Muscle Physiology

Exercise A: Contraction of Isolated Skeletal Muscle
Exercise B: Physiology of the Myoneural Junction (Demonstration)

Contraction and movement are basic properties of all animal cells. They are evident in ameboid movement, cytoplasmic streaming, movement of cilia and flagella, spindle fiber contraction during mitosis, and muscle cells (where in muscle contraction they reach their highest expression). There is a wide vanety of muscle types in animals, and they perform a wide variety of functions, such as body movement, maintenance of posture, gastrointestinal tract movements, and circulatory movements. All, however, operate through the same basic sliding filament mechanism, using proteins such as actin and myosin. These interact with calcium ions and ATP as the energy source to bring about shortening of the protein machinery.

Vertebrate skeletal muscle is composed of specialized muscle cells organized into sturdy, compact bundles. These and all other muscle cells are often referred to as muscle **fibers**. Skeletal muscle is also known as **striated muscle** because it appears under the microscope as transversely striped, with alternating light and dark bands (Fig. 1). This is the type of muscle making up a large fraction of our body mass. It is attached to skeletal elements and is responsible for the movements of appendages, trunk, respiratory organs, eyes, mouthparts, and so forth. It is also called **voluntary mus**cle because it is innervated by motor fibers from the spinal cord, which are under conscious cerebral control.

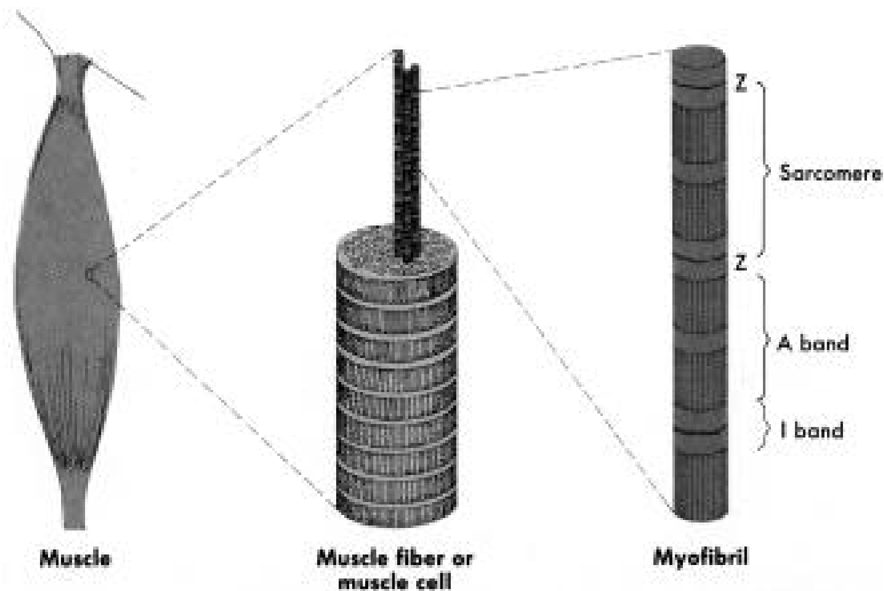


FIGURE 1 Organization of skeletal muscle

◆ EXERCISE A

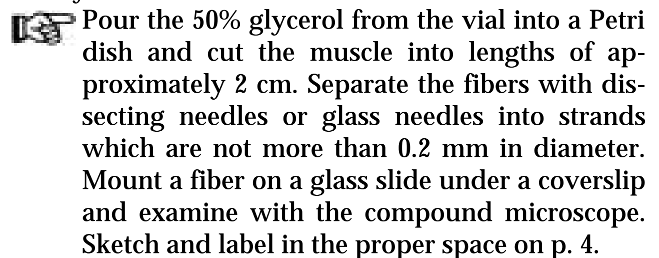
Contraction of Isolated Skeletal Muscle

The muscle fibers that make up muscle tissue are themselves composed of numerous subunits called **myofibrils**—perhaps 1000 to 2000 myofibrils in each fiber, or muscle cell (Fig. 1). The myofibrils are elongate strands of contractile protein that extend from one end of the fiber to the other. The myofibrils are subdivided into units called **sarcomeres**, which give the muscle its striated appearance. Within each sarcomere is an array of thick and thin **filaments**, consisting of bundles of myosin molecules and actin molecules, respectively.

Chemical and electron microscope studies have shown that the myosin molecules have globular heads that serve as cross-bridges to link them to the thinner actin filaments. When the muscle is stimulated, and in the presence of magnesium, calcium, and ATP, the crossbridges swing rapidly back and forth, puffing the filaments toward each other and shortening the sarcomere. This is the **sliding filament** model of muscle contraction, which is strongly supported by experimental evidence.

Glycerinated muscle preparations can be used to demonstrate and study muscle contraction. Small pieces of muscle were removed from a rabbit and the soluble constituents extracted with 50% glycerol at low temperature. This leaves the fibrous contractile proteins intact. Studies with glycerinated muscle are important to scientists who need to control the composition of the intracellular environment without destroying the contractile machinery.

In this simple experiment you will be able to witness and measure the contraction of muscle fibers in the presence of essential ions and with ATP as a source of energy. Strips of skeletal muscle have been extracted with 50% glycerol, tied to a stick, and stored in a vial of glycerol. You are also provided with (1) 0.25% ATP in triply distilled water, (2) 0.25% ATP plus 0.05 M KCl plus 0.001 M $MgCl_2$ in distilled water, and (3) 0.05 M KCl plus 0.001 M $MgCl_2$ in distilled water. Glassware and dissecting instruments should be cleaned thoroughly and rinsed in distilled water. The KCl solution is used to restore the normal ionic environment of the muscle cell, since both potassium and chloride are major ions in intracellular fluid. Magnesium is an ion that is necessary to activate the enzyme (ATPase) that catalyzes the action of ATP.



Pour the 50% glycerol from the vial into a Petri dish and cut the muscle into lengths of approximately 2 cm. Separate the fibers with dissecting needles or glass needles into strands which are not more than 0.2 mm in diameter. Mount a fiber on a glass slide under a coverslip and examine with the compound microscope. Sketch and label in the proper space on p. 4.

Place several thinly separated filaments on a glass slide in a minimum amount of glycerol, straighten them out parallel to one another, place on the stage of a compound microscope, and measure their lengths with a transparent millimeter rule. Record in your report.

Flood the fibers with a few drops of the solution of ATP plus KCl and $MgCl_2$; observe carefully for about 30 seconds; repeat the measurements and calculate the degree of contraction. Examine the contracted fibers Under a cover glass with the compound microscope. Make a sketch in the proper space on p. 4.

Repeat with clean slides and fresh fibers, using only ATP. Repeat with KCl plus $MgCl_2$ but with no ATP. Explain the results in your report.

◆ EXERCISE B

Physiology of the Myoneural Junction

The contraction of muscle fibers is triggered by impulses arriving at a synapse where a motor nerve makes contact with the muscle fiber. This functional connection is called a **myoneural junction** (= motor endplate). At this narrow gap between nerve ending and muscle membrane the arriving nerve action potential stimulates the release of acetylcholine from synaptic vesicles in the synaptic bulb (Fig. 2). In less than a millisecond the acetylcholine diffuses across the 20 nm space that separates the nerve and muscle membranes. It then combines with special receptor sites on the postsynaptic (muscle) membrane, leading to an abrupt drop in muscle membrane permeability. This creates a large muscle action potential. The electrical depolarization sweeps through the muscle fiber, generating a muscle contraction. It all happens within a few hundreths of a second.

There are a number of inhibitors and poisons that interfere with this elegant chain reaction. One such inhibitor is curare, long used by South American Indians as an arrowhead poison. An animal or

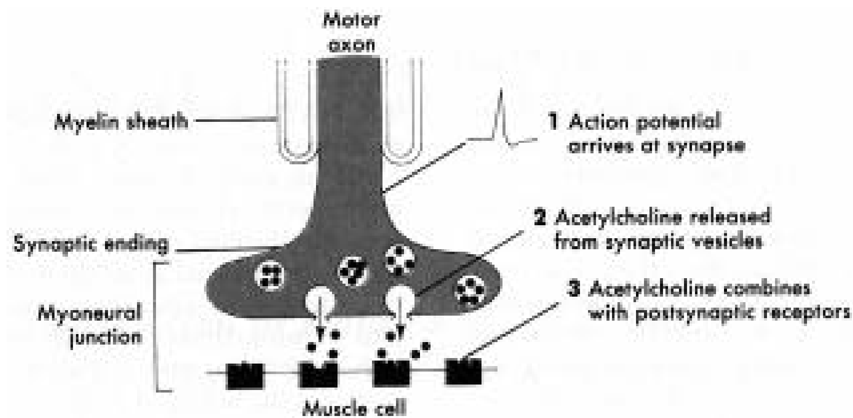
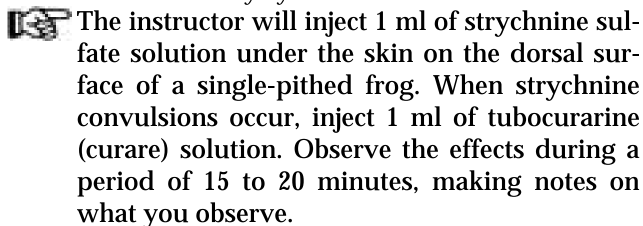


FIGURE 2 A myoneural junction, showing how acetylcholeine (the transmitter substance) released from synaptic vesicles diffuses across the synaptic cleft to bind with receptors on the postsynaptic membrane of the muscle cell.

human struck with such an arrow is soon paralyzed, though remaining fully conscious. Curare (the purified form is *d*-tubocurarine) competes with acetylcholine, the neurotransmitter substance, for receptor sites on the postsynaptic membrane, thus blocking muscle contraction. In this demonstration you will observe competitive inhibition of postsynaptic receptor sites and blockage of motor impulses across the myoneural junctions.

Effect of Strychnine and Curare

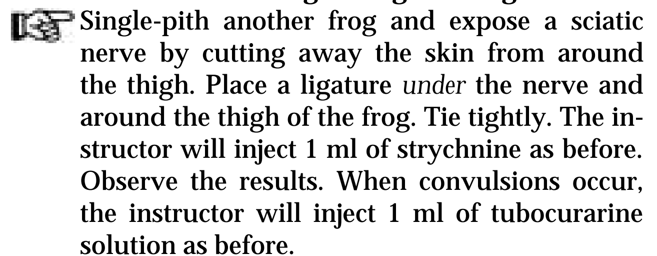
The exercise should be treated as a demonstration. *Curare and strychnine, both potent alkaloids that are extracted from various plants, are poisonous substances that should be handled only by the instructor.*



The instructor will inject 1 ml of strychnine sulfate solution under the skin on the dorsal surface of a single-pithed frog. When strychnine convulsions occur, inject 1 ml of tubocurarine (curare) solution. Observe the effects during a period of 15 to 20 minutes, making notes on what you observe.

Strychnine is used in this experiment to block inhibitory synapses in the central nervous system. When this happens, the slightest stimulus sets off an enormous chain reaction of neuronal activity. In the normal animal this catastrophic spread of excitation is prevented by inhibitory activity, which modulates all central nervous system activity.

Effect of Curare after Ligaturing One Leg



Single-pith another frog and expose a sciatic nerve by cutting away the skin from around the thigh. Place a ligature *under* the nerve and around the thigh of the frog. Tie tightly. The instructor will inject 1 ml of strychnine as before. Observe the results. When convulsions occur, the instructor will inject 1 ml of tubocurarine solution as before.

Wait several minutes for the effect of the curare. The tied leg should now be the only part of the frog to show convulsive behavior. If an electronic stimulator has been provided, stimulate the exposed nerve of the *untied* leg with single shocks. Repeat with the nerve of the tied leg. Now directly stimulate the muscle of the untied leg. Repeat with the muscle of the tied leg. Explain all of your observations in your report on p. 5.

Name ____

Date ___________ **Section** ______________________

Make a sketch below of the muscle fibers as they appear under high power. Label.

Record your measurement of muscle fiber length before and after the addition of ATP plus KCl and $MgCl_2$.

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Make a sketch below of the muscle fibers as they appear after the addition of ATP plus KCl and $MgCl_2$.

What happens when only ATP is applied? Explain. ____

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____

What happens when only KCl and $MgCl_2$ are applied? Explain. ____

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PHYSIOLOGY OF THE MYONEURAL JUNCTION

EFFECT OF STRYCHNINE AND CURARE

Briefly describe the effect of the strychnine injection. ______________________________

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Explain why curare quieted the convulsive frog. ______________________________

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____

Explain why this experiment would not have worked had the spinal cord been destroyed. ______________

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EFFECT OF CURARE AFTER LIGATURING ONE LEG

Briefly describe the results of this experiment. ______________________________

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____

Why does the tied leg still show convulsive behavior after the curare injection? ______________

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____

Does the muscle of the *untied* leg respond when electrically stimulated indirectly through the nerve? __________

Of the *tied* leg? Explain. ______________________________

____

____

Does the muscle of the *untied* leg respond to direct stimulation? __________ Of the *tied* leg? ______________

Explain. ______________________________

____

____

Circulation and Respiration

Exercise A: Study of Human Blood
Exercise B: Capillary Circulation in the Frog
Exercise C: Small Mammal Respiration

◆ EXERCISE A

Study of Human Blood

Blood is a complex liquid suspension composed of plasma and suspended formed elements that include red blood cells (erythrocytes), white blood cells (leukocytes), and platelets (thrombocytes). It is a medium that serves many transportation functions, including the movement of nutrients, the transport of oxygen and carbon dioxide between the cells and the respiratory organs, the movement of wastes to the organs of excretion, and the shuttling of hormones from endocrine glands to target organs. The blood protects the body from the invasion of foreign bodies through phagocytosis and the production of antibodies. It also distributes body heat and provides for its own (and the body's) protection through hemostasis (mechanisms that prevent blood loss).

In this exercise you will demonstrate some of the unique physiological properties of blood by examining your own blood and comparing the values you obtain with the norm for the human population.

I. ABO AND RH BLOOD GROUP SYSTEMS

The human red blood cell contains more than two dozen antigens on its surface. If blood containing one or more of these antigens is transfused into a recipient whose blood contains antibodies to the donor's blood antigens, a serious agglutination reaction may occur. Agglutination is the clumping of red blood cells together. Fortunately only two of these antigen-antibody systems are potentially troublesome in transfusions; these are the ABO system and the Rh system.

The ABO blood group system, discovered by Karl Landsteiner in 1900, is the best known of the blood antigen systems. It is an inherited immune system in which the antigens A and B are inherited as dominant genes. A person with genes *A/A* or *A/O* develops A antigen and is said to have type A blood. If the genotype is *B/B* or *B/O*, the person develops B antigen on the red blood cells and has type B blood.

The peculiar feature of the ABO system (Table 1) is that the person's plasma will *always* contain the noncomplementary antibodies to the blood cell antigens. This is unlike most antigen-antibody responses; normally antibodies develop only after an antigen is introduced into the system. Thus people with type A blood always carry anti-B antibodies in their plasma, and those with type B blood always carry anti-A antibodies. Type AB persons have both A and B antigens but have *neither* anti-A nor anti-B antibodies. Type O persons have no antigens on their red blood cells but have *both* anti-A and anti-B antibodies in their plasma. Thus the blood group name (O, A, B, or AB) identifies the *antigen* content of the blood. Type O persons are called "universal donors" because, having no antigens, their blood can be transfused into a person with any blood type. Type AB people are called "universal recipients" because they have no antibodies to the A or B antigens. But in practice blood types are always carefully

TABLE 1 Major blood groups

Blood type	Genotype	Antigens on red blood cells	Antibodies in serum	Can give blood to	Can receive blood from	Frequency in United States (%)		
						Whites	Blacks	Asians
O	*O/O*	None	Anti-A and anti-B	All	O	45	48	31
A	*A/A, A/O*	A	Anti-B	A, AB	O, A	41	27	25
B	*B/B, B/0*	B	Anti-A	B, AB	O, B	10	21	34
AB	*A/B*	AB	None	AB	All	4	4	10

matched for transfusions. (There are, in fact, other weak antigens present that make the matching of donor and recipient an important requirement for modem blood banking.)

The Rh blood group system is named for the rhesus monkey, in which it was first discovered (again by Karl Landsteiner, 40 years after his discovery of the ABO system!). The Rh system is complex. More than 40 different Rh antigens are known, but only one of these, the D antigen, is so strongly antigenic that it always requires close donor-recipient matching. People having the D antigen are called Rh+, and those lacking it are Rh–.

Unlike the situation in the ABO system, Rh– people do not have the corresponding antibody in their plasma *unless* they have been exposed to the D antigen by a previous transfusion. Then a subsequent transfusion of Rh+ blood (containing the D antigen) into the sensitized Rh– person may result in a serious immune reaction. Therefore blood banks always insist on the typing of donors for Rh factor as well as for ABO. Another aspect of the Rh factor, which is of great significance to expectant mothers, is the potential incompatibility between a mother and the fetus she is carrying. If an Rh-negative mother has an Rh-positive body (father is Rh-positive), she can become immunized by the fetal blood during the birth process. Anti-Rh antibodies can cross the placenta during a subsequent pregnancy and agglutinate the fetal blood.

Safety Procedures for Studies with Human Blood

To prevent contact with another person's untreated blood and the consequent risk of disease transmission, the following procedures must be rigidly followed.

- In blood-letting procedures, puncture and collect blood from your own finger, after cleaning the fingertip with alcohol. If for any reason you are to assist another student in blood collection, wear protective latex gloves.
- Use a sterile, disposable blood lancet for puncturing your finger and discard the lancet immediately after use in a disposable bag designated for this purpose by the instructor.
- Dispose of blood lancets, alcohol swabs, used blood test cards, blood smear slides, and any other supplies that have held or have come in contact with human blood in an autoclavable, disposable bag. At the end of the laboratory, the bag will be autoclaved before disposal.

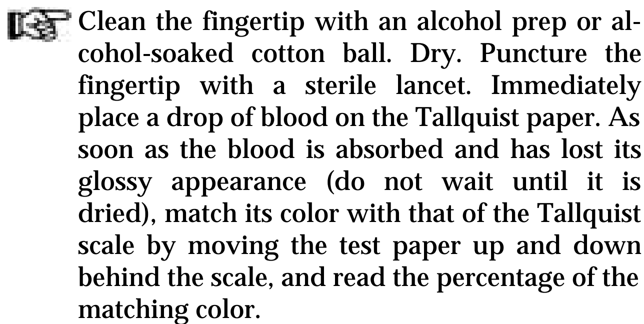
Obtain a blood test card, bottles of antiserums, an alcohol-soaked swab, a disposable sterile lancet, and an instruction sheet accompanying the antiserum kit. Follow these directions closely.

In brief, you will place drops of the anti-A, anti-B, and Rh serums on the blood test card, then add a drop of your fingertip blood to the circles adjacent to the antiserums. The blood and serum in adjacent circles are then mixed together and observed for occurrence of agglutination. Record the results of your own blood type in the blank provided on p. 11. When class results have been tabulated on the board, enter the percentages of persons having each blood type in the table on p. 11.

◆ PREPARATION FOR PARTS II AND III

Each student will be assigned to perform Part II *or* Part III on his or her own blood. If students are working in pairs, one student of the pair will be responsible for the hemoglobin determination and the other for the blood smear. Collect your materials before making the finger puncture: a clean lancet, alcohol prep, cotton balls, and either the Tallquist paper and scale for the hemoglobin test or clean slides for preparing the blood smear. Read over the procedure carefully.

II. AN ESTIMATION OF HEMOGLOBIN CONTENT


Clean the fingertip with an alcohol prep or alcohol-soaked cotton ball. Dry. Puncture the fingertip with a sterile lancet. Immediately place a drop of blood on the Tallquist paper. As soon as the blood is absorbed and has lost its glossy appearance (do not wait until it is dried), match its color with that of the Tallquist scale by moving the test paper up and down behind the scale, and read the percentage of the matching color.

One of the functions of red blood corpuscles is the transport of oxygen, which is dependent on hemoglobin, a red pigment. Hemoglobin is a complex protein combined with an iron-containing compound called heme. Males tend to have more hemoglobin than females, averaging 16 g per 100 ml of whole blood; females average 14 g. According to the Tallquist standard, 15.6 g of hemoglobin per 100 ml of blood equals 100%. What is your percent? To ascertain the grams of hemoglobin per 100 ml of your blood, multiply the percent shown on the chart by 15.6.

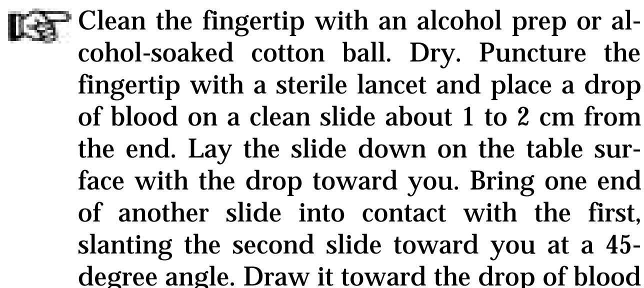
Anemia, which is a deficiency in the amount of circulating hemoglobin, may be caused by too few erythrocytes or by too little hemoglobin in the individual erythrocytes.

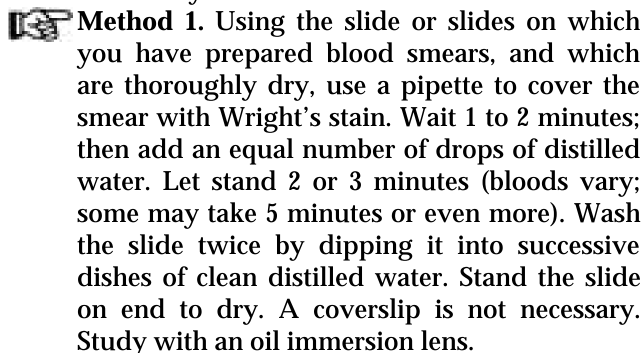
NOTE: Although the Tallquist method is a quick and simple means of estimating the blood hemoglobin, it is not considered accurate enough to be used by clinical laboratories, having a possible error of approximately 20%.

REPORT

Record the percentage reading for your blood on p. 11 and also the number of grams of hemoglobin per 100 ml. Record the latter also on the blackboard. If time permits, determine the average for the males and for the females in your laboratory section.

III. STUDY OF HUMAN BLOOD CELLS


Clean the fingertip with an alcohol prep or alcohol-soaked cotton ball. Dry. Puncture the fingertip with a sterile lancet and place a drop of blood on a clean slide about 1 to 2 cm from the end. Lay the slide down on the table surface with the drop toward you. Bring one end of another slide into contact with the first, slanting the second slide toward you at a 45-degree angle. Draw it toward the drop of blood till it just touches the drop. The blood should spread laterally along the edge of the second slide. Now push the slide *forward* so that it draws the blood along evenly and thinly over the length of the horizontal slide to form a thin, uniform smear. Wave the slide with the smear gently in the air for a moment to speed drying; then set it aside to complete air drying. You are now ready to stain the slide.


Method 1. Using the slide or slides on which you have prepared blood smears, and which are thoroughly dry, use a pipette to cover the smear with Wright's stain. Wait 1 to 2 minutes; then add an equal number of drops of distilled water. Let stand 2 or 3 minutes (bloods vary; some may take 5 minutes or even more). Wash the slide twice by dipping it into successive dishes of clean distilled water. Stand the slide on end to dry. A coverslip is not necessary. Study with an oil immersion lens.

Method 2 (quick method). After the blood smear has air dried, add 10 to 12 drops of Camco Quick stain. Leave 30 seconds; then, without draining off the stain, lay the slide into a dish of distilled water and rock the dish to rinse. Rinse again in clean water; then carefully blot the slide *(do not rub)* with two sheets of filter paper. Stand on end and allow to air dry without a coverslip. This method stains the cytoplasm an orange pink and the nuclei purple. Study the cells with an oil immersion lens.

If you prefer to cover the slides, use only a synthetic or neutral balsam mounting medium.

DRAWING

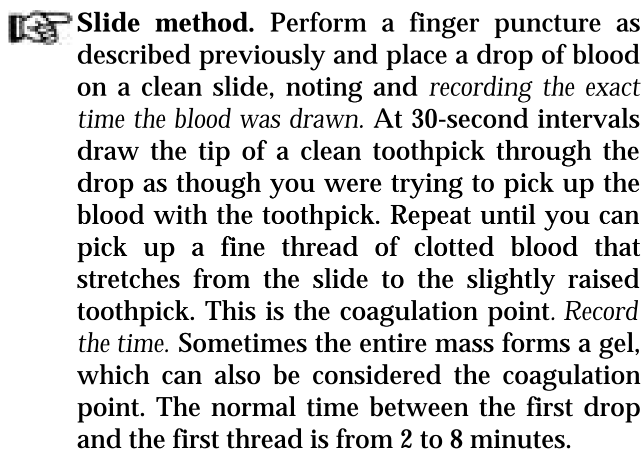
Sketch and label on p. 11 examples of as many types of blood cells as you can identify. Use colored pencils is you wish.

IV. SOME OPTIONAL BLOOD TESTS

Coagulation Time

Blood clotting, or **coagulation**, is a protective device of the body for preventing hemorrhage from a wound. It is a complex physiological process by which fibrinogen, a large plasma protein, is converted into an insoluble threadlike protein called fibrin, which entangles the blood cells, forming a gel-like clot. Read about the entire process in your text.

The process of coagulation (clotting) should not be confused with agglutination (clumping), as seen in the exercise of blood typing.



Slide method. Perform a finger puncture as described previously and place a drop of blood on a clean slide, noting and *recording the exact time the blood was drawn.* At 30-second intervals draw the tip of a clean toothpick through the drop as though you were trying to pick up the blood with the toothpick. Repeat until you can pick up a fine thread of clotted blood that stretches from the slide to the slightly raised toothpick. This is the coagulation point. *Record the time.* Sometimes the entire mass forms a gel, which can also be considered the coagulation point. The normal time between the first drop and the first thread is from 2 to 8 minutes.

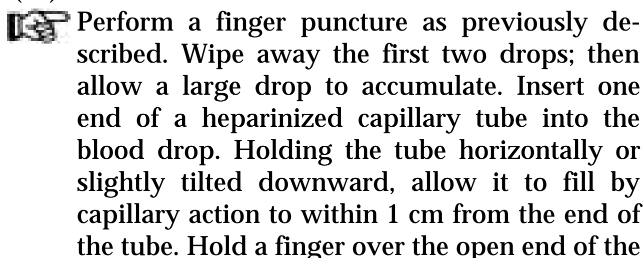
Capillary tube method. Perform a finger puncture as described previously and wipe off the first drop of blood. When the next drop appears, *record the time,* and place a non-heparinized capillary tube in the drop, holding the tube lower than the drop, so that it becomes nearly filled with blood. At each 30-second interval break off a small section of the tube. Continue until a small fibrin thread forms between the two ends of the tube. This is the coagulation point. *Record the time.*

REPORT

Record your clotting time on p. 13 and also on the blackboard. When all class members have recorded individual clotting time, determine the average time for the group and also the maximal and minimal times in the class.

Hematocrit (Micromethod)

The **hematocrit** is the volume of red blood cells in whole blood. It is obtained by centrifuging whole blood, then measuring the relative proportion of red cells to fluid (plasma). For example, a hematocrit of 46 means every 100 ml of whole blood has 46 ml of red blood cells and 54 ml of plasma. The normal hematocrit for males is 47.0 (±7) and for females 42.0 (±5).



Perform a finger puncture as previously described. Wipe away the first two drops; then allow a large drop to accumulate. Insert one end of a heparinized capillary tube into the blood drop. Holding the tube horizontally or slightly tilted downward, allow it to fill by capillary action to within 1 cm from the end of the tube. Hold a finger over the open end of the tube, withdraw the tube from the blood drop, and seal the blood-filled end with wax. Place the sealed tube into one of the grooves of the microhematocrit centrifuge, *with the sealed end of the tube at the outer circumference of the centrifuge.* Spin the centrifuge by hand a few times to make sure the tubes are properly placed; then close the lid and centrifuge for 5 minutes.

Remove the tube and determine the hematocrit ratio by using the hematocrit scale. If no scale is available, measure the height of the red cell column and the total height of the blood column. To find the percentage of cells, divide the height of the cells by the total height and multiply by 100.

REPORT

Record your hematocrit on p. 14. If time permits, compute the class average for both males and females.

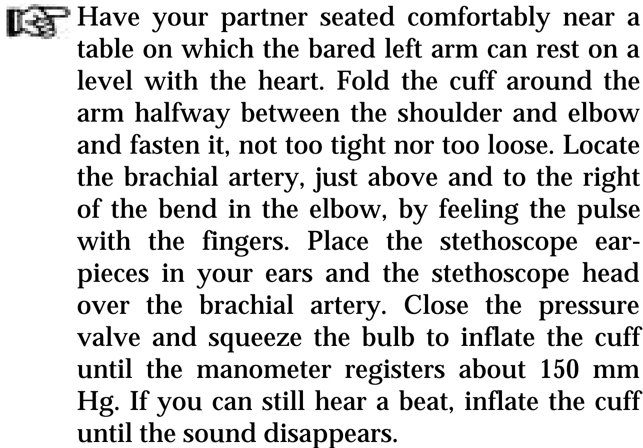
V. BLOOD PRESSURE DETERMINATION

Blood pressure is the force the flowing blood exerts against the walls of the arteries. When the wall of the left ventricle contracts and forces blood into the aorta and its branches, the pressure in the arteries increases. This maximum pressure is called the **systolic pressure.** When the heart rests—when the left ventricle is relaxing and the aorta is decreasing in diameter—the pressure in the arteries is reduced and is called the **diastolic pressure**. Many factors, such as exercise, state of mind, age, or even the time of day, can influence the blood pressure.

Both systolic and diastolic pressure are measured by a device known as a **sphygmomanometer** (sfig' mo-man-om'et-er), which consists of an inflatable arm cuff, an inflating bulb for inflating the cuff, a screw valve for controlling the rate of inflation or deflation, and a manometer or a calibrated gauge for reading the pressure. A stethoscope is used to detect the sounds of the heartbeats. The cuff, placed around the arm just above the place where the brachial artery branches into the radial and ulnar arteries, is inflated until the pressure stops the circulation in the area. Air is slowly released until a thumping sound detected by the stethoscope indicates that blood is again flowing in the artery. The pressure reading at this point is the systolic pressure. As more air is released, the sound dies away, and that point is the diastolic pressure.

Average ranges in blood pressure

	Female		Male	
Age	**Systolic**	**Diastolic**	**Systolic**	**Diastolic**
20	100-130	60-85	105-140	60-80
30	102-135	60-88	110-145	68-92
40	103-150	65-92	110-150	70-94
50	110-165	70-100	115-160	70-98
60	115-175	70-100	115-170	70-100



☞ Have your partner seated comfortably near a table on which the bared left arm can rest on a level with the heart. Fold the cuff around the arm halfway between the shoulder and elbow and fasten it, not too tight nor too loose. Locate the brachial artery, just above and to the right of the bend in the elbow, by feeling the pulse with the fingers. Place the stethoscope earpieces in your ears and the stethoscope head over the brachial artery. Close the pressure valve and squeeze the bulb to inflate the cuff until the manometer registers about 150 mm Hg. If you can still hear a beat, inflate the cuff until the sound disappears.

Slowly open the valve to release air graduallyuntil you hear a tapping sound for at least two consecutive beats. *Record the pressure (systolic) at this point.* Continue releasing air slowly. The sounds will become more distinct, then fade and disappear. *Record the pressure (diastolic) at the instant the sound ceases.*

If you miss the change in sounds at any point, deflate the cuff entirely and wait 1 or 2 minutes before trying again. It is well to take two or three readings and record the average.

DATA

Record your blood pressure and that of your partner on p. 14

A STUDY OF BLOOD

Name ____

Date ___________ **Section** ______________________

I. BLOOD TYPES

What is your blood type? _____________

Determine the percentage of persons of each type in your laboratory section and, if there is time, in all the sections combined:

Blood group	Your section (%)	All sections (%)
O		
A		
B		
AB		

Why is it important to know your blood type? ____

____

To what type might you be able to give a transfusion? ____________________________________

From what type might you be able to receive a transfusion? ______________________________

II. ESTIMATION OF HEMOGLOBIN

What is your Taliquist reading? _________ % _________ g Hb/100 ml

What is the class average? _________% _________ g Hb/100 ml

In your laboratory section what is the average g Hb/100 ml for males? ___________________________

For females? _________

III. STUDY OF BLOOD CELLS

Sketch and label as many types of blood cells as you can identify.

Name ______________________________

Date __________ **Section** ______________

IV. BLOOD TESTS

Coagulation time

What is the coagulation time for your blood? __________

What was the average coagulation time for your section? __________

What was the maximal time in your section? __________

What was the minimal time in your section? __________

What is the difference between coagulation and agglutination? ______________________________

____

____

What is the importnance of coagulation? ______________________________

____

____

What is the source of thromboplastin? ______________________________

____

____

What is its function? ______________________________

What is thrombin, and what is its function? ______________________________

____

____

What is hemophilia? ______________________________

____

Hematocrit

What is your hematocrit? ______________________________

In your laboratory section what is the average hematocrit for males? ______________________________

For females? ______________________________

How do these values compare with normal hematocrit values? ______________________________

____

How does your hematocrit compare with your hemoglobin estimate? ______________________________

____

____

If a hematocrit is low, would you expect to find the hemoglobin content high or low? ______________

Why? ______________________________

____

If the hemglobin content of blood is below normal in an individual, would the hematocrit necessarily be below normal also? __________ Why or why not? ______________________________

____

V. BLOOD PRESSURE

What is your blood pressure? __________Your partner's? __________

Why is high (systolic) blood pressure often associated with arteriosclerosis (hardening of the arteries)?

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If you undergo physical exercise strenuous enough to increase your pulse, how might this affect your blood pressure? ______________________________

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◆ EXERCISE B

Capillary Circulation in the Frog

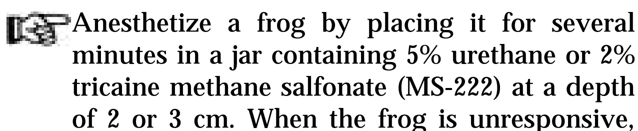
Capillaries, the extensive network of small vessels that permeate the body's tissues, are the raison d'etre of the circulatory system. Through these tiny tubes, seldom more than 1 mm in length and only 10 to 25 µm in diameter, flow the oxygen and nutrients body cells require. So vast is the network that any single body cell is not more than 2 or 3 cells away from a capillary. High arterial pressures are required to force blood through the millions of capillaries because resistance to fluid flow increases dramatically as vessel diameters narrow. Despite their delicate dimensions and appearance, capillaries are remarkably tough structures that cannot be easily ruptured with surges of pressure.

Blood flow to tissues depends on need, and control is achieved by the alternate constriction and dilation of vessels. Most important are the arterioles because even small changes in their diameters can effect large changes in resistance to blood flow. Most arterioles receive sympathetic nerves, which release noradrenaline at their endings in smooth muscle fibers composing most of the walls of the arterioles. Increased sympathetic discharge to these arterioles will constrict them, squeezing off blood flow to the capillaries beyond. Nervous control ensures that only a limited number of capillaries are open to receiving blood at one moment, for if all opened at once, the blood pressure would drop dramatically, impairing the crucial flow of blood to the brain. Indeed, only 3% to 5% of all capillaries are open at any time, and not more than 7% of the total blood volume is contained within capillaries.

Precapillary sphincters are located where arterioles enter the capillary bed; the sphincters are under local rather than nervous control. These sphincters, together with the smallest arterioles and the capillaries, are immersed in the metabolic environment of the tissues they serve. Active tissues use up oxygen and produce metabolites (for example, CO_2 and H^+) that cause the sphincters to dilate, increasing blood flow and flushing out metabolites while bringing in needed oxygen.

In this exercise you will examine the capillary circulation of the frog's tongue and observe blood cells circulating through arterioles, capillaries and venules.

I. STUDY OF CAPILLARY CIRCULATION

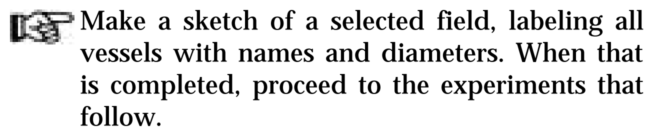


Anesthetize a frog by placing it for several minutes in a jar containing 5% urethane or 2% tricaine methane salfonate (MS-222) at a depth of 2 or 3 cm. When the frog is unresponsive, rinse with tap water and place it ventral side down on the frog board. Extend the tongue and spread it over the opening in the board. Pin the edges to the board so that the tongue is flat and spread evenly. Keep the tongue wet with water. Wrap the frog's body with a wet paper towel and tie the frog onto the board with string. Place the board on the stage of a compound microscope and position it so that the substage light passes through the hole and the tongue.

Study the capillary circulation with low power. You should see a beautiful vascular network and be able to distinguish erythrocytes (red blood cells) coursing rapidly through the vessels. Identify arterioles, capillaries, and venules by their diameters and direction of blood flow. Note the relative speed of cell movement in the vessels. Can you distinguish the effect of the heartbeat in any of the vessels by rhythmic changes in flow velocity? Does flow rate become smooth in the smaller vessels? Why do cells travel faster in some vessels than in others? Is flow rate in large vessels faster in the center or along the edges of the vessel? Look closely at the individual erythrocytes. What is their shape? Do they contain nuclei? Compare with human erythrocytes. Can the erythrocyte bend? Look for white blood cells, which sometimes can be seen as small, colorless spheres moving slowly along the periphery of vessels. Sometimes they stick to vessel walls. What is the function of white blood cells?

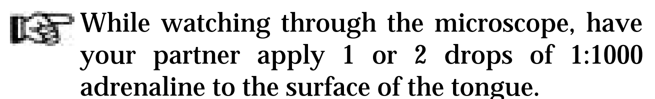
Now examine the capillary bed more closely. Can you locate the precapillary sphincters? Are all of the capillaries open to flow? Does blood always flow in the same direction through capillaries? Note whether some capillaries open or close over a period of several minutes. Why is a shifting flow of this kind necessary?

Make estimates of the diameters of arterioles, capillaries, and venules by relating to the dimensions of the red blood cells. A frog's erythrocytes are about 20 µm long, 15 µm wide, and 5 µm thick. Estimate the relative thickness of vessel walls.



Make a sketch of a selected field, labeling all vessels with names and diameters. When that is completed, proceed to the experiments that follow.

II. ACTION OF ADRENALINE



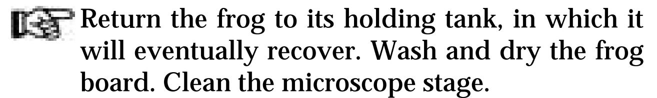
While watching through the microscope, have your partner apply 1 or 2 drops of 1:1000 adrenaline to the surface of the tongue.

Do vessels constrict? Do the precapillary sphincters change diameter? How is blood flow through the capillary bed altered? Record again the diameters of vessels that you measured before adrenaline was applied. When your observations are complete, thoroughly rinse the tongue with water.

III. ACTION OF METHYL SALICYLATE

When the circulation has returned to normal, pick up a very small amount of methyl salicylate on the tip of a dissecting needle and apply to the frog's tongue in the field of view.

What is the response of the vascular bed? Does local circulation increase or decrease? Which vessels change diameter and how rapidly? Why is methyl salicylate (oil of wintergreen) sometimes used in liniments?



Return the frog to its holding tank, in which it will eventually recover. Wash and dry the frog board. Clean the microscope stage.

Name ____

Date ____________ **Section** _____________________

1. Study of capillary circulation

Answer all of the questions in this section in brief essay form. Prepare a labeled sketch of the circulation you see, using a separate sheet of paper.

Action of adrenaline

What is the effect of adrenaline on vessel diameter? ____

____

Do all vessels respond alike? Explain. ____

Describe the effect of adrenaline on blood flow in the capillary bed. __________________________________

____

Record here the diameters of vessels measured before and after application of adrenaline.

Vessel	Diameter before adrenaline	Diameter after adrenaline

What is the effect of adrenaline on local capillary delivery of oxygen and nutrients? ____________________

____

Adrenaline does not constrict all vessels in the body; vessels of the heart (coronary circulation) and of skeletal muscles are dilated. Of what advantage is this? ____

____

3. Action of methyl salicylate

What is the effect of this drug on the circulation? ____

____

Why is this drug often used in liniments?____

____

◆ EXERCISE C

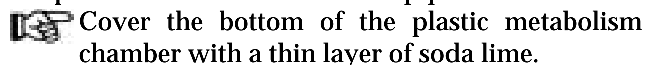
Small Mammal Respiration

Early eighteenth century biologists who wrote that "life is a combustion" were correct. Like the combustion of fuel in a fire, the metabolic reactions of aerobic organisms consume oxygen, "burn" fuel (foodstuffs), and release heat. But there is an important difference: metabolic combustions are controlled, flameless, and of low temperature. Furthermore, the energy released in metabolic reactions is coupled to a great variety of energy-consuming reactions.

The rapidity at which an animal consumes energy is called its **metabolic rate**. The metabolic rate can be thought of as the speed at which the body fires are burning, and indeed an animal's metabolic reactions all eventually end with the liberation of heat. Biologists are often very interested in knowing what the metabolic rate is because it provides a direct measure of how rapidly the animal is converting food into active metabolites, as well as how the energy metabolism of the organism is affected by body size, activity, and various environmental factors such as cold or heat. Of several methods that have been used to measure the metabolic rate (including the rather cumbersome one of actually measuring total heat production), the one most commonly used is the measurement of oxygen consumption.

The rationale for relating oxygen consumption to energy metabolism is that the amount of heat produced for each liter of oxygen consumed remains almost constant, no matter what the animal is eating. If the animal eats pure carbohydrates, it produces 5.0 kilocalories (kcal) of heat per liter of oxygen consumed.* The oxidation of fat produces 4.7 kcal of heat per liter of oxygen consumed. The oxidation of protein produces about 4.5 kcal of heat per liter of oxygen consumed (protein, unlike fat and carbohydrate, is not completely oxidized in the body, so yields a lower energy value). Thus the energy values of the different foodstuffs are not greatly different from each other. Because most animals in fact eat mixtures of carbohydrates, protein, and fat, we can accept an average energy value of 4.8 kcal. The largest possible error from using this figure is 6%.

The exercise described here will enable you to measure the oxygen consumption of a gerbil or small rat using a commercially available metabolism chamber (Fig. 1). The principle of operation is simple: oxygen is consumed and carbon dioxide is released by the animal. Because the carbon dioxide is absorbed by soda lime as it is released, the removal of oxygen from the air reduces the volume of gas space within the respiration chamber. This volume change is measured by timing the movement of a soap bubble within a volumetric pipette.


Cover the bottom of the plastic metabolism chamber with a thin layer of soda lime.

*A calorie is the amount of heat required to heat 1 g of water from 14.5° C to 15.5° C. 1 kcal = 1000 cal. Although the calorie has been the traditional unit for heat, it is not recognized by the International System of Units (SI System), in which the energy unit is the joule (J), defined as 1 cal = 4.184 J.

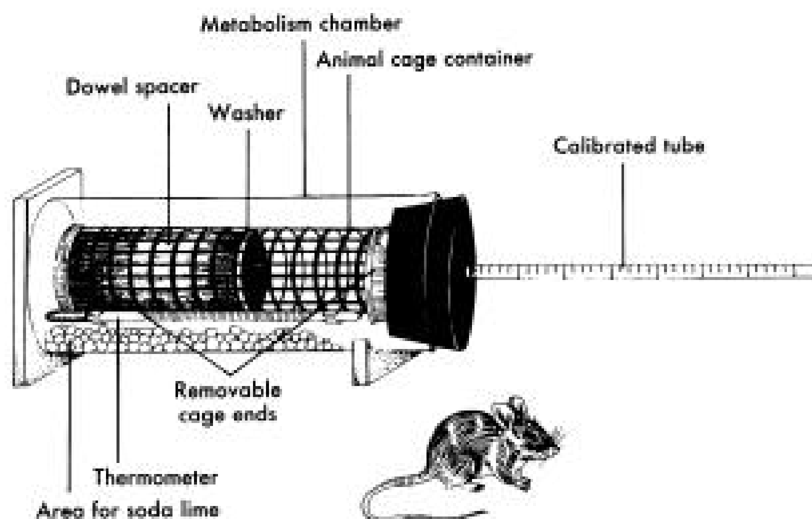
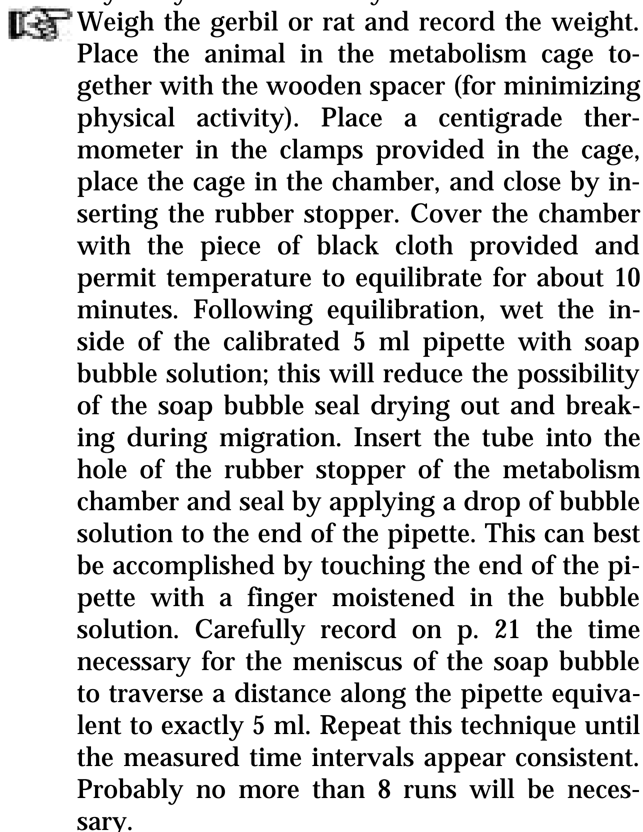


FIGURE 1 Apparatus for measuring the respiration of a small mammal.

Soda lime will absorb all expired carbon dioxide and allow for the measurement of oxygen consumption only. Why is this necessary?



Weigh the gerbil or rat and record the weight. Place the animal in the metabolism cage together with the wooden spacer (for minimizing physical activity). Place a centigrade thermometer in the clamps provided in the cage, place the cage in the chamber, and close by inserting the rubber stopper. Cover the chamber with the piece of black cloth provided and permit temperature to equilibrate for about 10 minutes. Following equilibration, wet the inside of the calibrated 5 ml pipette with soap bubble solution; this will reduce the possibility of the soap bubble seal drying out and breaking during migration. Insert the tube into the hole of the rubber stopper of the metabolism chamber and seal by applying a drop of bubble solution to the end of the pipette. This can best be accomplished by touching the end of the pipette with a finger moistened in the bubble solution. Carefully record on p. 21 the time necessary for the meniscus of the soap bubble to traverse a distance along the pipette equivalent to exactly 5 ml. Repeat this technique until the measured time intervals appear consistent. Probably no more than 8 runs will be necessary.

If your readings are not consistent look for the following problems: (1) leaks in the system, (2) insufficient CO_2 absorbent, (3) dirty or blocked pipette, (4) animals variably active in respirometer, or (5) changing temperature within chamber.

Calculate the volume of oxygen consumed per minute by dividing the total number of ml consumed during 4 *consistent* runs (20 ml) by the total length of time in seconds required to make the 4 measurements. Then:

ml oxygen consumed per minute =

In your calculations you must correct the measured volume of oxygen consumed in milliliters per minute to standard temperature and pressure (STP). It is conventional in all respiration studies to express oxygen consumed at 00 C and 760 mm Hg pressure. Use the following formula:

O_2 consumed/minute STP =

in which:

BP = barometric pressure in mm Hg
T° C = temperature inside metabolism chamber

Calculate heat production (energy metabolism) by your animal by assuming that a normal animal releases 4.8 kcal for every liter of oxygen consumed. To do this, multiply your corrected oxygen consumption (ml/min) by 0.0048. (Because oxygen consumption is expressed in ml instead of L, the 4.8 kcal must be divided by 1000; i.e., 4.8/1000 = 0.0048.) This yields the number of kilocalories of heat produced by the animal per minute. Since basal metabolic rate determinations are based on a 1-hour interval, the number of kilocalories produced must be multiplied by 60. Thus:

$$\text{kcal/hour} = \text{ml } O_2 \text{ consumed/minute}_{STP} \times 0.0048 \times 60$$

Finally, calculate the amount of food the gerbil or rat must eat to maintain a steady body weight. We know that 1 L of oxygen consumed is equivalent to about 4.8 kcal of energy production. Using the figure you obtained in your experiment for kilocalories produced each hour by your animal, and assuming the metabolizable energy value of the animal's food to be 3 kcal/g, calculate the amount of food it must eat each day.

Name ____

Date ___________ **Section** ______________________

Explain why it was necessary to use soda lime in the experiment. ____

____

____

What would be the effect on your results if chamber temperature were not in equilibrium before starting the experiment?____

____

Record below the time in seconds for the animal to consume 5 ml of oxygen for each run.

	Seconds	**chamber T**		**seconds**	**chamber T**
Run 1	____________	____________	Run 5	____________	____________
Run 2	____________	____________	Run 6	____________	____________
Run 3	____________	____________	Run 7	____________	____________
Run 4	____________	____________	Run 8	____________	____________

Total of 4 most consistent measurements = ____________ seconds

Calculate the millimeters of oxygen consumed per minute (show calculation)

ml O_2 consumed per minute =

Record barometric pressure. ______________________ Record average chamber temperature. ___________________

Calculate oxygen consumption corrected to standard temperature and pressure (write out complete formula and answer).

O_2 consumed per minute_{STP} =

Calculate heat production by your animal (show calculation).

kcal/hr =

Calculate the amount of food the animal must eat to maintain unchanging body weight (show calculation).

Food (g) consumed per day =

Comment on sources of error in this experiment. ____

____

____

If you were to repeat this experiment, what would you do to obtain more accurate results?_______________

____

____

Digestion

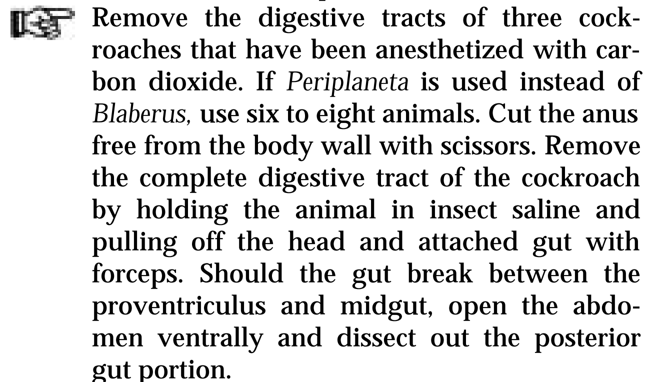
Exercise 26: Distribution of Digestive Enzymes

◆ EXERCISE A

Distribution of Digestive Enzymes

Among the vertebrates and many invertebrates, digestion is largely or completely an extracellular process through which complex foodstuffs are reduced to simple organic units suitable for absorption. The digestive tract of a cockroach (Fig. 1) is like that of most vertebrates: a tube modified into subdivisions that serve specialized digestive functions. These functions are food reception, conduction and storage, internal trituration (grinding), digestion, absorption, and conduction and formation of feces. Digestive enzymes are rather sharply localized along the digestive tract. In this exercise the activity of specific enzymes in different regions of the cockroach digestive tract will be determined through using suitable substrates.

The following parts of the cockroach digestive tract are to be tested for the presence of hydrolytic enzymes: salivary glands and reservoirs, crop, midgut with attached caeca, and hindgut. All four regions of the gut should be tested for the presence of sucrase, amylase, protease and lipase. Tabulate your results on the board so that all students have class results available for the report.



Remove the digestive tracts of three cockroaches that have been anesthetized with carbon dioxide. If *Periplaneta* is used instead of *Blaberus,* use six to eight animals. Cut the anus free from the body wall with scissors. Remove the complete digestive tract of the cockroach by holding the animal in insect saline and pulling off the head and attached gut with forceps. Should the gut break between the proventriculus and midgut, open the abdomen ventrally and dissect out the posterior gut portion.

Refer to the diagram and identify the salivary

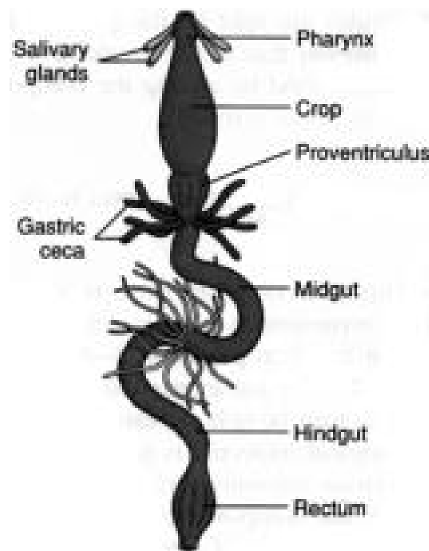
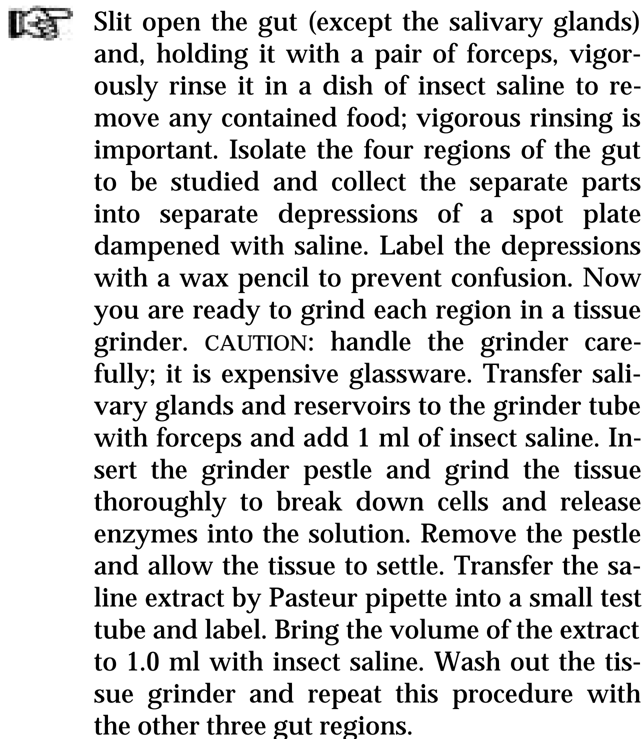


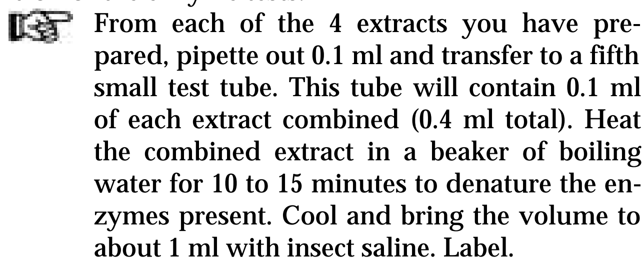
FIGURE 1 Insect digestive system (cockroach). The proventriculus is a gizzard containing chitinous teeth for grinding food.

glands, crop, proventriculus, midgut with attached caeca, hindgut with attached malpighian tubules, and rectum.



Slit open the gut (except the salivary glands) and, holding it with a pair of forceps, vigorously rinse it in a dish of insect saline to remove any contained food; vigorous rinsing is important. Isolate the four regions of the gut to be studied and collect the separate parts into separate depressions of a spot plate dampened with saline. Label the depressions with a wax pencil to prevent confusion. Now you are ready to grind each region in a tissue grinder. CAUTION: handle the grinder carefully; it is expensive glassware. Transfer salivary glands and reservoirs to the grinder tube with forceps and add 1 ml of insect saline. Insert the grinder pestle and grind the tissue thoroughly to break down cells and release enzymes into the solution. Remove the pestle and allow the tissue to settle. Transfer the saline extract by Pasteur pipette into a small test tube and label. Bring the volume of the extract to 1.0 ml with insect saline. Wash out the tissue grinder and repeat this procedure with the other three gut regions.

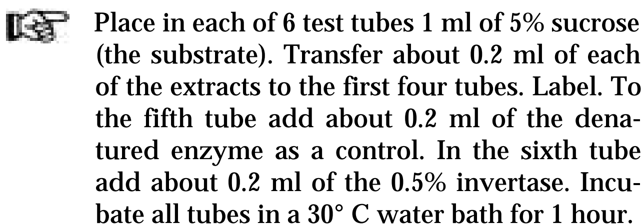
Before proceeding with the experimental sections, you need to make a suitable denatured enzyme control for the enzyme tests.



From each of the 4 extracts you have prepared, pipette out 0.1 ml and transfer to a fifth small test tube. This tube will contain 0.1 ml of each extract combined (0.4 ml total). Heat the combined extract in a beaker of boiling water for 10 to 15 minutes to denature the enzymes present. Cool and bring the volume to about 1 ml with insect saline. Label.

This is your enzyme control. Why is it needed? Do not confuse this denatured control with the four tissue extracts. You are now ready to test the tissue extracts for enzyme activity.

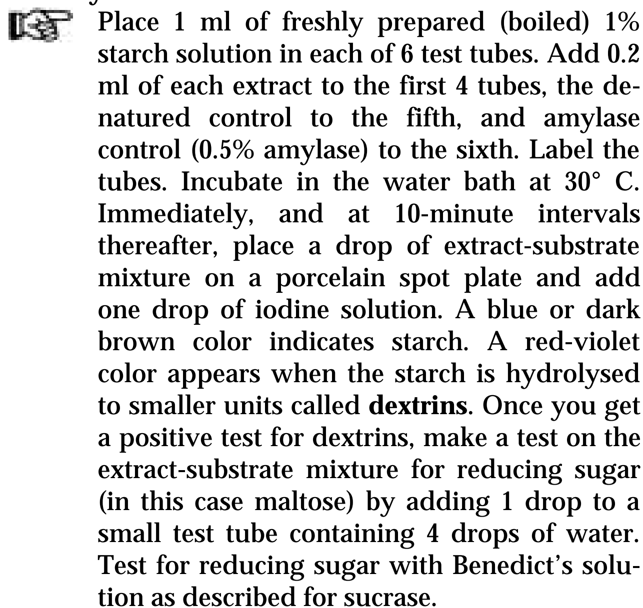
1. Sucrase



Place in each of 6 test tubes 1 ml of 5% sucrose (the substrate). Transfer about 0.2 ml of each of the extracts to the first four tubes. Label. To the fifth tube add about 0.2 ml of the denatured enzyme as a control. In the sixth tube add about 0.2 ml of the 0.5% invertase. Incubate all tubes in a 30° C water bath for 1 hour.

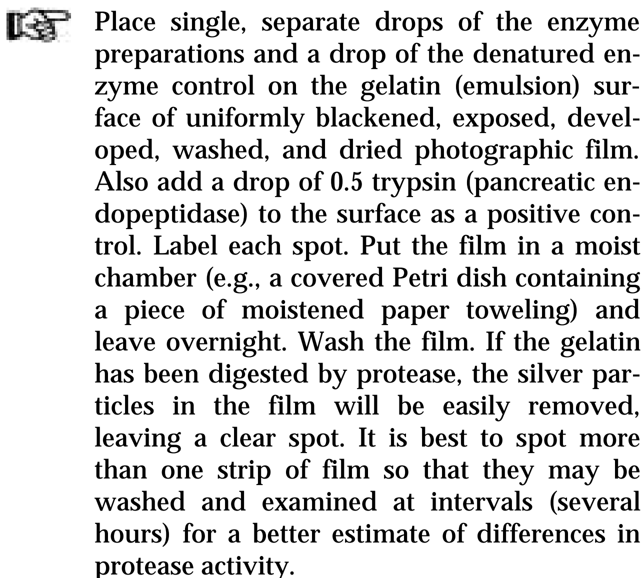
After incubation, test each tube for the presence or absence of reducing sugars by adding 1 drop of extract-substrate mixture to a small test tube containing 4 drops of water and 2 drops of Benedict's solution. Heat for 10 minutes in a beaker of boiling water. A reddish precipitate indicates the presence of a reducing sugar or sugars, such as glucose and fructose. What is meant by the term **reducing sugar**? Estimate visually the relative amounts of precipitate to compare sucrase activities of the different tissues.

2. Amylase



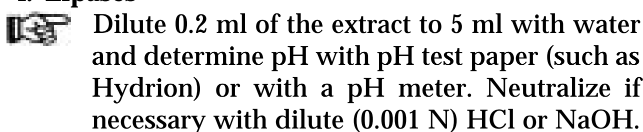
Place 1 ml of freshly prepared (boiled) 1% starch solution in each of 6 test tubes. Add 0.2 ml of each extract to the first 4 tubes, the denatured control to the fifth, and amylase control (0.5% amylase) to the sixth. Label the tubes. Incubate in the water bath at 30° C. Immediately, and at 10-minute intervals thereafter, place a drop of extract-substrate mixture on a porcelain spot plate and add one drop of iodine solution. A blue or dark brown color indicates starch. A red-violet color appears when the starch is hydrolysed to smaller units called **dextrins**. Once you get a positive test for dextrins, make a test on the extract-substrate mixture for reducing sugar (in this case maltose) by adding 1 drop to a small test tube containing 4 drops of water. Test for reducing sugar with Benedict's solution as described for sucrase.

3. Proteases



Place single, separate drops of the enzyme preparations and a drop of the denatured enzyme control on the gelatin (emulsion) surface of uniformly blackened, exposed, developed, washed, and dried photographic film. Also add a drop of 0.5 trypsin (pancreatic endopeptidase) to the surface as a positive control. Label each spot. Put the film in a moist chamber (e.g., a covered Petri dish containing a piece of moistened paper toweling) and leave overnight. Wash the film. If the gelatin has been digested by protease, the silver particles in the film will be easily removed, leaving a clear spot. It is best to spot more than one strip of film so that they may be washed and examined at intervals (several hours) for a better estimate of differences in protease activity.

4. Lipases



Dilute 0.2 ml of the extract to 5 ml with water and determine pH with pH test paper (such as Hydrion) or with a pH meter. Neutralize if necessary with dilute (0.001 N) HCl or NaOH.

Add 1 ml of olive oil and 10 drops of bile salt solution to emulsify the olive oil. Shake vigorously and quickly transfer 1 ml of the mixture to another small test tube, add a drop of phenolphthalein, and titrate with 0.005 N NaOH. Record the number of drops (or volume of titer) needed to turn the indicator pink. Incubate the remaining extract at 25° to 30° C for 1 hour. Remove a 1 ml sample and titrate as before. If time permits, repeat the titration of another 1 ml sample of the incubating mixture 1 hour later. As digestion proceeds, fats are hydrolyzed to fatty acids, and more NaOH is required to neutralize them. Run the lipase control and the denatured extract along with the other extracts, using this procedure.

WRITTEN REPORT

Tabulate your results and those of the class on pp. 26–27 and answer all of the questions.

Name ___________________________________
Date ___________ **Section** _________________

Tabulate your results and average class results below. Use a scale of 0 to + + + + to indicate relative enzyme concentration (0 for absence, + for weak reaction, and + + + + for strongest reaction).

	Your results				**Your class results**			
	sucrase	**amylase**	**proteases**	**lipases**	**sucrase**	**amylase**	**proteases**	**lipases**
Salivary glands and reservoirs								
Midgut and caeca								
Hindgut								

Write a brief interpretation of the results. ____

____

____

____

____

____

What is the function of the cockroach's salivary glands? ____

____

What is the function of the crop? ____

____

Why is it important to rinse out all gut contents before making enzyme tests? __________________________

____

What area of the cockroach gut is the major digestive area? ____

To what region of the vertebrate gut would this compare? ____

____

Write a brief statement on sources of error in your experimental procedure. What would you do differently were you to repeat the experiment? ____

____

____

____

____

Genetics

Exercise A: Inheritance in the Fruit Fly Drosophila
Exercise B: Human Inheritance
Exercise C: Problems in Genetics

Heredity is the transmission from generation to generation of physiological, physical, and psychological factors that cause offspring to resemble parents. The basis of the mechanism of heredity lies in the behavior of the chromosomes and the genes. The genes are believed to be arranged in a linear fashion on the chromosomes. Because there are two sets of chromosomes, there are also two sets of genes. Alternative forms of genes for a given trait (or characteristic) are called **alleles**. If the two alleles for a given characteristic are alike, the organism is pure, or **homozygous**, for that character. If the two alleles are unlike, the organism is hybrid, or **heterozygous**, for that character. Whenever the two alleles for a character are unlike, one of them usually gives expression to the visible character and is called **dominant;** the unexpressed allele is called **recessive**. Organisms of any size usually have both homozygous and heterozygous characters in their makeup.

The genetic constitution of an organixm, as determined by its particular combination of genes, (whether they are expressed or latent), is called its **genotype**. The visible and expressed character of an organism as controlled by the genotype is called its **phenotype**.

◆ EXERCISE A

Inheritance in the Fruit Fly *Drosophila*

Because 2 or 3 weeks is required to complete a genetic experiment, it is usually convenient to run genetic experiments simultaneously with other laboratory exercises. The first matings will have been made previously by the instructor and the resulting F_1 generation will be provided for class use.

If time does not permit these experiments to be performed by the students, the instructor may prefer to make the second crosses as well and provide the F_2 generations for examination and counting.

THE WILD-TYPE DROSOPHILA

Examine a culture of the wild-type *Drosophila.* These will show the collection of trait expressions generally found in wild flies. Try to locate in the bottle the larvae, pupae, eggs, and flying adults.

Etherizing the Flies. Shake a few of the flies into an etherizing bottle and insert the stopper quickly. To kill the flies for leisurely examination, leave them in the bottle for several minutes. To anesthetize them, leave them for only a minute or less, and as soon as they are immobilized, empty them onto a piece of white paper.

CAUTION: *Because ether is dangerously explosive, there must be no flames or lighted cigarettes in the room.*

Kill some of the wild-type flies now for examination. Use a soft brush for handling them.

Examining the Wild-type Flies. Using a hand lens or dissecting microscope, note the size, shape, and color of the flies. Study the wings, noting their size, shape, and color. Notice the color of the compound eye. Examine also the legs, antennae, and balancers. You may consider the expression of traits found in the wild type of flies to be the base line, and any different expressions of these traits to be mutants. A gene for wing development is located on chromosome II in *Drosophila.* The occurrence of greatly reduced, or "vestigial," wings is a recessive mutant condition. A gene for eye color is located on chromosome I. White eye color (from lack of pigmentation) is a recessive mutant condition. A great many such mutations are known to occur fairly

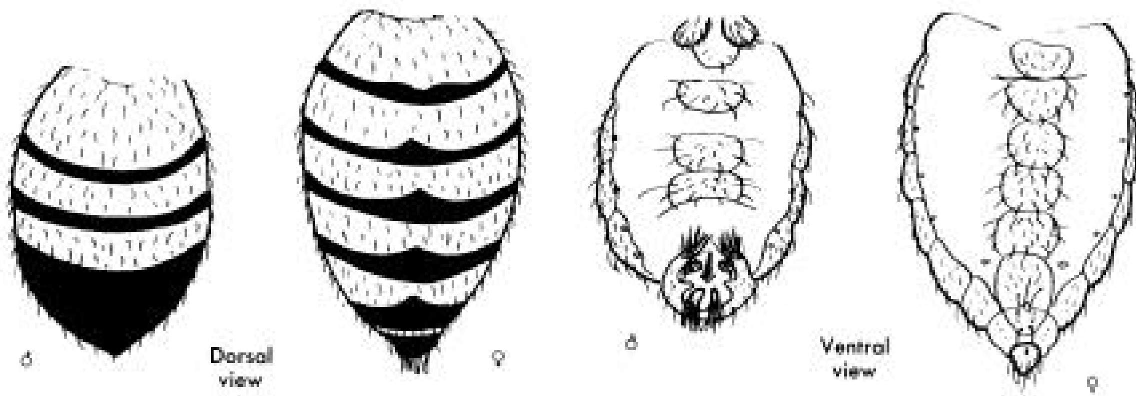


FIGURE 1 Sexual differences in abdomen of *Drosophila* as shown in dorsal and ventral veiews. In each view: **left**, male; **right**, female.

regularly in *Drosophilia.* You may also examine some flies that show these mutant characteristics.

Distinguishing the Sexes. The abdomen of the female is larger, more pointed, and less pigmented than that of the male. It also bears several pigmented stripes evenly spaced on the dorsal side (Fig. 1). The male abdomen is more blunt, and the posterior portion of the dorsal side is dark. On the ventral side the males have an area of dark bristles around the genital opening near the posterior end, and the females do not. With a soft brush, separate the males into one pile and the females into another.

Procedure for Experiments

Each team will receive a bottle that contains the offspring from a mating between a male and female that were different in one easily recognizable characteristic. The parents (P_1) might have been a homozygous long-winged fly (wild type) and a fly with the mutant nonfunctional (vestigial) wings. We call these offspring the F_1 (first filial) generation. The parents of these offspring were removed from the bottle before the emergence of the young to prevent their mating with any of the offspring.

Various symbols can be used to denote certain characteristics. For example, in a one-trait cross in which each individual has one pair of alleles, we might use one of the following:

E = gray body	*e* = ebony body
V = long wings	*v* = vestigial wings
W = red eyes	*w* = white eyes

Here the capital letters indicate dominant, or wild-type, alleles, and the small letters indicate recessive, or mutant, alleles.

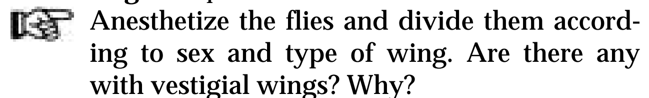
Using *v* for the vestigial-winged parent and *V* for the long-winged parent, one can state this first mating in the following way:

Cross I P_1: $V/V \times v/v$
Gametes: $V \; V \quad v \; v$

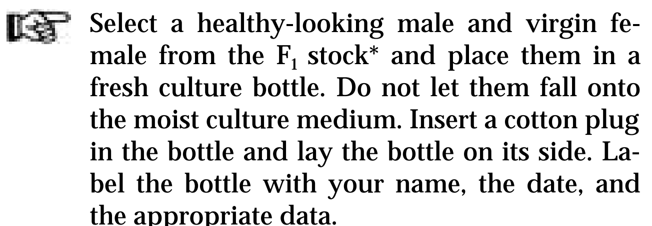
The slash mark between the allele symbols indicates that the genes are on different chromosomes.

What genotypes (genetic makeup) would you expect to find in these offspring? What phenotypes?

Mating the F_1 Flies


Anesthetize the flies and divide them according to sex and type of wing. Are there any with vestigial wings? Why?

REPORT
Record the results of your count under Breeding Experiment 1A on p. 36.


Select a healthy-looking male and virgin female from the F_1 stock* and place them in a fresh culture bottle. Do not let them fall onto the moist culture medium. Insert a cotton plug in the bottle and lay the bottle on its side. Label the bottle with your name, the date, and the appropriate data.

The mating you have just made may be represented symbolically as:

P_2: $V/v \times V/v$

What kinds of gametes can result from this union? What genotypes do you expect among the

*Females do not mate for the first 12 to 18 hours after emergence from the pupa. To obtain virgins, remove all flies from a culture bottle and within 12 hours remove all flies again. Females of this second group are assumed to be virgin.

offspring? What phenotypes? What proportion of each would you expect?

Eggs should appear within a few days after mating; the larvae appear in about 5 days, and the pupae in 7 or 8 days. The flies should emerge in about 11 or 12 days.

> Examine the bottle daily to be sure the parents are alive, replacing them if necessary. On the eighth day remove the parents from the bottle. Two weeks after mating, etherize (kill) this F_2 generation and tabulate your count under Breeding Experiment lB on p. 36. Place team counts in the box, and class totals at the right.

Other Suggested Breeding Experiments

In addition to the preceding mating, the following are suggested, but other crosses may be substituted, depending on the material available. Space for tabulation of results is found on pp. 37–39.

Making a Testcross. Because the long-winged condition is dominant over other wing types, long-winged flies may be either homozygous or heterozygous. A testcross, using a homozygous recessive, can be used to determine the genotype of an individual. Half the teams will receive long-winged flies known by the instructor to be homozygous, and the other half will receive long-winged flies known by the instructor to be heterozygous. Each group will mate long-winged with vestigial-winged flies and in 2 weeks record the results on p. 37 and attempt to determine, by the proportions of the offspring, which group had homozygous parents.

Dihybrid Cross to Show Random Assortment. From the F_1 flies of a cross between a homozygous long-winged ebony-bodied fly *(V/V e/e)* and a homozygous vestigial-winged gray-bodied fly *(v/v E/E),* mate a male and female and note the results. Compare the results obtained by all the teams. You should get the following phenotypes: long-gray, long-ebony, vestigial-gray, and vestigial-ebony. Does the ratio approach 9 : 3 : 3 : 1? Record on p. 38.

Sex-linked Inheritance. In the crosses listed above, it makes no difference in either the F_1 or F_2 generation whether a given characteristic is introduced by the male or by the female. However, some recessive characters are apparently carried on the X chromosome and there are no corresponding alleles on the Y chromosome. White eyes in *Drosophila* is such a character.

Half the teams will receive the F_1 generation of a mating between a white-eyed female *(w/w)* and a red-eyed male *(W/—).* Count and record the results on p. 39. Then mate a white-eyed male *(w/—)* and a red-eyed female *(W/w)* from this generation and 2 weeks later record the results.

The rest of the class will receive the F1 generation of a mating between a red-eyed female *(W/W)* and a white-eyed male *(w/—).* Count and record the results on p. 39. From this generation mate a red-eyed male *(W/—)* and a red-eyed female *(W/w)* and 2 weeks later record the results

◆ EXERCISE B

Human Inheritance

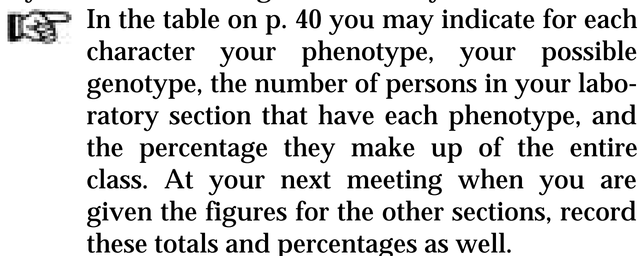
In this exercise you will be able to identify in yourself some of the common heritable traits, compare your results with those of the rest of the class (a sample of the population), and learn some biometrical methods for using and interpreting the statistics you compile. Biometry is the science of statistics as applied to biological observations.

SOME MONOHYBRID TRAITS

The study of human inheritance is complicated by the fact that most inherited characteristics are influenced by the interaction of more than one pair of genes, sometimes many pairs. However, studies have shown many physical characteristics in which variations in a single pair of genes will result in two distinct phases of expression, or **phenotypes.** Following are several of these mendelian traits that you will be able to recognize in yourself or in your friends. Remember, however, that modifying or cumulative factors may be involved in the development of many traits.

You can easily determine your phenotype in each of the following examples. Your phenotype will be the expression of either a **dominant** or a **recessive** gene. If the gene is recessive, you know that you are **homozygous** for that trait—you must be carrying two recessive genes. However, if your phenotype is that of a dominant gene, you may be either homozygous or **heterozygous** for that trait; that is, you may carry two dominant genes or one dominant and one recessive gene. For example, a straight chin is a dominant characteristic; a receding chin is the expression of a recessive character. If you have a receding chin, you can describe your genotype as cc. But if your phenotype is the straight chin, you do not know whether your genotype is *C/C* or *C/c*; so you may indicate this by writing *C/—*, using the dash

(—) to represent the unknown gene. The letters used to represent characters below were chosen arbitrarily. Other authors might use other symbols.



In the table on p. 40 you may indicate for each character your phenotype, your possible genotype, the number of persons in your laboratory section that have each phenotype, and the percentage they make up of the entire class. At your next meeting when you are given the figures for the other sections, record these totals and percentages as well.

Widow's Peak. When the hairline dips down to a point in the center of the forehead, it is called a widow's peak. This condition is caused by a dominant gene *(W)*, whereas the continuous hairline is recessive *(w)*. (The hairline may be altered if the gene for baldness has affected the front part of the head.) (See Fig. 2*A*.)

Convex (Roman) Nose. A high convex bridge, often called a Roman nose, seems to be dominant over a straight or concave nose. The letter *N* may be used to express the dominant gene.

Dimpled Chin. A distinct depression, or dimple, in the chin apparently results from a dominant gene *(D)*. The depth of the dimple is probably controlled by multiple factors.

Rolling the Tongue. Some persons can roll the tongue into a U-shape when it is extended. Others cannot do this. The ability to roll the tongue results from a dominant gene *(R)* (Fig. 2*F*).

Taster of PTC. The ability to taste phenylthiocarbamide seems to be inherited as a simple mendelian character. Taste a piece of treated paper. If you taste nothing, chew the paper. If you are a taster, you will know it—the taste will be very distinct. If you merely detect the taste of the paper itself, or if you are doubtful about the taste, you are a nontaster and are homozygous for a recessive gene (*t*).

Hypermobility of the Thumb Joints. Loose-jointedness, or the ability to put the thumb out of joint, is an inherited characteristic caused by a dominant gene *(H)* (Fig. 2*B*).

Bent Little Finger. Lay your hands flat on the table, muscles relaxed. In some persons a dominant gene *(B)* causes the little fingers to bend toward the ring fingers (Fig. 2*C*). Straight little fingers result from homozygous recessive genes *(b/b)*.

Long Palmar Muscle. Clench your fist and flex

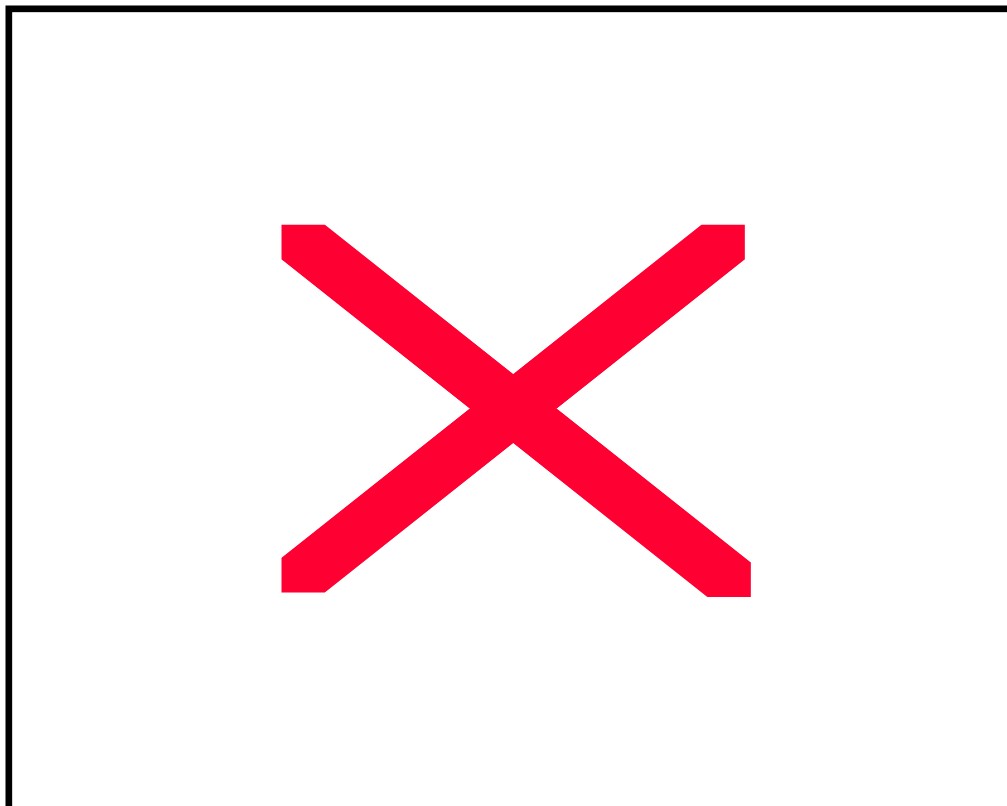


FIGURE 2 Dome inherited characteristics. **A,** Widow's peak. **B,** Hypermobility of thumb joint. **C,** Little finger bent toward other fingers. **D,** Attached small earlobe. **E,** Free earlobe. **F,** Ability to roll tongue.

your hand. Now feel the tendons in your wrist. It there are three tendons there, you have the long palmar muscle and are homozygous for a certain recessive gene *(l)*. If there are only two, you lack this muscle and have the dominant gene *(L)*.

Color of the Eyes—Pigmented Iris. The inner lining of the iris usually appears a deep blue or purple. If there is no pigment in the outer layer of the iris, the eyes appear blue or sometimes gray as a result of the reflection of this purple lining. This lack of pigment is caused by a homozygous recessive gene (*p*). If pigment is deposited in the outer layer, it tends to mask the blue and result in brown or hazel or green eyes, depending on the kind and amount of the pigment deposited. Other genes are responsible for the nature and density of the pigment, but the presence or absence of the pigment depends on the presence or absence of the dominant allele of the gene *(P)*. Consequently, brown, hazel, or green eyes (pigmented iris) are dominant to blue or gray eyes (nonpigmented iris).

Free Ear Lobes. Free ear lobes *(E)* are dominant over attached small ear lobes *(e)* (Fig. 2*D* and *E*).

Blood Groups. Blood groups are inherited. The determination of blood groups involve a gene (I^A) that produces the A antigen, a gene (I^B) that produces the B antigen, and a gene (*i*) that produces neither A nor B antigens. The genes I^A and I^B are both dominant to the gene *i* but intermediate when both are present. A person carrying either I^A/I^A or I^A/I (I^A/—) has type A blood. One carrying I^B/I^B or I^B/I (I^B/—) has type B blood. The combination (I^A/I^B) produces type AB blood, and *i/i* produces type O blood. Record your phenotype and genotype.

APPLICATION OF STATISTICAL METHODS TO GENETICS (BIOMETRY)

Calculating Gene Frequencies

Knowing the percentage of homozygous recessives in any population, one can compute both the gene frequency in that population and the distribution of the genes into the three possible genotypes by using the Hardy-Weinberg law. This law states that in a large, stable population not undergoing mutation, immigration, emigration, or genetic drift and where there is random mating, the population tends to remain in genetic equilibrium, in which the gene frequencies are not appreciably altered from generation to generation. So if we know the gene frequencies of one generation, we can compute the frequencies of the next. The Hardy-Weinberg formula, $p^2 + 2pq + q^2$, is based on the 1 : 2 : 1 ratio of the mendelian monohybrid.

Let us suppose, for example, that 16% of the students in your laboratory section (which we will consider as a sample population) are unable to roll the tongue. Because this is a recessive trait, this 16% must be homozygous recessives (indicated by the genotype *r/r*). We want to find out (1) the proportion (frequency) of the alleles *R* and *r* in this population and (2) the proportion (frequency) of the genotypes *R/R* and *R/r*.

Let the symbol *p* represent the frequency of the *R* allele, and the symbol *q* represent the frequency of the *r* allele. Because there are only 2 alleles present at this locus, $p + q = 1$. Now if 16 students out of 100 are homozygous recessive for the tongue-rolling trait, their frequency is $16/100 = 0.16$. Letting q^2 in the Hardy-Weinberg formula stand for *r/r*, we can write

$$q^2 = 0.16$$

The frequency of the allele *r*, then, is the square root of 0.16:

$$\sqrt{\quad}$$

Now, having found that the frequency of the allele *r* is 0.4, we know that the frequency of *R* must be 0.6 because the two frequencies must always add up to 1 (i.e., $p + q = 1$). So our hypothetical population contains 40% recessive alleles *(r)* and 60% dominant alleles *(R)*.

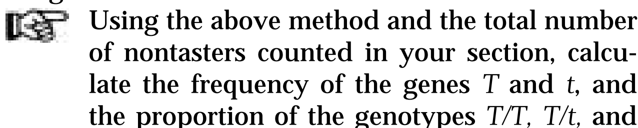
We can show how these alleles come together in the following table:

		Female gametes	
		*p***R** = 0.6	*q***r** = 0.4
Male gametes	*p***R** = 0.6	p^2 **R/R** = 0.36	*pq* **R/r** = 0.24
	*q***r** = 0.4	*Pq* **R/r** = 0.24	q^2 **r/r** = 0.16

Or, using the Hardy-Weinberg formula, we can compute the frequencies as follows:

$$\mathbf{p^2} + \mathbf{2pq} + \mathbf{q^2} = \mathbf{1}$$
$$(0.6)\,(0.6) + 2(0.6)\,(0.4) + (0.4)\,(0.4) = 1$$
$$0.36 + 0.48 + 0.16 = 1$$

Thus in this sample population we would have 36% homozygous dominants, 48% heterozygotes, and 16% homozygous recessives, with a gene frequency of 60% dominant and 40% recessive alleles for the genes controlling the ability to roll the tongue.



Using the above method and the total number of nontasters counted in your section, calculate the frequency of the genes *T* and *t*, and the proportion of the genotypes *T/T*, *T/t*, and

t/t in your section. When the count is in for the total of all the sections, use that count to calculate again. Record your results on p. 41.

In a sample made by L.H. Snyder of nearly 4000 persons, the ratio of tasters to nontasters was found to be 7:3; in other words, 70% were tasters and 30% were not. How closely do your results resemble Snyder's? Which set of your figures is closer to his? Is there a logical explanation for this?

Chi-square Test of Significance

How closely do the results of a test such as the one above agree with those predicted in a total population? Obviously in any sample of the population there is almost sure to be some deviation from the expected ratio, because of the operation of the laws of chance. Certainly the size of the sample is likely to have some effect on the accuracy of figures. The larger the sample, the greater the chance of obtaining the expected ratio. For example, it would not be at all unusual for a couple to have 3 sons and 1 daughter, but if out of 400 children born 300 were reported to be boys, you might expect either an error in the figures or some most unusual cause. How great a deviation, then, is considered "normal"; that is, what deviation would have a high probability of occurring entirely because of chance? This is a problem frequently encountered by scientists: how to determine whether the deviations they observe in their experimental results are significant or not.

Let us consider an example. Suppose two groups of students were given the PTC test. In one group of 85 students, 53 were tasters and 32 were nontasters. In the other group of 30 students, 27 were tasters and 3 nontasters. In the sample made by L.H. Snyder, 70% were tasters and 30% were nontasters. Using this ratio as the expected value, how do our tests compare?

The deviation *(d)* is the difference between the observed value *(o)* and the expected value *(e)* and can be written

$$d = o - e$$

In the group of 85 students:

	Tasters	Nontasters
Observed *(o)*	53	32
Expected *(e)*	.7 × 85 = 59.5	.3 × 85 = 25.5
Deviation *(d)*	53 – 59.5 = –6.5	32 – 25.5 = 6.5

In the group of 30 students:

	Tasters	Nontasters
Observed *(o)*	27	3
Expected *(e)*	0.7 × 30 = 21	0.3 × 30 = 9
Deviation *(d)*	27 – 21 = 6	9 – 3 = 6

The deviation in one group is 6.5 and in the other 6. Is this a significant figure in either case? How does one evaluate the results of such an investigation?

Statisticians have devised many mathematical tests for evaluating observational data. One simple formula, the **chi-square test,** is very convenient for testing the significance of deviations, and is applicable to many genetic experiments, whether dealing with two classes (in this case the two phenotypes, tasters and nontasters) or more, such as peas that have three phenotypes—red, pink and white blossoms, or blood types having four classes—A, B, AB, and O.

Again, using *o* for the observed value, *e* for the expected value, *d* for the deviation, and the Greek letter sigma (k), meaning the "the sum of," we find that the chi-square (x2) value is the following:

So let us return to our figures:

	Tasters	Nontasters
o	53	32
e	59.5	25.5
d	6.5	6.5
d^2	42.25	42.25
	0.71	1.65

Determining the chi-square value for the group of 30:

	Tasters	Nontasters
o	27	3
e	21	9
d	6	6
d^2	36	36
	1.71	4

Now we know the ^{2} values of each group and that each group dealt with two classes (two phenotypes). When dealing with only two classes in a sample of, for example, 100 and knowing that 60 of them belong to one phenotype, we automatically know that 40 of them belong to the other. So there is one independent class (i.e., the class of 60), and once the number of individuals for that class is known, the number of individuals in the remaining class is also known; it is dependent on the first. Thus, in two classes, one is **independent** and the other **dependent**.

TABLE 1 Probabilities for certain values of chi-square

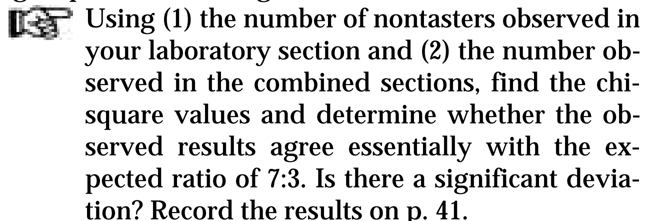
Degrees of freedom	0.50 (1 in 2)	*p* = 0.20 (1 in 5)	*p* = 0.10 (1 in 10)	*p* = 0.05 (1 in 20)	*p* = 0.01 (1 in 100)	*p* = 0.001 (1 in 1000)
1	.46	1.64	2.71	3.84	6.64	10.83
2	1.39	3.22	4.60	5.99	9.21	13.82
3	2.37	4.64	6.25	7.82	11.34	16.27
4	3.36	5.99	7.78	9.49	13.28	18.46
5	4.35	7.29	9.24	11.07	15.09	20.52
6	5.35	8.56	10.64	12.59	16.81	22.46
7	6.35	9.80	12.02	14.07	18.48	24.32
8	7.34	11.03	13.36	15.51	20.09	26.12
9	8.34	12.24	14.68	16.92	21.67	27.88
10	9.34	13.44	15.99	18.31	23.21	29.59

Based on a larger table in Fisher, R.A. 1946. Statistical methods for research workers, ed. 10, Edinburgh, Oliver & Boyd, Ltd.

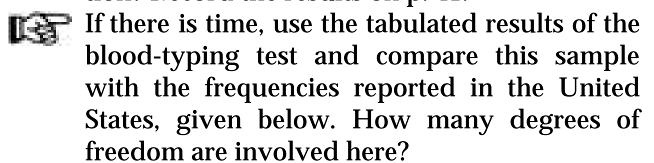
It is customary in statistical work to refer to the number of independent classes in a chi-square table as "degrees of freedom." Because we have one independent class, we have one degree of freedom. If we were dealing with three phenotypes and knew the number of individuals exhibiting two of them, we would automatically know the number in the third class; there would be two independent classes, or degrees of freedom. *The degrees of freedom are always one less than the number of classes involved.*

Tables of chi-square values have been worked out that give the probability of certain deviations occurring by chance alone. Table 1 shows a portion of such a table. Statisticians have decided arbitrarily that if a deviation has a chance probability as great as or greater than 0.05 (1 in 20), it will not be considered statistically significant. In Table 1 you will notice that, for one degree of freedom, the chi-square value given that has 1 chance in 20 is 3.84. In our larger group we found the chi-square value to be 2.36, which has a probability greater than 1 in 20, in fact, even greater than 1 chance in 10 of occurring entirely by chance. So the deviation here is not significant and can be ignored. Our results agree well enough with predicted results.

In the smaller group our chi-square value is 5.71, which would have a smaller chance (between 1-in-20 and 1-in-100) of actually happening normally. So we need to reexamine these results. If it is found that there has been no error in conducting the experiment or in recording the results, then perhaps the smaller size of the group prevented getting the predicted results. Note that although the actual deviations in these two experiments were similar (6.5 and 6), they had quite a different significance in the smaller group than in the larger one.



Using (1) the number of nontasters observed in your laboratory section and (2) the number observed in the combined sections, find the chi-square values and determine whether the observed results agree essentially with the expected ratio of 7:3. Is there a significant deviation? Record the results on p. 41.

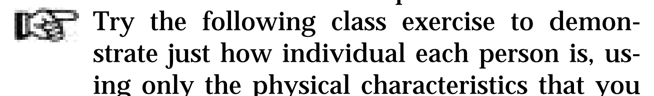


If there is time, use the tabulated results of the blood-typing test and compare this sample with the frequencies reported in the United States, given below. How many degrees of freedom are involved here?

	O	A	B	AB
Blacks	48%	27%	21%	4%
Whites	45%	41%	10%	4%
Asians	31%	25%	34%	10%

Genetic Variability in Humans

No two persons are exactly alike in their genetic constitution, with the exception of identical twins, who are the nearest alike of any two individuals. Even in identical twins certain features may be mirrored in the two members rather than duplicated.



Try the following class exercise to demonstrate just how individual each person is, using only the physical characteristics that you

Recorded earlier on p. 40. Record the results in the table on p. 42.

One person will be selected to stand and read his or her list of characteristics. As the first phenotype on the list is read, all members of the class who share this characteristic will also stand. When the second characteristic is read, only those who also share this phenotype will remain standing; the others **will** sit down. Continue the reading until only the reader remains standing—an individual genetically different from all others in the class. Have one member of the class count the number of persons left standing for each trait. How many traits must be compared before all members are seated except the reader? Repeat this by having three or four other persons read their lists.

EXERCISE C

Problems in Genetics

To understand the fundamentals of genetics, there is no better way than working out genetic problems. In answering these questions, make free use of your textbook. Be sure to show how you arrived at your answers. Use capital letters to represent the dominant gene; its recessive allele should be indicated by the same lowercase letter. Start with the genotypes of the parents and then form the gametes of the parents of the cross. Next, form all possible crosses by the Punnett square. Finally, show the phenotypic ratio. Place your answers in the blanks provided and your explanations on other paper.

1. Usually brown eyes in humans are dominant to blue eyes.

a. The child of brown-eyed parents has blue eyes. What is the genotype of each parent?_________

b. If a blue-eyed man marries a brown-eyed woman whose mother had blue eyes, what proportion of their children might be expected to have blue eyes?

____

2. In dogs, wire hair is dominant to smooth hair. If a homozygous wire-haired dog is bred to a smooth-haired dog:

What will be the hair condition in F_1? _____________

____

b. If litter mates of this cross are bred, what will be the hair condition? ___________________________

c. If a dog of the F_1 of *a* is crossed to a smooth-haired dog, what are the possible results? _________

____

3.

a. If each parent has a genotype of *A/a B/b,* what will be their gametes?___________________________

b. What proportion of their offspring could be expected to have the genotype *a/a b/b?* ____________

4. The ability to taste the drug phenylthiocarbamide (PTC) is attributable to a dominant gene. A non-taster man marries a taster woman whose father was a nontaster:

a. What will be the expected genotypes of their four children? _________________________________

b. What could have been the genotype of this woman's mother? ______________________________

5. The gene for yellow coat color in mice is lethal in a homozygous condition. Yellow coat is dominant to gray coat.

a. What will be the ratio of viable phenotypes in a cross between two yellow-coated mice? _________

____

b. What are the phenotypes and their ratio in a cross between yellow coat and gray coat? _________

____

6. In humans, suppose brown eyes are dominant over blue eyes and right-handedness is dominant over left-handedness. A brown-eyed, right-handed man marries a blue-eyed, right-handed woman. If their first child is blue-eyed and left-handed, what are the genotypes of the parents?

____

7. Two sets of factors, called complementary, are responsible for flower color in sweet peas. These genes may be represented by *P* for purple pigment and *E* for the activator of *P.* These two dominant genes must be present to get purple color. If either one or both of these genes are absent, the flower color is white.

a. If two white varieties of genotypes *P/P e/e* and *p/p E/E* are crossed, what is the color of the F_1?

____

b. If members of F_1 are crossed, what will be the genotypes and phenotypes? ___________________

8. In humans, hemophilia (failure to clot) is sex linked and recessive. If a normal woman whose father had hemophilia marries a normal man whose father also had hemophilia, what can be expected in their children, two boys and two girls? ___________

____

9. Suppose a certain disease in humans is caused by a dominant gene with 10% penetrance. If a heterozygous man with this disease married a normal woman, what percentage of their children would be expected to have the disease? __________________

10. In Ayrshire cattle, a genotype of *A/A* produces a mahogany color, whereas a genotype of *a/a* is red; however, the genotype *A/a* is mahogany in males and red in females. If a calf born to a mahogany cow is red, what is its sex?__________________

Name ______________________________

Date ________**Section** ________________

BREEDING EXPERIMENT 1—MONOHYBRID CROSS

A.. Mating homozygous long-winged fly *(V/V)* with vestigial-winged fly *(v/v)*

Resulting F_1 generation: long-winged males ______________ vestigial-winged males ________________

long-winged females __________________ vestigial-winged females ___________________________

B. Mating long-winged male *(V/v)* and long-winged female *(V/v)* of F_1 generation

Resulting F_2 generation:

Team	Male		Female	
	Long-winged	**Vestigial-winged**	**Long-winged**	**Vestigial-winged**
1				
2				
3				
4				
5				
6				

Class totals

Long-winged ______________

Vestigial-winged ___________

Ratio ____________________

Ratio expected _____________

Males expected _____________

Males ____________________

Females __________________

Ratio_____________________

Ratio expected______________

BREEDING EXPERIMENT 2—TESTCROSS

Mating long-winged (*V/V* or *v/v*) with vestigial-winged (*v/v*) to determine genotype of the long-winged parent

Predicted proportions of offspring if:

Long-winged parent is homozygous — Long-winged ______ Vestigial-winged ______

Long-winged parent is heterozygous — Long-winged ______ Vesitigial-winged ______

Team	Male		Female	
	Long-winged	**Vestigial-winged**	**Long-winged**	**Vestigial-winged**
A1				
A2				
A3				
A4				
A5				

Actual count:

A. Total long-winged ______

Total vestigial-winged ______

Conclusion: The long-winged parent was ______

______

Team	Male		Female	
	Long-winged	**Vestigial-winged**	**Long-winged**	**Vestigial-winged**
B1				
B2				
B3				
B4				
B5				

B. Total long-winged ______

Total vestigial-winged ______

Conclusion: The long-winged parent was ______

______

Name ________________________________

Date ________**Section** ____________________

BREEDING EXPERIMENT 3—DIHYBRID CROSS

Mating long-winged, gray-bodied male (*V/v E/e*) and long-winged, gray-bodied female (*V/v E/e*) (from F_1 generation from union of long-winged, ebony-bodied male [*V/V e/e*] and vestigial-winged, gray-bodied female [*v/v E/E*])

Team	Male				Female			
	Long-gray	Long-ebony	Vestigial-gray	Vestigial-ebony	Long-gray	Long-ebony	Vestigial-gray	Vestigial-ebony
1								
2								
3								
4								
5								
6								
Total								

Total for all teams participating

Long-gray ____________ Long-ebony ____________ Vestigial-gray ____________ Vestigial-ebony ____________

Ratio? _______________ Ratio expected? _______________

BREEDING EXPERIMENT 4—SEX-LINKED INHERITANCE

A. First cross
P_1: Red-eyed male *(W/—)* × white-eyed female *(w/w)*

Team	F_1 males		F_1 females	
	Red-eyed	White-eyed	Red-eyed	White-eyed
A1				
A2				
A3				
A4				
Total				

Expected ratio ______________________

Actual ratio ______________________

B. First cross
P_1: White-eyed male *(w/—)* × red-eyed female *(W/W)*

Team	F_1 males		F_1 females	
	Red-eyed	White-eyed	Red-eyed	White-eyed
B1				
B2				
B3				
B4				
Total				

Expected ratio ______________________

Actual ratio ______________________

A. Second cross
P_2: White-eyed male *(w/—)* × red-eyed female *(W/w)*

Team	F_2 males		F_2 females	
	Red-eyed	White-eyed	Red-eyed	White-eyed
A1				
A2				
A3				
A4				
Total				

Expected ratio ______________________

Actual ratio ______________________

B. Second cross
P_2: Red-eyed male *(W/—)* × red-eyed female *(W/w)*

Team	F_2 males		F_2 females	
	Red-eyed	White-eyed	Red-eyed	White-eyed
B1				
B2				
B3				
B4				
Total				

Expected ratio ______________________

Actual ratio ______________________

Name ___________________________________

Date ____________ Section _______________

Phenotypes and their frequency

	Characteristics	Your phenotype	Possible genotype	Your section		All section	
				No.	%	No.	%
1.	Widow's Peak						
	Continuous hairline						
2.	Convex nose						
	Straight or concave nose						
3.	Dimpled chin						
	Chin not dimpled						
4.	Can roll tongue						
	Cannot roll tongue						
5.	Taster of PTC						
	Nontaster						
6.	Hypermobility of thumb						
	Normal mobility						
7.	Bent little finger						
	Straight little finger						
8.	Long palmar muscle						
	Muscle absent						
9.	Pigmented iris						
	Nonpigmented iris						
10.	Free ear lobes						
	Attached ear lobes						
11.	Blood type A						
	Blood type B						
	Blood type AB						
	Blood type O						

Gene and genotype frequencies

Population	Phenotype		Gene frequency		Genotypes		
	% tasters	% nontasters	% ***T***	% ***t***	% ***T/T***	% ***T/t***	% ***t/t***
Your section							
All sections							
United States	**70**	**30**					

THE CHI-SQUARE TEST

My section

	Tasters	Nontasters
o		
e		
d		
d^2		
—		

All sections

	Tasters	Nontasters
o		
e		
d		
d^2		
—		

What do the ^{2} values you have computed indicate about the results of these two experiments?

Name ______________________________
Date ____________ **Section** ______________

Record in each column the number of persons in the class whose phenotype for each trait agrees with that of the reader.

Trait number	1st reader	2nd reader	3rd reader	4th reader	5th reader
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					
11					

Find the average number of characteristics that it was necessary to consider before a genetic difference was found

____

If a survey was made with any group of relatives of the same number as your class, would you expect similar or different results? ____________________ Explain.