plate with Sarcina
2. Be sure to flame (heat sterilize) the loop before and after using it.
3. Expose the plate to ultraviolet (UV) light for 15 minutes. Be sure to remove the top cover; UV light will not penetrate glass or plastic. Cover ½ of the dish with an opaque covering. The covered part will serve as a control.
4. After irradiation replace the top cover and incubate for 48-96 hours. Label the petri dish on the bottom to indicate the control and irradiated sections.
5. After incubation, observe for variation in color types.
6. Make a stained smear of each colony type and make a diagram of each type of bacteria.

Effect of Irradiation on E. coli

Introduction: When E. coli is placed on EMB agar (Eosin-Methylene Blue) it takes up the dyes and the colonies become purple to red in color. This is due to its ability to ferment lactose by means of an enzyme β-galactosidase. In the absence of the enzyme, lactose cannot be broken down. As a result, the cell will give rise to a white colony.

Procedure:

1. Using an inoculating loop, spread a loopful of E. coli broth culture evenly over the agar surface. Be carful to heat (flame) sterilize the loop before and after use.

2. Expose the plate, uncovered, to UV rays for 15 minutes. Immediately after exposure to radiation remove the plate and replace cover. Incubate for 48-96 hours. Cover ½ of the plate to serve as a control. Be sure to label the control and irradiated sections.

3. Make a stained smear of each colony type and make a diagram of each type of bacteria.

Serratia marcesans

	Color of Colony	Gram Reaction	Morphology	Change or Alteration with UV-light
Control				
Irradiated				

E. coli

	Color of Colony	Gram Reaction	Morphology	Change or Alteration with UV-light
Control				
Irradiated				

Sarcena lutea

	Color of Colony	Gram Reaction	Morphology	Change or Alteration with UV-light
Control				
Irradiated				

LABORATORY INVESTIGATION 10C — Inducing Ovulation - Frog Egg Development

Objectives:

1. To induce ovulation in the frog by injecting pituitary extract.
2. To inseminate frog eggs with a viable sperm suspension.
3. To differentiate between the fertilized and unfertilized eggs.
4. To observe and identify stages of development from the fertilized egg to the multicellular stage.

Inducing Ovulation in Female Frogs

Introduction: In vertebrates the pituitary gland influences the activity of the gonads by means of hormones called gonadotrophins. In the female frog, the seasonal changes of spring stimulate the pituitary to secrete large quantities of gonadotropins into the bloodstream. These hormones cause the eggs to be released into the oviducts. After these eggs are laid, the ovaries build up a new supply of eggs during the summer. When the female frog goes into hibernation in the autumn, the eggs to be laid the following spring need only the stimulus of gonadotropin to be released from the ovaries. Therefore, if gonadotropic hormones are injected into the female frog during the winter, eggs can be obtained.

Artificial increase of the pituitary level can be produced by the injection of whole frog pituitaries or a pituitary suspension. This causes normal ovulation and the eggs can be stripped from the uterus with mechanical pressure.

Procedure:

A. Inducing Ovulation

1. Inject either the pituitaries or pituitary extract intraperitoneally into a female frog. It is best to inject through the wall of the lower abdomen, directing the needle anteriorly. Avoid damage to the ventral abdominal and lateral veins and the internal organs. The number of pituitary glands required to induce ovulation varies with the season. In October, six (6) pituitaries should be injected. The number injected may be decreased 1 per month thereafter to a minimum of two (2) pituitaries. Return the injected female to an individual container and leave for 48 hours at room temperature. Be sure to label - name, date, etc.

B. Insemination of Eggs

1. Forty-eight hours after injection, the female should be ready to ovulate. A test should be made for the presence of eggs by stripping the female.

2. The female frog is held firmly in the palm of the hand while the legs are held in the other hand. A firm pressure with a milking motion is applied to the abdomen with the thumb to force the eggs from the uterus. With a firm steady milking motion, the eggs will soon appear. They should be milked into a clean culture dish into which the sperm suspension has been placed.

3. To prepare the sperm suspension, pith the male frog and dissect out the testes. These are fairly large oval yellowish bodies on the dorsal surface of the peritoneal cavity near the posterior end of the kidneys. The testes should be macerated thoroughly in about 10 cc. of pond water. The sperm will become active about 5 minutes after they are released from the testes. Prepare a wet mount of sperm suspension and observe the sperm for movement.

4. Pipette the sperm suspension over the eggs so that they will be exposed. Bathe the eggs in the sperm suspension.

5. Allow the inseminated eggs to stand for about 5 minutes, and add about 50 cc. of pond water. In 20 minutes change the water, adding enough to just cover the eggs.

6. If the eggs are fertilized, they will rotate so that the animal hemisphere (dark side) is up in about one (1) hour.

7. As soon as the jelly membrane has enlarged, the eggs may be separated. They should be distributed into culture dishes in lots of about 25 to 30 eggs each. Add pond water to the dish to a depth of about ½ inch. This should be done before the first cleavage. If the eggs are too crowed abnormalities will begin to appear at about the third cleavage.

8. Locate and identify all the developmental stages from the fertilized egg stage - to - the gastrula. Make a large drawing of each stage. Compare the living stages to the preserved ones.

Development Begins with a Series of Cell Division Called Cleavage

1. The Fertilized Egg - The animal hemisphere appears dark because of the presence of pigment granules in this region. The lighter vegetal hemisphere contains yolk. The beginning of the cleavage furrow can be seen.

2. Two-Cell Stage - The first division occurs within two to three hours after fertilization.

3. Four-Cell Stage - The second division follows quickly and at right angles to the first division.

4. Eight-Cell Stage - The third division occurs horizontally to the first and second division.

5. Late Cleavage - Many divisions now occur and the plane of divisions becomes irregular. The individual cells are smaller, but the total size of the developing individual is no larger than the single fertilized egg. Growth in size has not begun. The cells in the animal hemisphere are smaller and are called micromeres. The larger ones in the vegetal hemisphere are macromeres.

6. Blastula - A hollow ball of cells is formed as a result of the continuing cell divisions. It contains a fluid-filled cavity, the blastocoel. This stage in development is called the blastula.

7. Gastrula - The groove continues to fold toward the inside to form a cavity, the archenteron, which later forms the gut of the embryo.

Approximate Rate of Development at 20-25 C° (68-77° F)	
Fertilization	1 hour
2 cells	2 hours
4 cells	2.5 hours
8 cells	4.5 hours
16 cells	5.5 hours
32 cells	5 hours
Crescent Blastula	21 hours
Yolk Plug	36 hours

LABORATORY INVESTIGATION 11C Endocrine Glands

Objectives:

1. To locate and identify the thyroid and parathyroid gland in the white rat.
2. To identify the type of tissue comprising the thyroid and parathyroid glands.
3. To locate and identify the adrenal glands in the white rat.
4. To differentiate between the medulla and cortex of the adrenal glands.
5. To locate and identify the pancreas in the white rat.
6. To locate and identify the islets of Langerhans.
7. To differentiate between the type of tissue in the pancreas.
8. To locate and identify the gonads in the white rat.
9. To locate and determine where sex hormones are produced in the gonads.
10. To observe the pituitary gland.
11. To differentiate between the anterior and posterior pituitary gland.

Endocrine Glands

Introduction: The endocrine system consists of a group of glands that release their secretions directly into the blood stream. The secreations, known as hormones, exert regulatory actions on the entire body.

Thyroid and Parathyroid Glands: The thyroid gland consists of two large lobes which are connected by the isthmus. The isthmus lies across the anterior surface of the trachea.

The parathyroid glands are composed of four (4) small round bodies attached to the posterior surface of the lateral lobes of the thyroid gland.

Procedure:

1. Dissect out the thyroid gland on the rat.
2. Remove the gland and observe it under the dissecting microscope. Locate the parathyroid glands attached to the thyroid.
3. Observe under the high power objective a prepared slide of a section of thyroid. Make a diagram of the type of tissue and label.

Adrenal Glands: The adrenals are two small masses of tissue lying above the kidney. The gland is composed of two distinct parts: (1) cortex, outer layer; and (2) medulla, inner layer. These two parts differ in both structure and function.

Procedure:

1. Dissect out the adrenal gland on a rat.
2. Remove one gland and make a sagittal section, exposing the interior.
3. Observe under the dissecting microscope and identify the cortex and medulla.
4. Observe under the high power objective a prepared slide of a section of the adrenal gland. Make a diagram of the type of tissue and label.

Pancreas: The pancreas is a long gland that lies directly beneath the stomach. It extends from the duodenum to the spleen. It is composed of two types of tissue: (1) the acini, secrete digestive juices into the intestines and (2) the islets of Langerhans, which secrete insulin.

Procedure:

1. Dissect out the pancreas of the rat. Use the seeker to remove the connective tissue and expose the gland. Be careful in the dissection not to destroy the gland. It is a very soft spongy-like tissue.
2. Observe under the high power objective a section of pancreas. Locate, identify, and make a diagram of the islets of Langerhans.

Gonads: The ovaries and testes both produce gametes and secrete sex hormones.

1. Locate and identify the testes and ovary on a male and female rat respectively.

2. Observe sections of both the testes and ovary under the microscope. Determine which tissue is secreting the hormones and make a diagram of the tissue.

Pituitary: The pituitary gland lies in the sella turcica, which is the saddle-shaped depression in the sphenoid bone. A stemlike portion, the pituitary stalk, attaches the gland to the undersurface of the brain. The pituitary consists of two separate glands: (1) the anterior pituitary and (2) the posterior pituitary. The two glands secrete different hormones and have different microscopic structures.

Procedure:

1. Observe the pituitary gland dissected out of a preserved specimen.

2. Locate and identify the anterior and posterior portion.

3. Observe sections of the gland under the microscope and make a diagram of the type of tissue in both the anterior and posterior pituitary.

LABORATORY INVESTIGATION 1D Organs of Special Sense

Objectives:

1. To determine the taste areas of the tongue.
2. To detect the major tastes.
3. To locate and identify taste buds on the tongue.
4. To determine the sensitivity of various areas of the skin.
5. To locate and identify the parts of the eye.
6. To locate and identify the parts of the inner and middle ear.
7. To differentiate between pitch and amplitude.
8. To determine how the ear detects and hears sounds.

The Taste Sensation

Introduction: There are two major cranial nerves that control the taste sensations of the tongue. The facial nerve receives impulses from the anterior portion of the tongue, the glossopharyngeal from the posterior portion of the tongue.

Four major tastes can be detected by the tongue: (1) sweet (2) salty (3) sour (4) bitter. Certain areas of the tongue are more sensitive to one taste than the others.

Procedure:

1. Dip one clean cotton-tipped applicator into each solution.
2. Touch the applicator to the following regions of the tongue: (a) tip, (b) middle, (c) back, (d) sides.
3. Record the region where most taste sensation can be detected and the region where it is least or absent.
4. Using separate applicators, repeat the above tests with each of the available solutions.
5. Observe taste buds through a section of tongue. Make a large drawing and label the parts.

Results:

Solution	Region of the Tongue	
	Most Intense	Least Intense or Absent
10.% Sucrose Solution		
1.% Salt Solution		
1.% Vinegar		
.1% Quinine		

Two Point Sensitivity

Introduction: The power of adjustment to its environment is an important characteristic of a human being. Specialized nerve endings, called sense receptors, enable a more highly organized form of life to adjust itself effectively to environmental changes. The stimulation of a receptor may evoke a sensation. The result is the impulse from the sensory surface is conveyed through neurons to the cerebrum.

Touch receptors are limited almost entirely to the skin. The stimulus for touch sensations is the distortion of the skin by unequal pressure.

This experiment will measure the two-point sensitivity of five parts of the body. The ends of two toothpicks are applied to the skin simultaneously. If the points of the toothpicks are far enough apart, the subject perceives two points. However, as the points are brought closer together, a place will be reached where the two points are perceived as one.

Procedure:

1. Have the subject seated with his eyes closed during the experiment.
2. The experimentor will then use the ends of two toothpicks for applying two tactile stimuli to the skin simultaneously.
3. Start with the toothpicks far apart (1-2 inches) where the subject perceives two points. As the points are brought closer together, a setting will be reached where the two points are perceived as one. Measure this distance in mm.
4. Obtain the average of three readings and record your data.
5. Observe under the microscope, the pressure receptors in a section of skin.

Results: A. Data Sheet

Area	Distance Between Points of Stimulation			
	Trial 1	Trial 2	Trail 3	Average
1. Fingertip				
2. Palm				
3. Arm				
4. Cheek				
5. Neck				

B. Order of Sensitivity (Most Sensitive First)

Eye Anatomy

Procedure:

1. Examine a diagram of the eye and be able to identify each structure.

2. Make a large diagram of the cross section of an eye and label the following parts.

   (a) Cornea
   (b) Pupil
   (c) Iris
   (d) Lens
   (e) Retina
   (f) Optic Nerve

Ear Anatomy

Procedure:

1. Examine a diagram of an ear and be able to identify each structure.

2. Make a large diagram of the middle and inner ear and label the parts.

   (a) Ear Canal
   (b) Eardrum
   (c) Semicircular Canal
   (d) Cochlea
   (e) Eustachian Tube
   (f) Anvil, Hammer, Stirrup

Physiology of Hearing

Introduction: Sound waves in the air enter the external auditory canal. The vibrations strike against the tympanic membrane, setting it in vibration. The loudness or amplitude is determined by the height of the sound wave. The pitch is determined by the frequency of the vibration.

Procedure:

1. Each tuning fork has a certain pitch. It doesn't matter how hard you hit the tuning fork, the pitch will remain the same.

2. What are the lowest and highest rates of vibrations that the human ear hears?

3. What is sound and how is it produced?

4. What is the difference between pitch and loudness?

LABORATORY INVESTIGATION 2D The Skeletal System

Objectives:

1. To locate, identify, and name the bones on a human skeleton.
2. To describe the function of each group of bones.
3. To determine the composition and internal structure of long bones.
4. To observe and identify the composition of bone tissue.

Bone Tissue

Introduction: Bone tissue is composed of deposits of inorganic salts in an organic matrix. The two most abundant salts are calcium phosphate and calcium carbonate. The organic materials include fibers embedded in a matrix containing protein (collagen), bone cells, blood vessels, and cartilaginous substances.

Procedure:

1. Make a large diagram of the longitudinal section of a long bone. Label the marrow cavity, compact bone, spongy bone, and periosteum.

2. Examine a prepared slide of bone marrow under the microscope. Make a diagram and label the parts. What is the composition of bone marrow?

3. Examine a prepared slide of bone tissue. Label the Haversian canals. What makes up the Haversian system? Label all parts.

Bones of the Skeleton

Introduction: The human skeleton consists of two major divisions: (1) the axial skeleton composed of the bones which form the upright part or axis of the body and (2) the appendicular skeleton, composed of bones which attach to the axial skeleton as appendages.

Procedure:

1. Learn the names and number of bones in each division of the skeleton.

2. As you learn the name of each bone, locate it on your body and on the skeleton available in the laboratory.

3. List the name of each bone on the chart provided. Include the number and a brief description of each group of bones.

I. Axial Skeleton - 80 Bones

Part of Body	Name of Bone	Number (amount)	Description (function)
Skull Cranium Face			
Vertebral Column			
Sternum Ribs			

II. Appendicular Skeleton - 126 Bones

Part of Body	Name of Bone	Number (amount)	Description (function)
Upper Extremities			
Lower Extremities			

LABORATORY INVESTIGATION 3D Connective Tissue and Articulations

Objectives:

1. To locate and identify different types of connective tissue.
2. To differentiate between ligaments and tendons.
3. To locate, identify, and describe various types of cartilage.
4. To compare and contrast adipose tissue with other connective tissues.
5. To compare and contrast the various types of articulations.
6. To describe the function and location of the bursae.

Connective Tissue

Introduction: Connective tissue is found throughout the body. It helps to form the framework of the body and connects organs and various other structures. It is characteristic of connective tissues that the number of cells is minimal, but the intercellular substance is ordinarily abundant.

Fibrous Connective Tissue is a very strong, tough tissue. In tendons and ligaments the fibers are very densely packed together. There is no blood supply to this tissue.

1. Examine under the microscope fibrous connective tissue. Make a diagram and label the cells and fibers. What is the composition of the fibers?

	Location (Area of the Body)	Composition	Function
Tendons			
Ligaments			

Cartilage: There are three forms of cartilage in the body: (1) fibrous, (2) hyaline and (3) elastic. They are distinguished by the character of their fibers and the relative proportions of fibers and matrix.

Hyaline cartilage is the most abundant and most widely distributed type of cartilage. This type of cartilage is found in the embryo which later develops into bone.

1. Examine prepared slides of cartilage under the microscope. Describe the structure. How is cartilage supplied with nourishment?

Type of Cartilage	Location (Where is it Found)	Description
1. Hyaline		
2. Yellow Elastic		
3. White Fibrous		

Adipose tissue is often considered as a type of connective and supporting tissue. It differs from other types, in that it does not form intercellular fibers or matrix. The adipose tissue is specialized for the storage of fat. They form a reserve of food as well as supporting pads of tissue.

1. Examine a prepared slide of adipose tissue under the microscope. Draw and label several cells.

2. Explain the shape and location of the nucleus of each cell.

Articulations and Bursae

Introduction: The bones are joined together to form the skeleton by a series of articulations, the structure of each varies with the degree of movability of the joint. Immovable joints are composed of bones which are fitted together by an interlocking arrangement. In slightly movable joints the bone shape and joint structure make only slight movement possible. Freely movable joints consist of the ball-and-socket type, which is the most freely movable to permit rotary movements. The hinge joints permit movement in two directions.

Bursae are sacs of fibrous connective tissue lined with synovial membrane. They are commonly located at joints to prevent the friction of one surface moving upon another. They are found also between tendons or between muscles and bones, wherever friction is likely to occur.

Procedure: Bursae

1. Make a list of the different kinds of bursae and explain their location and function.

Bursae	Location	Function

Procedure: Articulations

1. Locate and identify the different types of joints. Locate them first on your body and then on the skeleton.

2. Complete the following summary chart for the various types of joints.

Results: Data Chart

I. Immovable Joints

Examples	Bones Involved	What Holds Them Together	What is Found Between the Bones

II. Slightly Movable Joints

Examples	Bones Involved	What Holds Them Together	What is Found Between the Bones

III. Freely Moval Joints

Examples Type of Movement	Bones Involved	at Holds em Together	What is Found Between the Bones

LABORATORY INVESTIGATION 4D Gross and Microscopic Anatomy of Muscles

Objectives:

1. To observe and identify the fine structure of muscle tissue.
2. To differentiate between smooth and skeletal muscle.
3. To differentiate between striated and cardiac muscle.
4. To differentiate between a transverse and a longitudinal section of muscle tissue.
5. To locate and identify the origin and insertion of the major skeletal muscles.

The Muscular System

Introduction: The muscles of the body are responsible for movement. Skeletal or striated muscles cause the skeletal framework to move, as in walking. The heart or cardiac muscle enables the heart to beat and to supply blood to all parts of the body. The third kind of muscle is the smooth muscle which makes up the viscera.

I. Smooth Muscle: Under the microscope, smooth muscle has a characteristic appearance. The individual cells are elongated with a central nucleus. The muscle fibers normally occur in sheets closely packed together, and an entire cell can seldom be seen. Longitudinal sections are best studied at the edge of a band or sheet where the muscle shades off into the surrounding connective tissue, and individual cells may be distinguished.

In transverse section, the smooth muscle cells appear as discs of cytoplasm having various diameters.

Procedure:

1. Examine smooth muscle tissue under the microscope using low and high power. Make a large diagram and label the parts. Specify whether it was a transverse or longitudinal section.

II. Cardiac Muscle: This type of tissue is unique in that it is found only in the heart. Under the microscope, this tissue can be identified by its structure. The fibers branch freely. The nuclei are centrally located like those of smooth muscle. In some preparations of cardiac muscle transverse markings on the fibers are visible but they are different from the striations of skeletal muscle. In transverse, as in longitudinal section, the position of the nuclei differentiates cardiac from skeletal muscle.

Procedure:

1. Examine cardiac muscle tissue under the microscope. Make a large diagram and label the parts. Can you distinguish between a transverse and a longitudinal section? How?

III. Skeletal or Striated Muscle: In the development of a striated muscle fiber, nuclear division occurs without cytoplasmic division. Therefore, the adult skeletal muscle is multinucleate under the microscope, skeletal muscle appears to be composed of many fibers extending along the length of the muscle. Each fiber is finely striated with alternating light and dark bands.

Procedure:

1. Observe under the microscope a longitudinal section of striated muscle. Make a large drawing and label the muscle fibers, striations, and nuclei. Can the striations be seen in a transverse section of striated muscle? Why?

2. For the gross anatomy of skeletal muscles, fill in the data sheet. List only the major muscles of each of the body regions listed. Ordinarily, the more stationary attachment of a muscle is called the origin, and the more movable attachment is called the insertion. List the origin, insertion, and function for each major muscle.

Results: Data Sheet for Gross Anatomy of Skeletal Muscles

Body Area	Major Muscles	Origin	Insertion	Function
Head				
Shoulder				
Arm				
Chest				
Abdominal Wall				
Thigh				
Lower Leg				

LABORATORY INVESTIGATION 5D Muscle Contractions

Objectives:

1. To calculate the percent of muscle contraction after the application of the following: (1) ATP, (2) ATP plust salt, (3) salt alone.
2. To explain how ATP enables muscles to contract.
3. To record a tracing of a skeletal muscle twitch.
4. To determine subminimal, threshold, and maximal stimulus.
5. To identify the contraction and relaxation phase of a single muscle contraction.
6. To record a tracing of the staircase or treppe phenomenon.
7. To place a muscle in a state of tetany.
8. To determine the causes of tetany.

Contraction of Glycerinated Muscle with ATP

Introduction: Glycerinated muscle from the rabbit is excellent material for classroom studies of the microanatomy and mechanism of muscle contraction. Clearly defined striations of skeletal muscle are easily observed. When a bundle of freshly dissected muscle fiber is stored in cold glycerol, the striations are unchanged.

Procedure:

1. Remove from the test tube the stick to which the bundle of skeletal muscle has been tied. Pour the glycerol into a finger bowl.

2. Cut the muscle bundle into pieces about 2 cm. in length. Drop these into the glycerol in a Syracuse dish. One piece is sufficient for each individual.

3. Using dissecting needles, tease the segment of muscle into very thin groups of myofibers (muscle cells); single fibers. The thinnest fibers will demonstrate the greatest contraction. Strands of muscle exceeding 0.2 mm. in cross-sectional diameter should not be used.

4. Mount one of the strands on a microscope slide without a cover glass. Examine under low and high magnification. Note the striations in the fibers and the smooth walls. Do not use the oil immersion lens.

5. Transfer three or more of the thinnest strands to a second microscope slide. Position the strands straight and parallel to each other. Keep the strands moist with a small amount of glycerol.

6. Place the slide under a dissecting microscope and measure the length of the fibers with a millimeter scale held beneath the slide. Record these lengths.

7. Flood the fibers with several drops of the solution containing ATP plus potassium and magnesium ions. Observe the reaction of the fibers.

8. After 30 seconds or more, remeasure the fibers and calculate the degree of contraction. Have the fibers changed in width?

9. Remove one of the contracted strands to another slide. Examine under a compound microscope and compare the fibers with those seen in Step 4. What differences do you see?

10. Repeat the experiment using clean slides, new myofibers, and the solutions of ATP alone and salts alone. What conclusions may be drawn from your results?

11. To calculate percent of contraction:

$$\frac{\text{(Original length) minus (length after treatment)}}{\text{Original Length}} \times 100 =$$

Results:

	Fiber Length (mm.)	Percent Contraction	Expected Results
Untreated Muscle Fiber			
After Application of ATP + Salt			
After Application of ATP Alone			
Salt Alone			

Discussion:

1. What is the effect of ATP upon muscle contraction?

2. What is the role of $MgCl_2$ and KCl in muscle contraction?

Kymograph Tracing of Skeletal Muscle Contractions

Introduction: Experimentally, much can be learned about the nature of contractions in an isolated skeletal muscle by arraning for a muscle to record its contractions on graph paper. The instrument you will use is the kymograph. It makes a recording in ink of the contractions and relaxations of the muscle preparation.

Procedure:

1. First make the frog insensitive to pain. This can best be done by pithing the frog or by injecting 1.0 ml. of 10% urthane into the neck or corner of the mouth.

2. Muscle preparation. Skin the hind leg of a frog. Locate the gastrocnemius (calf) muscle. It arises from the lower end of the femur forming a thick belly in the back of the tibia. Tie a fine string or thread around the achilles tendon before cutting it from the leg. Cut off the femur above the knee leaving about 1 cm. of femur attached to the gastrocnemius muscle. This is used to hold the preparation in the muscle clamp. Be sure to remove the tibia bone.

3. It is important to keep the muscle moist with saline or Ringer's Solution. Save the other leg for a second preparation.

4. Set up the preparation so that the femur is secured in the femur clamp and tie the achilles tendon to the ink-writing lever.

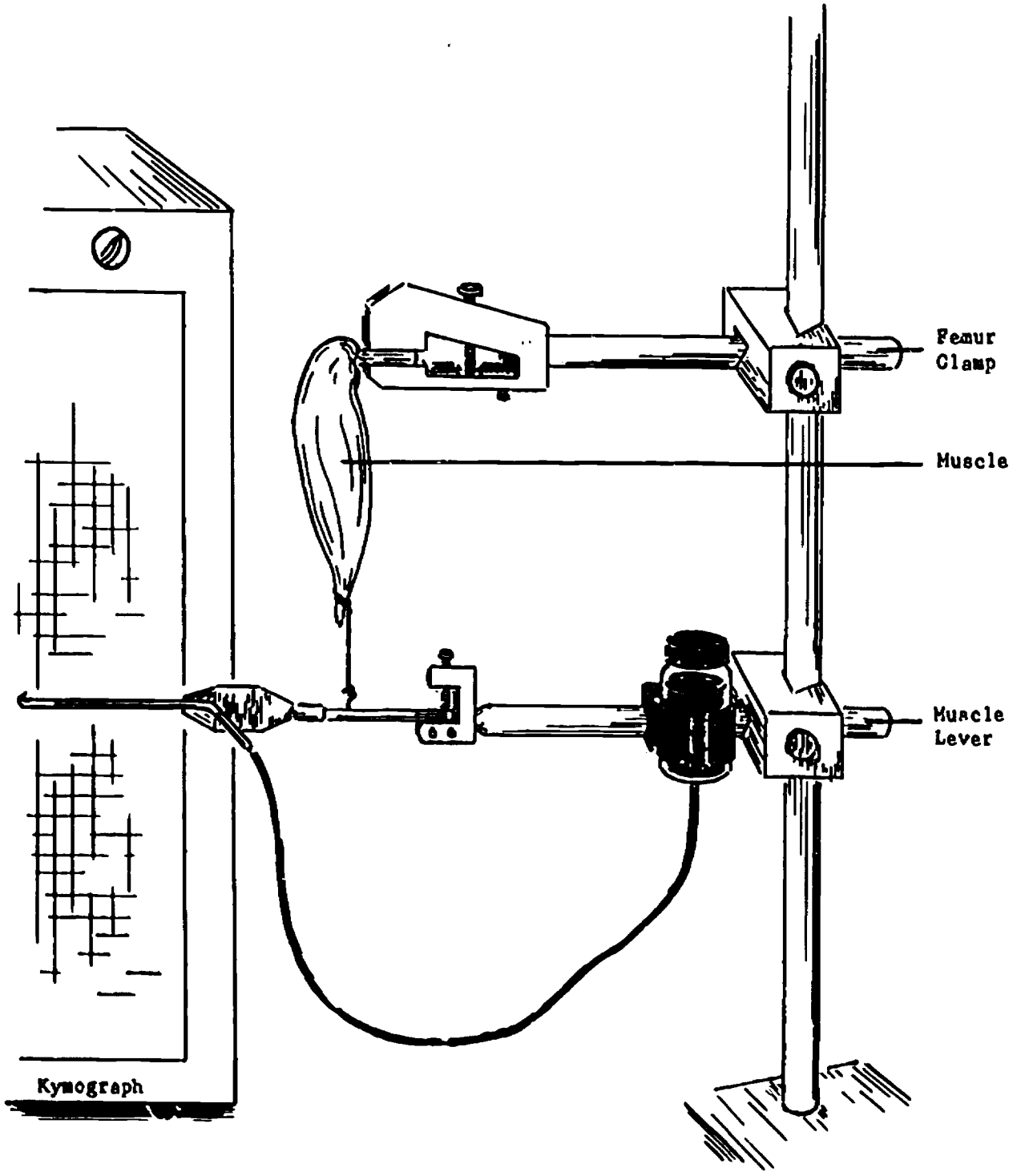
5. Make sure that ink is coming through the writing pen and when the lab exercise is completed, clean the pen. Ink left in the pen will clog it and prevent it from being used again.

6. Save a good recording of each experiment. Each graph must be labeled.

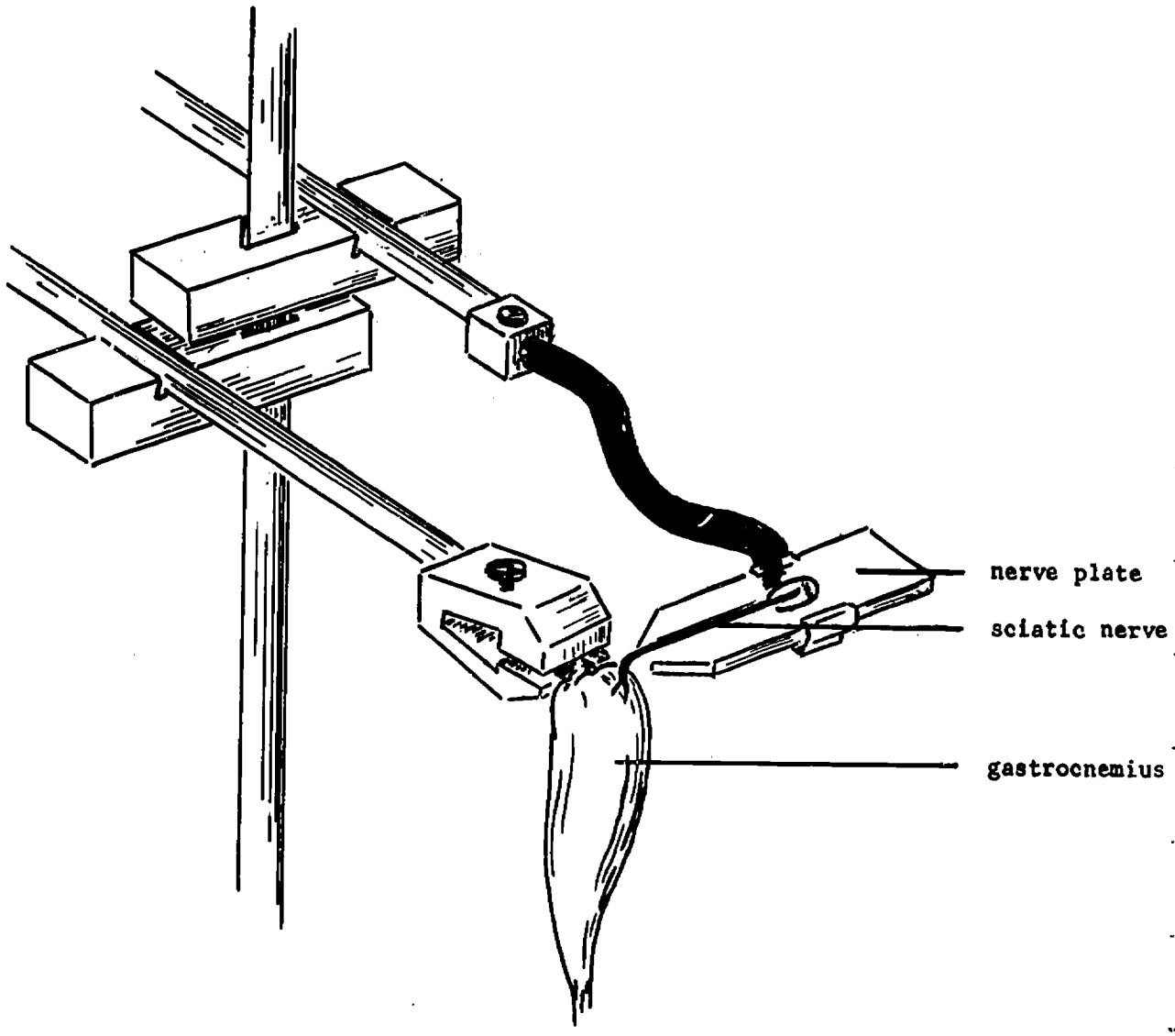
A. Intensity of a Stimulus: To provoke a response, a stimulus must possess a certain intensity. A weak stimulus will elicit no contraction; such a stimulus is said to be subminimal. Should the intensity of the stimulus be increased gradually, a point will be reached at which the muscle will barely respond; this is called a threshold stimulus. If the intensity of the stimulus is gradually increased further, the extent of contraction will also increase gradually until a point is reached beyond which further increase in intensity of the stimulus produces no increase in the extent of contraction; this is called a maximal stimulus.

a. With the chart stopped, stimulate the muscle with a single shock with the least amount of current (0.1 volts). Set the frequency to 50.
b. In order to stimulate the muscle, touch the middle of the muscle with the electrode; be certain that both prongs of the electrode touch the muscle. After each stimulation increase the current by 0.5 volts and move the chart about 2 cm.
c. Have the chart stopped so that the contracting muscle writes a vertical line for all contractions.
d. Wait at least one minute between each stimulation.
e. Increase the stimulus by 5 volts after the threshold is reached.
f. Record the height of each contraction.
g. Keep the muscle preparation moist with saline or Ringer's Solution. Use an eye dropper to apply the saline. A moist muscle will conduct the electrical stimulation better than a dry one. As a result, you will obtain a better muscle contraction.

MUSCLE LEVER SET-UP



MUSCLE-NERVE SET-UP



Results:

	Volts	Height of Contraction
Record the Voltage of First Observable Contraction		
Voltage Giving Greatest Contraction		

Muscle Contractions	Voltage	Height of Contraction
1. Subminimal Stimulus =		
2. Threshold Stimulus =		
3. Maximal Stimulus =		

B. A Single Muscle Contraction: A single complete muscle contraction is often referred to as a muscle twitch.

a. Keep the muscle moist with saline at all times.
b. Set voltage at 10 volts. Set the frequency to 1.
c. With the chart moving at fast speed, record a single muscle twitch.
d. Wait 5 seconds and record a second muscle twitch.
e. Label the contraction period and relaxation.
f. Record a third muscle twitch, but stimulate the muscle a second time before it relaxes, (stimulate the muscle at the end of the contraction period.)
g. What effect does this second stimulation have upon the normal muscle contraction.

Results:

Trial	Length of Contraction Phase	Length of Relaxation Phase
1		
2		
3		

C. Staircase or Treppe: Following the application of repeated stimuli the contractions are successively higher even though there is no increase in the strength of the stimulus.

a. Have the chart moving slowly.
b. Set the voltage to 10 volts above threshold.
c. Stimulate (with the single shock stimuli) as rapidly as possible for 10-15 seconds. Gradually increase the frequency from 1-100.
d. Record and observe staircase phenomenon.
e. Make as many trials as needed to obtain a good recording.
f. Save the graph and label it.

D. Tetanus: This condition results from rapid, repeated stimulation. As the rate of stimuli is increased, the muscle no longer relaxes to its original length. However, the individual contractions remain visible. This is known as "incomplete tetanus". If the rate is increased more, there will be a complete fusion of contractions, and individual contractions are no longer visible. This is known as "complete tetanus".

a. Keep the muscle moist with saline.
b. Have the chart moving at medium speed.
c. Start with 10 volts above threshold and the lowest frequency. Increase the frequency until there is a constant stimulus.
d. Record the number of stimuli per second required both for incomplete and complete tetanus.
e. Save the graph illustrating a good example of tetanus.
f. Is this condition permanent or temporary?
g. How can it be corrected?

LABORATORY INVESTIGATION 6D Muscle Work and Fatigue

Objectives:

1. To record muscle contractions caused by electrical stimulation to the sciatic nerve.

2. To calculate the amount of work performed by a skeletal muscle.

3. To determine the cause of muscle fatigue.

4. To record contractions of human muscle contraction and observe variations during the fatigue process.

5. To determine the effects of stress upon a muscle as to the amount of work performed.

Work - Muscle-Nerve Preparation

Introduction: Before starting the experiment, read the entire exercise. Be sure you understand the nature of the experiment and the results to be obtained.

In this experiment, you will be using a muscle-nerve preparation. The nerve will conduct the stimulation to all fibers of the muscle at one time. This will enable the entire muscle to contract, thus it can do more work. If only a few fibers of a muscle contract, the entire muscle does not contract. A constant weight on a muscle will cause it to stretch and do more work. Compare the amount of work done in Procedure A with Procedure B.

Procedure A: Weight not supported by the resting muscle.

1. Dissect out the sciatic nerve and make sure you leave it attached to the gastrocnemius muscle. Be sure you know how to do the dissection before you begin.

2. Attach the femur bone in the femur clamp and tie the achilles tendon to the lever on the kymograph.

3. Be sure to place the sciatic nerve in the nerve plate. Attach the nerve plate directly above the femur clamp.

4. Be sure the sciatic nerve is kept moist with Ringer's Solution. In this experiment you will be stimulating the sciatic nerve directly.

5. Use a stationary chart and move it between the periods of stimulation.

6. Set the voltage to 10 volts with the frequency set at 1. If this voltage fails to give a response, adjust the voltage until you get a maximal contraction.

7. Begin with 5 grams and progressively increase the load by adding weights until the muscle fails to lift the total load.

8. Label your records completely. Indicate the number of grams lifted in each case.

9. Stimulate the sciatic nerve in the nerve plate. Be sure to keep both the nerve and muscle moist with Ringer's Solution.

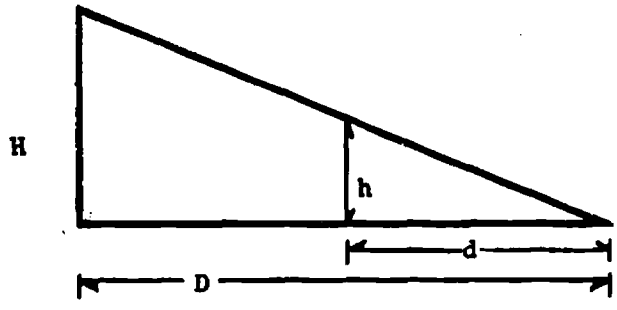
10. With increasing work, the muscle will stretch; therefore, you will need to take up the slack. Keep a slight tension on the lever at all times.

11. Be sure to give the muscle a brief rest between work loads. In this way, it will be able to lift more weight before being fatigued.

Results: Work = Weight X Distance

1. The height that the ink-writer moves is not the distance the weight is lifted. You must calculate the height the weight is lifted. This corresponds to the amount of muscle contraction or how much the muscle shortens.

$$H \times d = D \times h$$



H =

D =

d =

h =

H - height of contraction on ink-writing graph

h - actural amount of muscle contraction, this must be calculated

D - length of ink-writing lever, from fulcrum (pivot) to tip of ink-writing point

d - length from fulcrum (pivot) to where the muscle is attached. Where the string is tied on.

Procedure B: Weight is supported by the resting muscle.

1. Adjust the setscrew on the lever arm so that the lever arm and weight are supported by the resting muscle.

2. Keep the weight and tension on the muscle. The muscle tends to stretch after successively lifting weight. Therefore, it may be necessary to raise the muscle clamp to take up for the stretching.

3. If possible use the same muscle as you used for Procedure A. Follow the same procedure as before. If the muscle fails to respond, use another muscle-nerve preparation.

4. Keep adding weights until the muscle fails to respond.

Results:

1. What was the maximum work done?

2. Under which situation did the muscle accomplish the most work? Explain why.

Human Muscle Fatigue

Introduction: Muscle fatigue will develop very rapidly in muscles doing work. The fatigue depends upon the amount of work, the speed that it is done, and the muscles involved. In this experiment the ergograph will be used. It is a trigger type instrument for recording the strength of muscle contractions.

Procedure:

1. Connect the ink-writing pen to the erograph.
2. Make sure ink is making a fine line on the kymograph.
3. Using your index finger, pull the trigger as far back and as many times as possible. Do not stop for a rest or pause between contractions.
4. Have the chart moving slowly while you are pulling the trigger.
5. Count the number of times the trigger was pulled and record the length of time required to induce muscle fatigue.
6. Rest for 15-20 seconds and repeat the experiment using the same finger. Compare the results.

Results:

	Trial 1	Trial 2
Time - Contraction Started		
Time at Fatigue		
Length of Time Required to Induce Fatigue		
Height of Contraction First Contraction		
Height of Contraction Last Contraction (Fatigue)		
Total Number of Contractions		

LABORATORY INVESTIGATION 7D Brain and Cranial Nerves

Objectives:

1. To locate and identify the meninges of the brain.
2. To locate and identify the regions of the brain.
3. To explain the function of the various parts of the brain.
4. To locate, identify, and explain the function of the twelve cranial nerves.

The Central Nervous System

Introduction: The nervous system of vertebrates may be divided into three parts. The brain and spinal cord are sometimes called the central nervous system, the best known part of this system because it is relatively easy to locate, dissect, and study. The nerves and nerve processes that lead to and from the brain and spinal cord, the cranial and spinal nerves, are referred to as the peripheral nervous system. This system carries sensory impulses in to the central nervous system and motor impulses away from the central nervous system. The third system is the autonomic nervous system. Its structural components are largely a part of the central and peripheral nervous systems and it exercises control over the viscera and the blood vessels and certain other structures in all parts of the body.

The brain and the first ten cranial nerves which pass out to the tissues are much the same in all vertebrates so far as their arrangement, structure, and functions are concerned. The parts of the brain may vary consideraly in size, however, from on group to another. Also the proportions between different parts will vary greatly from group to group. Cranial nerves originate in some part of the brain and pass out directly through small openings in the cranium to the part innervated. In the various vertebrates, each corresponding pair of cranial nerves serves essentially the same function hence the same names are used in the various groups.

Procedure:

1. Each student will receive one-half of a preserved sheep brain.

2. Locate and identify as many of the following structures as possible.

3. Be able to explain the function of each structure.

4. Make a large diagram of the brain and label the structures.

A. Meninges

1. pia mater
2. arachnoid
3. dura mater

B. Cerebrum

1. Lobes
2. Convolutions
3. Corpus callosum

C. Diencephalon

1. thalamus
2. hypothalamus

D. Mid brain

E. Pons

F. Medulla

G. Cerebellum

Results: Complete the following chart.

Cranial Nerves	Location	Function
1. Olfactory		
2. Optic		
3. Oculomotor		
4. Trochlear		
5. Trigeminal		
6. Abducens		
7. Facial		
8. Audiotry (Acoustic)		
9. Glossopharyngeal		
10. Vagus		
11. Spinal Accessory		
12. Hypoglossal		

LABORATORY INVESTIGATION 8D Nerves

Objectives:

1. To observe and identify the parts of a nerve cell.
2. To observe and identify the internal structure of the spinal cord.
3. To identify the structure of an axon.
4. To locate and identify the myelin sheath and the neurilemma.
5. To differentiate between myelinated and unmyelinated nerves.
6. To locate and identify a synapse.
7. To diagram a spinal nerve entering and leaving the spinal cord.

Nerve Cells

Introduction: Neurons consist of a cell body; one or more branching processes known as dendrites, which transmit impulses toward the cell body; and one efferent process known as the axon, which conducts impulses away from the cell body.

A synapse is a region where the axon of one neuron comes in close proximity to the dendrites or succeeding neurons.

Procedure:

1. Observe under the microscope various types of neurons.
2. Make a large diagram of each type and label the parts.
3. Make a large diagram of the axis cylinder (axon). Label the (1) myelin sheath (2) node of Ranvier and (3) the neurilemma.
4. Make a large diagram of a synapse and label the parts.

Spinal Cord

Introduction: The spinal cord lies within the spinal cavity. It consists of gray matter which forms the inner core. It takes on the appearance of a "butterfly". The white matter is the outer part. The white matter is composed of the axis fibers of neurons. Nearly all are myelinated, but they have no neurilemma. They are concerned with transmission of nerve impulses both up and down the cord.

Procedure:

1. Observe the cross-section of the spinal cord under low power.

2. Make a large diagram and label the parts.

Spinal Nerves

Introduction: Thirty-one pair of nerves have their origin on the spinal cord. Unlike the cranial nerves, they have no special names but are merely numbered according to the level of the spinal column at which they emerge from the spinal cavity. There are eight cervical, twelve thoracic, five lumbar, and five sacral pairs and one coccygeal pair of spinal nerves.

Procedure:

1. Determine whether the spinal nerves are myelinated or unmyelinated.

2. Make a diagram illustrating how the spinal nerves join the spinal cord.

LABORATORY INVESTIGATION 9D Nerve Membrane Potentials

Objectives:

1. To observe a nerve action potential on the oscilloscope.
2. To differentiate between a membrane potential and an action potential.
3. To determine the threshold stimulus for an action potential.
4. To determine the effects of K^+ ions upon the action potential.
5. To determine the effect of temperature upon the action potential.
6. To determine the composition of the sciatic nerve.
7. To calculate the number of fibers in the sciatic nerve.
8. To estimate the length of a neuron.

The Resting and Action Potentials of Neurons

Using the Oscilloscope

Introduction: All cells, animal and plant, exhibit a transmembrane potential. If the inside of any cell is connected to the outside through a voltage indicator a potential of about 90 to 120 mV will be shown. All cells preferentially accumulate some ions and activity exclude others; the asymmetrical distribution of ions thus resulting produces the membrane potential.

The distribution of ions within and outside the cell results in the outside being positive to the inside and therefore the membrane is said to be polarized for each side of it exhibits a difference in electrical charge. If a sufficiently intense stimulus impinges on this polarized membrane the distribution of charge will be upset resulting in depolarization and even a reversal of polarity. However, the resting polarity is soon re-established after depolarization and the cell can again respond to the depolarizing stimulus.

The nerve cell or neuron is a highly elongated cell specialized for conduction of disturbances in polarization; hence, the nerve impulse is a wave of depolarization traveling along the length of the neuron. This wave is referred to as the "action potential" to distinquish it from the "resting potential" (membrane potential) of the unstimulated cell.

The action potential of the neuron may in turn result in stimulation of another neuron or may activate a gland or muscle. Activated muscle exhibits an action potential quite similar to the neuron.

In the laboratory, the sciatic nerve will be placed across a pair of electrodes and stimulated at one end while the response is picked up further down the nerve by the recording electrodes.

While making observations by extracellular recording from nerve trunks and neurons, three conditions must be kept in mind: the recording (1) is extracellular; (2) is a comparison between two places on the tissue surface; and (3) the potentials recorded represent the difference in voltage between the two electrodes.

Stimulation of the nerve could be brought about by mechanical, chemical, thermal, or electrical changes impinging on the nerve. The stimulus of choice, however, is electricity; for precise quantities can be generated and controlled with ease.

Procedure: For Excision of the Sciatic Nerve

Lay the frog ventral side up. With forceps raise the skin at the midline of the body between the forelegs and with scissors snip the skin. Beginning at this snip make a continuous cut through the skin until you have encircled the entire body just below the forelegs. Be careful not to cut into the body cavity.

Now with one hand tightly grasp one forepaw and with the other hand strip the skin down and off the hind legs with one quick pull.

Place the skinned frog dorsal side up and locate the urostyle. Free the posterior end of the urostyle of the tissues underlying it so that it can be raised, and then cut loose the tissues just inside and along the two ilium bones until the urostyle and musculature above it can be raised like a lid, with the sacrum as a hinge, to reveal the path of the sciatic nerve trunk from the vertebral column to the gastrocnemius. Tightly tie a white thread around the sciatic nerve where it joins the gastrocnemius and cut the nerve away from the muscle. Keeping very slight tension on the nerve by pulling on the thread, raise the nerve out of its cavity by freeing it from all its connective tissue attachments up to the column. Thus, the nerve can be manipulated by holding it by the thread only. Note: Excise both sciatic nerves, then place them in Ringer's Solution.

Procedure: For Determination of Artifacts

1. Before proceeding with the characteristics of a nerve impulse, the nature of the stimulus artifact should be examined. Moisten a piece of thread in Ringer's Solution. Place the thread in the nerve chamber in such a manner that it covers the stimulating electrodes and recording electrode. When the wet thread, which acts as a conductor, is "stimulated", a wave of electricity will be viewed on the screen. Adjust the magnitude on the stimulus until a visible trace appears on the screen. Measure the size of this artifact.

2. Compare the response of the thread at three different stimulation intensities. (Voltages)

3. Move the recording electrodes to different positions and note the effect on the waveform.

Procedure: For Study of Live Sciatic Nerve

The action potential can be elicited and its characteristics studied by mounting an excised live nerve in a suitable moist chamber, stimulating the nerve, collecting the action potential on recording electrodes which are connected to the input on the IMPScope, and then displaying the impulse on the oscilloscope. The nerve should be wetted frequently even when within the moist nerve chamber.

A. Threshold Stimulus

1. Place the nerve in the chamber and adjust its position to be certain that adequate contact is made with the stimulating and recording electrodes.

2. Measure the threshold stimuli, the summation of sub-threshold, and the response is now viewed as a tracing on the screen rather than as a muscle twitch. Be sure to keep the nerve moist throughout the experiment. Use 10-15 second intervals between each stimulus applied.

3. At maximal stimulation (you may have to reduce the vertical gain), observe and draw the action potential on graph paper.

B. Effect of Increasing Extracellular K^+

1. Soak the nerve in 1M KCl for 5 minutes.
2. Drip 1M KCl on the recording electrodes.
3. Drip 1M KCl on the stimulating electrodes while stimulating and recording.
4. Draw the wave on graph paper.

C. Effects of Temperature

1. Determine the effect of temperature on conduction velocity at various temperatures. Use ice cold saline.

2. Draw the wave on graph paper.

Results:

1. What was the threshold stimulus?_____

2. What was the maximal stimulus?_____

3. What was the effect of KCl upon the action potential?

_____

4. What was the effect of a decrease in temperature upon the action potential?_____

Procedure: For Microscopic Study of the Sciatic Nerve

1. Dissect (preferably under a dissecting microscope) the sheath away from the nerve trunk by using a very sharp dissecting needle and tearing at the sheathing with a motion starting from about 3 mm. from the cut end and tearing toward this end. Dissection with the nerve under water will aid in fiber separation.

2. Try to obtain one fiber about 1 mm. or more in length for observation under a compound microscope.

3. Does microscopic examination of a short nerve segment reveal any characteristic structures? Explain.

4. How many fibers would you estimate make up the nerve trunk?

5. What specific part of the neuron do these fibers represent? Where are the cell bodies of these neurons? How long, is the neurons of the frog? Compare this with the maximum length of human neurons.

6. Make a large drawing of a nerve fiber.

LABORATORY INVESTIGATION 10D Reflexes - Response to Stimuli

Objectives:

1. To trace the pathway of a reflex action.
2. To observe reflex action on a frog.
3. To stimulate the body to obtain a reflex action.
4. To identify the types of neurons involved in a reflex action.
5. To observe behavior and determine the response to various stimuli.

Reflex Actions

Introduction: In this experiment, simple spinal reflexes will be observed. A simple reflex arc involves a receptor organ or cell and a sensory neuron, one or more connector neurons in the spinal cord, a motor neuron, and an effector organ (muscle).

Procedure: Frog Reflexes

1. Destroy the brain and spinal cord of a frog by pithing.
2. Tie the frog to a ring stand so that it is suspended by the neck.
3. Using a disposable pipette, apply a weak acid (acetic acid) to various parts of the frog's body. Start applying it to the legs and then over the back. Be sure to have a pan or beaker under the frog to collect the acid that runs off the frog.

Results:

1. Explain what happened.
2. The reflex arc consists of at least five fundamental parts. Specify what these are:
    1. Receptor -
    2. Sensory Transmitter -

3. Motor Transmitter -

4. Neuro-Effector Junction -

5. Effector -

Procedure: Perform and Observe the Following Reflexes on Your Own Body.

1. Babinski Reflex - stimulate foot from heel to toes
2. Patellar Reflex - knee jerk
3. Planter Reflex - stimulate sole of foot
4. Achilles Reflex - stimulate the achilles tendon
5. Corneal Reflex - stimulate with different intensities of light

Animal Behavior

Introduction: The purpose of this experiment is to observe the effects of various stimuli upon animal behavior.

Procedure:

A. Observe a newt (salamander) swimming in a finger bowl for five minutes while subjected to each stimuli.

B. After each stimuli return the newt to a finger bowl containing tap water. Allow enough time for the newt to adjust back to normal.

C. With each type of stimuli indicate the nerves involved (types) and the region of the brain involved. Describe observable behavior. What receptors are receiving the stimuli?

D. Stimulus:

1. Pond water at room temperature.
2. Cold water at 0° C. Use ice water.
3. Hot water at 45° C.
4. Hypertonic solution (use concentrated salt solution).
5. Weak acid (acetic acid)
6. Alcohol (ethyl at 50 proof)
7. Use a painful stimulus, such as sticking it with a dissection needle.

Results:

Stimuli	Observable Behavior	Region of the brain and nerves affected
1. Normal Pond Water		
2. Cold Water (0° C)		
3. Hot Water (45° C)		
4. Hypertonic Solution		
5. Acid		
6. Alcohol		
7. Pain		

LABORATORY INVESTIGATION 11D Microbiology of Infections

Objectives:

1. To locate and identify the T. spiralis larvae.
2. To diagram the life cycle of the pork worm.
3. To identify bacteria causing conjunctivitis.
4. To observe a prepared slide of Clostridium tetani and identify the spores.
5. To observe and identify meningococcus meningitis.
6. To locate and identify bacteria found in the spinal fluid.

Trichinosis

Introduction: Trichinosis is a disease caused by a roundworm, Trichinella spiralis. The source of human infection is the consumption of animal meat, principally pork that are infected with T. spiralis. After the infected meat is digested in the stomach, the released larvae pass into the intestines, where maturation of the adult worm occurs. The female which becomes embedded in the wall of the intestine, releases from 1000 to 1500 larvae. These larvae are carried to the skeletal muscles. The muscles most commonly affected are the greater pectoral, parts of the deltoid, gastrocnemius, biceps, and diaphragm.

Procedure:

1. Observe under the microscope a section of skeletal muscle containing the encysted T. spiralis larvae.
2. Make a drawing of a larvae and the surrounding muscle fibers. Label the parts.

Conjunctivitis

Introduction: Conjunctivitis may result from bacterial infection; such as pneumono-coccus and gonococcus. There is an acute purulent conjunctivitis that occurs in newborn babies that is due to gonococcus. The infection occurs at the time of birth from the infected birth canal of the mother. It is a serious disease, often causing blindness. As soon as the baby is born, the eyes are cleansed with a 1% solution of silver nitrate.

Procedure:

1. Examine prepared slides of gonococcus under the microscope. Make a large drawing of the organism.

Tetanus

Introduction: Tetanus is caused by the bacterium Clostridium tetani. This organism is anaerobic gram-positive bacillus. Spores are formed at one end. Infection may occur whenever a deep wound is contaminated with soil.

Procedure:

1. Observe under the oil immersion a prepared slide of Clostridium tetani.
2. Observe and locate the spores.
3. Make a large drawing of an organism containing a spore and another drawing without spores.
4. Streak an agar plate from a soil sample. Identify the type of bacterial colonies in the soil.

Questions:

1. Where are the spores located?
2. What color of stain did the bacteria take? Why?
3. What stain did the spore take? Why?
4. How do anaerobic organisms survive both inside and outside the body?

Meningococcus Meningitis

Introduction: Meningococci are gram-negative diplococci. They form no spores and have no flagella. This organism first enters the respiratory tract of the new host; from there they invade the blood. In the blood, the meningococci reach the meninges causing meningitis.

They may be diagnosed by examination of the cerebrospinal fluid. On direct microscopic examination, pus cells as well as intra and extra-cellular gram-negative diplococci may be found. The intracellular organisms are phagocytized.

Procedure:

1. Observe under high power a smear containing meningococci. Locate the organisms both inside and outside the pus cells.

2. Make a large drawing under oil immersion of the meningococci.

Spinal Fluid

Introduction: The spinal fluid is obtained by making a spinal puncture. This is performed by a physician. In a routine examination a smear may be made. In the case of disease, the spinal fluid may contain the following organisms: (1) staphylococci, (2) streptococci, (3) meningococci, (4) tubercle bacilli, and (5) influenza bacilli.

Procedure:

1. Observe under oil immersion as many of these organisms that are available. Be able to identify each kind. Make a large drawing of each and label the parts.

APPENDIX

RECORDING LABORATORY DATA AND RESULTS

A. Recording microscopic observations

1. Make large diagrams or drawings under observation.
2. Do not draw the entire viewing field.
3. Use only <u>one</u> side of unlined paper.
4. Space all diagrams so there is a margin and space between them.
5. Place one or two diagrams per page, depending upon the size of each diagram. <u>Never</u> place more than three (3) diagrams on one page.

B. Labeling Diagrams

1. Label each page with the appropriate title.
2. Label the parts of each diagram.
3. <u>Print</u> all labels.
4. Make all diagrams and labels in pencil, <u>not</u> ink.

C. Making Gross Observations

1. Record all <u>Data</u> directly in the laboratory manual.
2. Make permanent records of the results.

AUDIO-TUTORIAL LABORATORY

PERMANENT EQUIPMENT AND SUPPLIES / BOOTH

EQUIPMENT

1. Compound Microscope
2. Tape Recorder with Earphones
3. 8 mm. Film Loop Projector
4. Individual Slide Viewer (2x2 slides)
5. Bunsen Burner

SUPPLIES

1. Stains (1-bottle of each)
   a. methylene blue
   b. carbol-fuchsin
   c. safranine
   d. gram iodine
2. Staining Pans (2)
3. Solutions (1-bottle of each)
   a. 95% alcohol
   b. distilled water
   c. saline
4. Washing Bottle
5. Immersion Oil
6. Wooden Slide Box with Slides
   a. plain (5)
   b. depression (2)
   c. cover glass (10)
7. Lens Paper (10 sheets)
8. Test Tube Rack
9. Test Tube Clamp (holder)
10. Ring Stand with Wire Asbestos Mesh
11. Innoculating Loop
12. Glass Stirring Rod (2)
13. Atlas of Diagnostic Microbiology
14. Matches (1 box)
15. Ruler (plastic)
16. Hand Lens
17. Dissecting Kit
    a. scalpel
    b. scissors
    c. probe
    d. seeker
    e. dissecting needle
    f. eye dropper

FIRST TERM

MATERIAL FOR LABORATORY INVESTIGATIONS

LAB NO.	SUPPLIES NEEDED	MATERIAL TO PREPARE OR ORDER
1A	Cotton Strands Onions Prepared Slides 1. newsprint 2. frog's blood Dilute Methylene Blue Tooth Picks	
2A	Prepared Microscope Slides 1. Mixed bacteria 2. E. coli 3. Sarcina lutea 4. Bacillus subtilis	Nutrient agar slants Sub-culture living bacteria 1. E. coli 2. B. subtilis 3. S. lutea 4. Rhodospirillum rubrum
3A	Prepared Slides (X-sect) 1. esophagus 2. stomach 3. small intestine 4. large intestine	White Rats
4A	Prepared Slides 1. Salmonella 2. Shigella Antiseptics (4 oz. bottle) Lavoris Tincture of Zephrian 2% Lysol 70% Alcohol Zephriam Chloride Phisohex Cidex	Nutrient agar plates Nutrient broth tubes Sub-culture living bacteria 1. E. coli 2. P. vulgaris

FIRST TERM

LAB NO.	SUPPLIES NEEDED	MATERIAL TO PREPARE OR ORDER
5A	Biuret Solution Benedict's Solution Carbohydrates glucose — powdered milk fructose — potatoes sucrose — crackers lactose — onion starch Fats olive oil corn oil coconut mineral poly-unsaturated Protein albumin gelatin casein Glassware 1. test tubes 2. beakers 3. graduate cylinder Mortar and Pestle Spoons and Spatula	2% iodine in CCl_4
6A	Dialyzing Tubing - 150 ft. NaCl Glucose Starch Glass Beakers Spoons and Spatula	NaCl - 10% Solution Elodea Leaves $AgNO_3$ (2% solution) Benadict's Solution

FIRST TERM

LAB NO.	SUPPLIES NEEDED	MATERIAL TO PREPARE OR ORDER
7A	Burettes Blue Litmus Paper Red Litmus Paper pH Indicator Paper Flasks for Titration Filter Paper Graduate Cylinders Vinegar Acetic Acid (Dilute)	Indicators 1. Phenolphthalein 2. Bromthymol Blue 3. Litmus Solution Acid .10N HCl .05N HCl Base .15 N NaOH
8A	Thermometer Prepared Slides 1. Liver 2. Salivary Gland 3. Pancreas Benedict's Solution Blue Litmus Solution Test Tubes Beakers Graduate Cylinders	HCl (pH-2) Pepsin 5% Starch Solution 5% Pancreatin 5% Hard Boil Eggs Skim Milk (gal.) Cream or Half-Half Enzyme - Rennet Tablets

FIRST TERM

LAB NO.	SUPPLIES NEEDED	MATERIAL TO PREPARE OR ORDER
9A	Finger bowl Prepared Slides 1. Trypanosomes 2. Plasmodium vivax 3. Endamoeba histolytic 4. Taenia saginata 5. Trichinella spiralis 6. Necator americanus 7. Trematode 8. Enterobius vermicularis	Frogs (live)
10A	Prepared Slides 1. Clostridium botulinum 2. C. tetani 3. C. perfringens 4. B. anthracis 5. Salmonella typhosa	Agar Plates Ground Meat Milk
11A	Antibiotic Discs 1. Penicillin 2. Streptomycin 3. Aureomycin 4. Chloramphenical 5. Terramycin 6. Actinomycin	Agar Plates Cultures of mold 1. Rhizopus nigricans 2. Penicillium notatum 3. Streptomyces antibroticus 4. Streptomyces venezullae 5. Streptomyces griseus Cultures of living bacteria 1. Aerobacter aerogens 2. Staphyloccus aureus Media for mold growth 1. Bread 2. Fresh Fruit

SECOND TERM

LAB NO.	SUPPLIES NEEDED	MATERIAL TO PREPARE OR ORDER
1B	70% Alcohol Cotton balls Lancet Hemoglobin test paper and color charts Blood typing kits Capillary tubes Dextrostix	Blood (3 pints) to make 100 samples (3 ml) Living amoeba (10 cultures)
2B	Heprinized capillary tubes and plugs (100) 70% alcohol and cotton Lancet (200) Wrights Blood Stain (10) Prepared Slides (10) Centrifuge	Make 100 samples of blood
3B		Preserved Pig Hearts
4B	Sphygmomanometer Stethoscope Prepared Slides 1. Artery (X-sect) 2. Vein (X-sect) 3. Capillary (X-sect)	Frogs - living Frog boards to observe web 10% urethane

SECOND TERM

LAB NO.	SUPPLIES NEEDED	MATERIAL TO PREPARE OR ORDER
5B	Kymographs with heart lever Adrenaline Acetylcholine Digitalis Atropine	Frogs (living) 10% urethane Frog Ringer Solution (5 liters)
6B	Oscilloscope (2) Electrodes (350) 70% alcohol and cotton balls	Graph Paper
7B	Kymograph with heart lever Electrical stimulator	Living turtles 10% urethane
8B	Prepared Slides 1. lung tissue 2. broncial tube	Rats (living) Paramecium (10 cultures) Frogs (living)
9B	Tongue depressor Cotton swabs for throat Prepared Slides D. pneumoniae K. pneumoniae M. tuberculosis C. diptheriae S. aureus S. pyogenes B. antharacis P. pestis	Blood agar plates

SECOND TERM

LAB NO.	SUPPLIES NEEDED	MATERIAL TO PREPARE OR ORDER
10B	Spirometer Disposable mouth pieces Paper bags Straws Beakers	Blue Litmus Solution Lime Water Solution
11B	Candy Bars Cotton swabs Smith Fermentation tubes Blue litmus solution Litmus paper	Agar plates Milk Grape juice Yeast suspension Living cultures: E. coli B. subtilis S. lactis Beef broth

THIRD TERM

LAB NO.	SUPPLIES NEEDED	MATERIAL TO PREPARE OR ORDER
10	Prepared Slides 1. skin 2. hair follicle 3. nerve ending	Frogs (living)
20	Prepared Slide 1. kidney tissue	Preserved Pig Kidney
30	Prepared Slides 1. ureter 2. urethra 3. urinary bladder Centrifuge Glassware Beaker Test tubes Disposable pipettes	Urinary Sediment Stain
40	Glassware Beaker (small) Beaker (large) Test tubes Stirring rods Graduate cylinder Urinometer	Ketostix Combistix Bilirubin test kit Benedict's Solution Acetic acid (dilute)

THIRD TERM

LAB NO.	SUPPLIES NEEDED	MATERIAL TO PREPARE OR ORDER
5C	Prepared Slides 1. Neisseria gonorrhoeae 2. gonorrhea smear 3. Treponema pallidum 4. E. coli 5. Staphylococcus aureus 6. Streptococcus faecalis 7. Pseudomonas aerugrnosa 8. Streptococcus veridans 9. Trichomonas vaginalis 10. Phthirus pubis	
6C	Prepared Slides 1. testes (seminiferous tubules) 2. epididymis (x-sect) 3. vas deferens (x-sect) 4. seminal vesicles 5. prostate 6. sperm 7. penis (x-sect)	
7C	Prepared Slides 1. Ovary illustrating a. corpus luteum b. corpus albicans c. follicle and ovum 2. fallopian tube (x-sect) 3. uterus endometrium	
8C	Prepared Slides 1. Stages of mitosis	

ERIC

THIRD TERM

LAB NO.	SUPPLIES NEEDED	MATERIAL TO PREPARE OR ORDER
9C	Radiation Box (UV-irradiation)	Living Bacteria Cultures 1. Sarratia marcesans 2. Sarcina lutea 3. E. coli Nutrient Agar Plates EMB agar plates Nutrient Broth Cultures
10C	Disposable Syringe Finger Bowls Syracuse watch glass	Living Frogs males (4 doz.) females (4 doz.) Pituitary extract Preserved frog egg (various stages)
11C	Prepared Slides 1. thyroid 2. parathyroid 3. ovary and testes 4. pancreas 5. adrenal 6. pituitary	Living Rats

FOURTH TERM

LAB NO.	SUPPLIES NEEDED	MATERIAL TO PREPARE OR ORDER
1D	Cotton swabs Tooth picks Tuning Forks Prepared Slides 1. Taste buds 2. Tongue sections	Solutions 1. Sucrose 10% 2. NaCl 1% 3. Vinegar 1% 4. Quinine .1%
2D	Skeleton - Bones Prepared Slides 1. Bone Marrow 2. Bone Tissue	
3D	Skeleton - Bones Prepared Slides 1. Fibrous Connective Tissue 2. Hyaline Cartilage 3. Elastic Connective Tissue 4. Adipose Tissue	
4D	Prepared Slides 1. Smooth Muscle 2. Cardiac Muscle 3. Skeletal Muscle	
5D	Glassware Finger Bowl Syracuse Dish Kymograph Stimulator	Glycerinated Muscle with ATP Ringer Solution

FOURTH TERM

LAB NO.	SUPPLIES NEEDED	MATERIAL TO PREPARE OR ORDER
6D	Kymograph 1. Muscle-nerve plate 2. Femur clamp 3. Pan and weights Ergograph	Ringer Solution
7D		Sheep Brain (halves)
8D	Prepared Slides 1. Neuron 2. Spinal Cord	
9D	Oscilloscope Nerve Chamber	Frogs (live) Ringer Solution 1 M Solution of KCl Graph Paper
10D	Glassware Finger bowl Beakers Thermometer	Frogs (live) Newts (live) Solutions 1 cohol (50 proof) etic Acid (dilute) Cl (10%)
11D	Prepared Slides 1. T. spiralis 2. Gonococcus 3. C. tetani 4. Meningococci	Ag te So

THE METRIC SYSTEM

UNITS OF WEIGHT

gram (g)	=	1000.	milligrams (mg)
milligram (mg)	=	.0001	gram (g)
kilogram (kg)	=	1000.	grams (g)

UNITS OF VOLUME

liter (l)	=	1000.	milliliter (ml)
milliliter (ml)	=	.001	liter (l)
milliliter (ml)	=	1.	cubic centimeter (cc)

UNITS OF LENGTH

meter (m)	=	100.	centimeters (cm)
centimeter (cm)	=	10.	millimeter (mm)
millimeter (mm)	=	1,000.	micron (μ)
millimicron (mμ)	=	10.	Angstroms (A)
Angstrom (A)	=	0.1	millimicrons (mμ)

Some Approximate Equivalents

1 inch	=	2.54	centimeters
1 pound	=	450.	grams
1 quart	=	1.1	liters
98.6 F	=	37	C
212. F	=	100	C
32.	=	32	C

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