Unit

1

CHRIST CHURCH FOUNDATION SCHOOL

CAPE Biology 2011-2012

Laboratory Manual

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Laboratory and Safety Guidelines

Emergencies

□□ Inform the teacher immediately of *any* mishap—fire, injury, glassware breakage, chemical spills, and so forth.

□□ Know the location of the fire extinguisher, safety shower, eyewash, fire blanket, and first aid kit. Know how to use this equipment.

□□ If chemicals come into contact with your eyes or skin, flush with large quantities of water and notify your teacher immediately.

Preventing Accidents

□□ Do NOT wear clothing that is loose enough to catch on anything. Do NOT wear sandals or open-toed shoes. Remove loose jewelry—chains or bracelets—while doing lab work.

□□ Wear protective safety gloves, goggles, and aprons as instructed.

□□ Always wear safety goggles (not glasses) in the laboratory.

□□ Wear goggles throughout the entire activity, cleanup, and handwashing.

□□ Keep your hands away from your face while working in the laboratory.

□□ Remove synthetic fingernails before working in the lab (these are highly flammable).

□□ Do NOT use hair spray, mousse, or other flammable hair products just before or during laboratory work where an open flame is used (they can ignite easily).

□□ Tie back long hair and loose clothing to keep them away from flames and equipment.

□□ Eating, drinking, chewing gum, applying makeup, and smoking are prohibited in the laboratory.

□□ Do NOT inhale vapors or taste, touch, or smell any chemical or substance unless instructed to do so by your teacher.

Working in the Laboratory

□□ Study all instructions before you begin a laboratory or field activity. Ask questions if you do not understand any part of the activity.

□□ Work ONLY on activities assigned by your teacher. NEVER work alone in the laboratory.

□□ Do NOT substitute other chemicals or substances for those listed in your activity.

□□ Do NOT begin any activity until directed to do so by your teacher.

□□ Do NOT handle any equipment without specific permission.

☐☐ Remain in your own work area unless given permission by your teacher to leave it.

☐☐ Do NOT point heated containers—test tubes, flasks, and so forth—at yourself or anyone else.

☐☐ Do NOT take any materials or chemicals out of the classroom.

☐☐ Stay out of storage areas unless you are instructed to be there and are supervised by your teacher.

Laboratory Cleanup

☐☐ Keep work, lab, and balance areas clean, limiting the amount of easily ignitable materials.

☐☐ Turn off all burners, water faucets, probeware, and calculators before leaving the lab.

☐☐ Carefully dispose of waste materials as instructed by your teacher.

☐☐ With your goggles on, wash your hands thoroughly with soap and warm water after each activity.

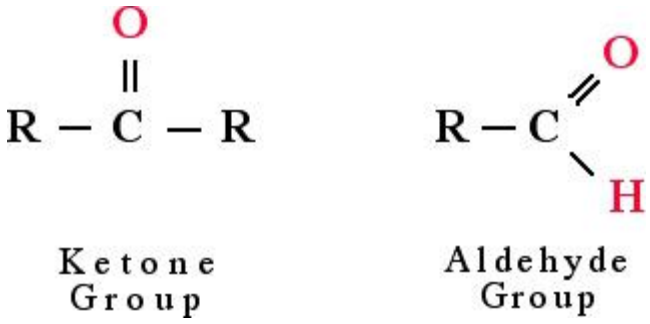
Experiment 1 – Carbohydrates

Aim: To investigate the three classes of carbohydrates using the Reducing sugars, Non Reducing Sugars and Starch tests.

Apparatus/ Materials:

Carbohydrates serve as energy sources and provide structural support as in the cell wall of plants. Carbon, hydrogen, and oxygen are the elements found in carbohydrates.

Part 1 - Benedict's Test for Reducing Sugars



Benedict's reagent is used as a simple test for reducing sugars. A reducing sugar is a carbohydrate possessing either a free aldehyde or free ketone functional group as part of its molecular structure. Recall from lectures that functional groups are the regions of a molecule that gives it particular properties. A single molecule can have more than one functional group as part of its structure. When a molecule with multiple functional groups is involved in a reaction all, some or none of the functional groups may be involved.
Glucose is a reducing sugar, while the disaccharide sucrose is not. As a result, glucose heated in Benedict's reagent reduces Cu^{2+} ions to form a green to brick-red precipitate depending on the amount of sugar present.

In the lab you prepared 3 tubes:
1. Water and Benedict's reagent
2. Glucose and Benedict's reagent
3. Sucrose and Benedict's reagent

Procedure:
- To 2 mL of Benedict's solution (aqueous solution of $CuSO_4$)
- Add 2 mL of the sugar solution, mix well and boil.
Try to see **the red precipitate of cuprous oxide** that forms at the end of the reaction.

Consider the following questions. For answers to the questions consult your lab manual, textbook and lecture notes. If you are still unsure consult with one of the instructors.

Questions:
- Which sugar is a reducing sugar?
- Write the reaction(s) involved in Benedict's Test.
- Some disaccharides such as maltose are reducing agents, whereas others, such as sucrose are not. Explain briefly by including the structures of the sugars.
- Which functional group is responsible for the difference?
- Why is the functional group not available in the non-reducing sugar?

- What is the purpose of each tube?

Part 2 b) Non Reducing Sugars Test: The Inversion of Sucrose:

Sucrose is a disaccharide, which means that it is a molecule that is derived from two simple sugars (monosaccharides).

Procedure:

- Add 5 mL of sucrose solution to two test tubes.
- Add 5 drops of conc. HCl to one test tube.
- Heat both tubes in boiling water bath for 10 min.
- Cool and neutralize with diluted NaOH (use litmus paper).
- Test both solutions for the presence of reducing sugar with Benedict's solution.

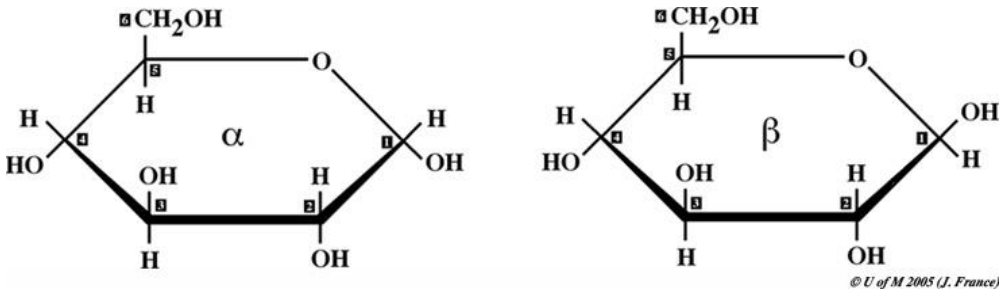
Questions:

- ✓ What monomers make up the sucrose molecule?
- ✓ What type of reaction has occurred to cause the conversion of the sucrose to its monomers?
- ✓ Why was the acid used in the reaction?
- ✓ Explain the result by giving the reasons and related reactions and drawing relevant structures.
- ✓ What would you expect from a similar reaction with starch?

Part 3 - Glucose Polymerization

Glucose & Glucose Polymers

Glucose is one of the most important biological carbohydrates. It is produced by plants during photosynthesis and as such it is a common food source for non-autotrophs. Glucose, once produced by the plant, or ingested by animals or fungi needs to be stored for later use. The are two main glucose polymers used for storage: **starch** by plants and **glycogen** by animals.



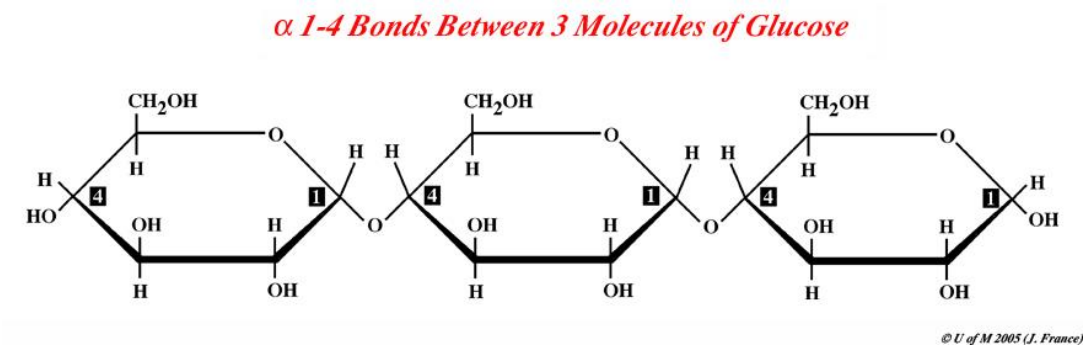
Note the location of each of the six carbons.

Test for starch

Cut a small section of potato and place it in a petri dish. Place a drop of IKI (iodine potassium iodide) solution on the surface of the potato. IKI is a stain routinely used to locate starch deposits. A dark blue-black colour indicates that starch is present.

Consider the following questions. For answers to the questions consult your lab manual, textbook and lecture notes. If you are still unsure consult with one of the instructors.

1. Does the potato tissue contain starch?
2. Why would the potato contain starch?



Keep in mind that a regular starch molecule found in a plant storage site like a potato can be 100s of thousands on glucose units long.

Consider the following questions. For answers to the questions consult your textbook and lecture notes. If you are still unsure consult with one of the instructors.

1. Explain the reaction between iodine solution and starch.
2. Name the complex which is formed
3. What molecule would be removed to make the connection (bond)?
4. What is this type of reaction called?
5. If you wanted to make cellulose, another glucose polymer, could you use the structure as the starch molecules?
6. Draw representative structures to represent cellulose.
7. How does the structure of cellulose cause its properties to be significantly different from those of starch and glycogen?

Lab due on _____.

Experiment 2 - Proteins

Aim: To carry out confirmative tests to determine the presence of proteins and fats in provide food samples.

Apparatus/ Materials:

Proteins are complex, specialized molecules composed of carbon, hydrogen, oxygen, and nitrogen. Many proteins also contain sulfur. The building blocks of proteins are the **amino acids**.

Biuret Test for Proteins: Biuret reagent is a light blue solution which turns purple when mixed with a solution containing protein. When the copper ions of the Biuret Reagent react with peptide bonds in the polypeptide chains, a purple color complex is formed.

Note: All groups will set up tubes 1-4 in Table V below. Groups 1, 3, and 5 should also set up tubes 5-8 for a total of eight tubes. Groups 2, 4, and 6 should also set up tubes 9-12 for a total of 9 tubes. Everyone is responsible for recording and knowing all results, so you should share information.

1. Label a set of tubes (see note above) with a wax pencil.
2. Add 2 mL (40 drops) of sample to each tube.
3. Add 2 mL of biuret reagent to each tube. What color is the biuret reagent?
4. Mix the contents of each tube using the vortex genie.
5. Wait 2 minutes.
6. Examine each tube carefully. Note the color.
7. Record your observations in the Table V.

Table V. Results of the Biuret Test for Proteins

Tube number	**The color of Biuret Reagent is**: _____	Initial color at mixing	Final color after 2 minutes at room temperature	Is this a positive test? Are peptide bonds present (Yes or no?)
1	*Negative control*: water			
2	*Positive control*: serum albumin			
	Do these foods contain proteins?			
3	whole milk			
4	skim milk			

5	regular soda			
6	diet soda			
7	red bull			
8	egg white			
9	egg yolk			
10	mashed up potato			
11	mashed up beans			
12	coffee			

Discussion Questions:

Summary Questions:

1. **What are Proteins?** (Include the elements which make up proteins.)
2. List five functions of proteins.
3. **Proteins are long chains of amino acids. Draw the structure of an amino acid (use the general formula).**
4. **Use two amino acids to show how the monomers may be used to form dipeptides.**
5. **Proteins are usually arranged in four possible structures. Name these four structures and give an example of a protein which has the named structure.**
6. **Give a brief summary of with each structure, making sure to include any chemical linkages which may be present.**
7. **Specifically, what does the Biuret reagent detect? Why would this test NOT detect an amino acid?**

Conclusion:

Lab due on _____.

Experiment 3- Lipids

The Ethanol Emulsion test determines the **presence** of a broad group of naturally occuring compounds known as **lipids**.

Other **Lipid Tests** include the **Grease Spot Test** and the **Sudan Stain Test**.

The Ethanol Emulsion Test is described as it is the most **commonly** used.

Procedure

Soild sample :

1. **Crush** the food sample and place in a dry test tube.
2. **Add ethanol** to about 2 cm3 above the level of the sample and shake thoroughly.
3. Allow the solid to **settle** (about 3 min) to allow the lipid to be extracted.
4. **Decant** the ethanol into another test tube.
5. Add 2 cm3 of **deionized water** to the second test tube.
6. Make observations.

Liquid sample:

1. Add a **few drops** of the liquid food sample to a dry test tube.
2. Add 2 cm3 **ethanol** and shake it thoroughly
3. Add 2 cm3 of **deionized (distilled) water**.
4. Make **observations**.

Results and Interpretation

	OBSERVATION	**INTERPRETATION**
POSITIVE TEST	A **layer of cloudy white suspension** forms at the top of the solution. (Upon close inspection you can see the tiny globules of fat suspended in the solution. This an emulsion. Foods with high lipid content have a 'higher' layer than foods with less).	Lipids are **present**
NEGATIVE TEST	Solution remains **colourless**. No emulsion is formed.	Lipids are **not present**

Food	*Appearance of filtrate when added to water*	*Interpretation*
Nuts		
Cheese		
Potato		
Meat		

Discussion Questions

8. **What are Lipids?**
9. **Most Dietary Lipids are triglycerides. Describe the structure of the triglycerides.**
10. **Account for the physical states of Fats and Oils.**
11. **Outline the function of dietary lipids in the body. Name 3 sources of dietary lipids.**
12. **Why is a fat-free diet unadvisable?**
13. **Define obesity. Name three diseases associated with obesity.**
14. **What is the significance of crushing the soild food sample and leaving it to stand in ethanol for a few minutes?**
15. **Describe the principle of the ethanol emulsion test.**
16. **State one precautions taken during the ethanol emulsion test**.

Conclusion:

Lab due on _____.

Experiment 4 - Macromolecules

Aim: To investigate and compare the reducing sugars, starch, lipid and protein content of three unknown solutions.

Apparatus/ Materials:

Prelab: THIS IS TO BE COMPLETED BEFORE THE START OF THE LAB AT HOME. Complete this in your lab notebooks.

1. Once again, read carefully which macromolecules are expected to give positive results for each test – and complete the table on Pg 4 – complete the 'PREDICT' columns for the controls you are bringing. Ideal positive control will be expected to give a '++++' and negative control a '0' on the test.

2. Glue down the results data table in your lab notebook – it is in this hand out on Pg _____.

Bring a positive and negative 'ideal control' from home for this lab FOR EACH TEST. Controls may overlap between tests – you may bring only 4 solutions in all. **Food has to be in liquid form – cannot be water.**

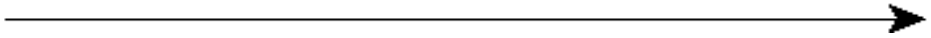
Your goal in the lab: Find the biochemicals in the three unknown powders/solutions given to you. Rate them based on the test results for which one has the maximum amount of each type of biochemical. Compare the test results from the unknowns to known positive and negative controls to confirm your results. Use the following tests and run them on the unknowns as well as your positive and negative controls.

Take one mini spoon of each of the unknown powders and add 10 ml of water – use this solution to test for Benedict's (reducing)sugars, Iodine test (polysaccharide) and Biuret's (protein). Use the emulsion test for fats.

A) Benedict's Test for Reducing Sugars

Which sugars will react in this test: The Benedict's test allows us to detect the presence of reducing sugars (sugars with a free aldehyde or ketone group

Rating Scale: Increasing amounts of reducing sugar



blue	green	orange	red	reddish brown
(-)	(+)	(++)	(+++)	(++++)

To Test For Reducing Sugars (monosaccharides and some disaccharides):

1) Add 2 ml of unknown/control solution

2) Add 1.5 ml of Benedict's solution (WEAR GLOVES + GOGGLES!!)

3) Mix gently. Make sure tube is labelled on the top – as the label will soak in water!

4) Place test tube in a boiling water bath

5) Record the color development in 3-5 min using the rating scale above.

B) Starch Test For Polysaccharides

To Test For Starch:

1) Add 1 ml of unknown/control in a test tube
2) Add 1 drop of Iodine solution
3) Record color development against a white paper placed behind the test tube

Brown (-) Blue-Black (++++)

Results from macromolecule lab: ++++ is highest, 0 is lowest. Shaded regions are tests that are not performed – you may perform them if you wish! Fill in your ratings here:

Unknown/Food Solution Tested	Benedict's Test		Starch Test		Biuret's Test		Fat Test	
	Predict	**Actual**	**Predict**	**Actual**	**Predict**	**Actual**	**Predict**	**Actual**
Unknown 1								
Unknown 2								
Unknown 3								
Unknown 4								
Carbohydrate test:								
Positive Control Monosachharide								
Positive Control Monosachharide								
Positive Control Polysachcharide								
Negative Control Polysachcharide								
Protein test:								

Positive Control Protein								
Negative Control Protein								
Lipid Test								
Positive Control Lipid								
Negative Control Lipid								

Food has to be a liquid other than water. Ideal Positive = ++++ ; Ideal Negative = 0

My choices	**Food I brought for this from home**	**Reason I believe this is an ideal choice**	**Did the lab confirm this choice?**	**If no, why not?**
Positive control-Monosachcharide				
Negative control-Monosachcharide				
Positive control-Polysachcharide				
Negative control-Polysachcharide				
Positive control-Protein				
Negative control-Protein				
Positive control-Lipid				
Negative control-Lipid				

Discussion Questions:

1) What are macromolecules?
2) What are 4 types of macromolecules?
3) Give the monomer and polymer names for each type.
4) What are the reactions that make polymers/macromolecules called?
5) When are these reactions used in the body?
6) What is the purpose of this lab?
7) What are the 4 tests that shall be used in this lab to test for sugars, starches, protein and fats (herein called the biochemicals).
8) **Specifically, what does the KI/I_2reagent detect?**
9) **Specifically, what does the Biuret reagent detect? Why would this test NOT detect an amino acid?**
10) Is this a qualitative or quantitative lab in terms of the results collected?
11) What is the difference between a positive and a negative control?
12) Why are the positive/negative controls used in this lab?
13) **Explain the limitations of the Benedict's Test in determining whether or not sugar is present in a certain food product.**
14) **Indentify the unknowns and give relative quantities of substances which are present in each sample.**

Conclusion: Identify the unknown

Lab due on _____.

Biological Drawings

The following guidelines should be used when doing a formal biological drawing of an object you view under the microscope:

1. Drawings, including labels, name and other info, must be done in pencil. (preferably soft lead that will not smudge).

2. The title of the drawing is simply the name of the object you are looking at.

3. If the drawing is a formal drawing to be handed in, use unlined white 81/2" x 11" paper or lab drawing paper.

4. The drawing should be as large as possible. (at least 1/3 of the page) and should be kept to the left of the center of the page.

5. All labels must be printed and are to be lined up on the right hand side of the drawing. Use a ruler for label lines.

6. The drawing should be an outline of what you see. Do not include additional structures just because you think you should see them.

7. Do not shade or sketch. All lines should be solid and complete.

Experiment 5 - Microscopy: Light and Electron Microscopes

Format:

- 1 X 1 hour lecture
- 1X 2 hour practical

Lecture:
Before the lecture and the lab takes place, ask students to prepare answers to the following questions.

- What is the definition of a cell?
- What are the different components of a cell?

Microscopy of Cells Lab

Aims

- To introduce the use of the light microscope for studying cells
- To measure cells using a calibrated eyepiece graticule

Objectives

You should be able to:

- accurately record what you see
- calibrate a light microscope using an eyepiece graticule and stage micrometer
- use the calibrated microscope to measure different cell types

Light Microscopy

Instructions

Calibration of the light microscope
You have already had some practice calculating the calibrations for different magnifications. Today you are going to calibrate the light microscope using the stage micrometer and eyepiece graticule.

- You will need:
- A light microscope
- An eyepiece graticule (in the eyepiece lens)
- A stage micrometer

1. Move the objective lens to the lowest power (X4).

Place the stage micrometer on to the stage, making sure that it is in the slide holder correctly.

2. Move the stage micrometer until it is directly under the objective lens, using the knobs at the right hand side of the stage to move it to the correct position.

3. Focus the stage micrometer until you see both scales, superimposed on one another.
4. Count the number of stage divisions equal to 100 eyepiece divisions.
5. Calculate the distance between each eyepiece division.
6. Repeat for X40 objective lens.

X4 magnification

- 100 eyepiece divisions = ____ *stage divisions
- We know that 100 stage divisions = 10mm
- 1 stage division = ____mm
- ____ *stage divisions = ____mm
- 100 eyepiece divisions = ____mm
- 1 eyepiece division = ____ mm or ____μm

X10 magnification

- 100 eyepiece divisions = ____ *stage divisions
- We know that 100 stage divisions = 10mm
- 1 stage division = ____mm
- ____ *stage divisions = ____mm
- 100 eyepiece divisions = ____mm
- 1 eyepiece division = ____ mm or ____μm

X40 magnification

- 100 eyepiece divisions = ____ *stage divisions
- We know that 100 stage divisions = 10mm
- 1 stage division = ____mm
- ____ *stage divisions = ____mm
- 100 eyepiece divisions = ____mm
- 1 eyepiece division = ____ mm or ____μm

The following 3 steps are the calculations you will use when doing work related to the microscope. Review each calculation; then click on the link at the bottom of the page to proceed to the practice worksheet.

Calculations Related to the Microscope:

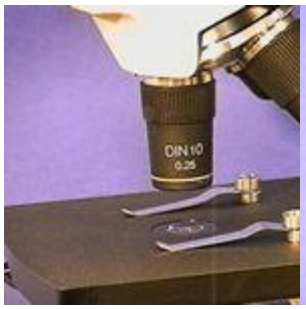
1) Calculating the magnification of a microscope:

Total magnification = magnification of eyepiece x magnification of objective lens

Ocular Lens - W10XD (Look for a whole number or a number followed by an X to determine the power.

Low Power Objective - DIN4

10X ocular x 4X objective = 40X total



Medium Power Objective - DIN10

10X ocular x 10X objective = 100X total

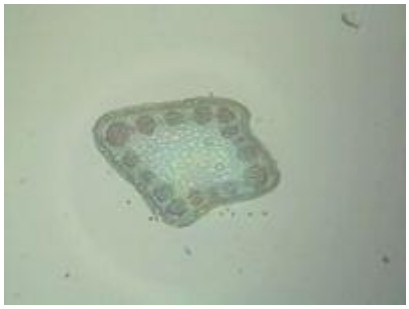
High Power Objective - DIN40

10X ocular x 40X objective = 400X total

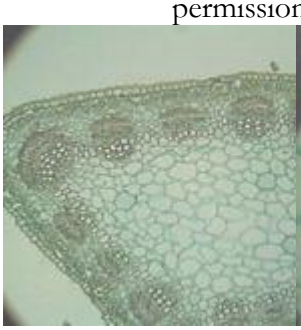
Microscope images used with permission.

The images to the right illustrate how the appearance of an image changes with magnification. As the magnification increases, the size of the image increases. However, because less of the image can be seen, the 'field of view' decreases.

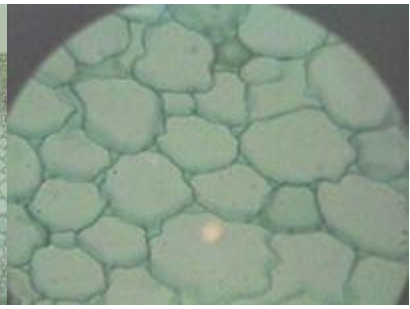
higher magnification = smaller field of view



alfalfa stem @ 40X



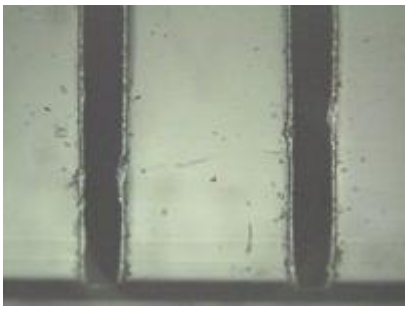
alfalfa stem @ 100X



alfalfa stem @ 400X

2) Measuring the microscope field of view on lowest power:

Place a clear plastic ruler with mm markings on top of the stage of your microscope. Looking through the lowest power objective, focus your image. Count how many divisions of the ruler fit across the diameter of the field of view. Multiply the number of divisions by 1000 to obtain the field of view in micrometers (μm). Record this in μm (1mm = 1000 μm).



Magnified at 40X, the lines of the ruler are clearly visible. However, in order to accurately measure the field of view, one line should be moved to the edge of the field of view, as shown in the image to the right.

Now that one of the lines has been moved to the extreme left, it is easier to determine the field of view:

2 mm x 1000 = 2000 μm

(image @ 40X)

In this example, the field of view would be calculated:

3 mm x 1000 = 3000 μm

3) Calculating the microscope field of view on other powers:

i. Using the above procedure, determine the field of view in micrometers (μm) for the lowest power on your microscope.

ii. After you have determined the field of view for low power, use the equation below to mathematically calculate the field of view on higher powers:

$$\frac{\text{total magnification on low power}}{\text{total magnification on other power}} = \frac{\text{field of view on other power}}{\text{field of view on low power}}$$

For Example:
If at 40X on low power, the field of view is 2000 μm, calculate the field of view under 100X magnification (medium power).

$$\frac{40X}{100X} = \frac{?}{2000\mu m} \qquad ? = (40X \times 2000\mu m) / 100X \; ; \; ? = 800\mu m$$

Microscope Calculations (continued)

4) Estimating the size of an object:

- You must first calculate the diameter of the field of view for the power of magnification you are using.
- Looking through the eyepiece, estimate how many times the object will fit across the field of view.
- Calculate the size of the object using the formula below.
- Remember to use *µ*m

$$\text{Size of Object} = \frac{\text{diameter of field of view (in } \mu\text{m)}}{\text{number of times object fits across field of view}}$$

Example:

The image of the amoeba to the left is seen under **medium** power (100X magnification).

If the diameter of the field of view on ***low*** power (40X) for this microscope is 4000 µm, first calculate the diameter of the field of view for ***medium*** power as follows:

$$\frac{40X}{100X} = \frac{?}{4000\mu m} = \frac{40X\ (4000\mu m)}{100X} = 1600\mu m$$

Second, estimate the number of times the object (amoeba) fits across the field of view. Unless otherwise instructed, estimate using the longest dimension of the object. In the image to the left, the amoeba would fit across the field of view approximately 2 times. Because the diameter of the field of view for medium power was calculated as 1600 µm, the size of the object would be calculated as follows:

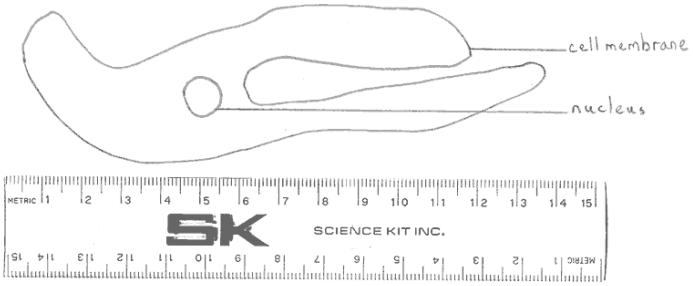
$$\text{Size of object} = \frac{1600\mu m}{2} = 800\ \mu m$$

Therefore, the approximate length of the amoeba is 800 µm or 0.8mm.

The final calculation you will need to use when using the microscope is to calculate the magnification of your drawing. (This is a way of calculating how many times larger your drawing is in relation to the estimated size of the object.)

5) Calculating the magnification of your drawing:

- First calculate the size of the object using the procedure outlined above.
- Using a ruler, measure the size of your drawing and convert units to *µ*m. (In the diagram below a ruler has been superimposed on the drawing to illustrate how to measure. The image in this particular example is 13.7cm or 137mm, which converts to 137 000 µm.)
- Calculate the magnification of your drawing using the formula below the diagram.



171 X

This number indicates how many times larger your drawing is relative to the actual size of the object.

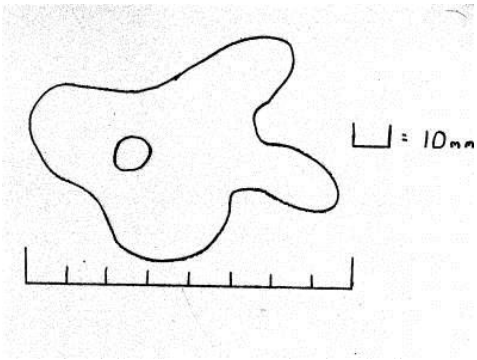
This number should appear at the bottom of your drawing.

$$\text{Magnification of drawing} = \frac{137\,000\ \mu m}{800\ \mu m} = 171\ X$$

$$\text{Magnification of drawing} = \frac{\text{size of drawing of object (in } \mu m)}{\text{size of object (in } \mu m)}$$

Worksheet 1 - Calculations Related to the Microscope

Anne viewed an amoeba under the high power 40X objective lens on her microscope. She drew the following picture of that amoeba:



She needs to calculate the magnification of her drawing. Other important information includes:

- Eyepiece lens = 5X Low Power Objective = 4X
- Low Power Diameter of Field of View = 4.2 mm
- Estimate of the number of times object fits across field of view (high power) = 4.5

  *Anne used the longest dimension to estimate.

a) Convert the diameter of field of view on low power to *u*m.

b) Calculate the total magnification under low power and high power.

c) Calculate the diameter of the field of view under high power.

d) Calculate the size of the object under high power.

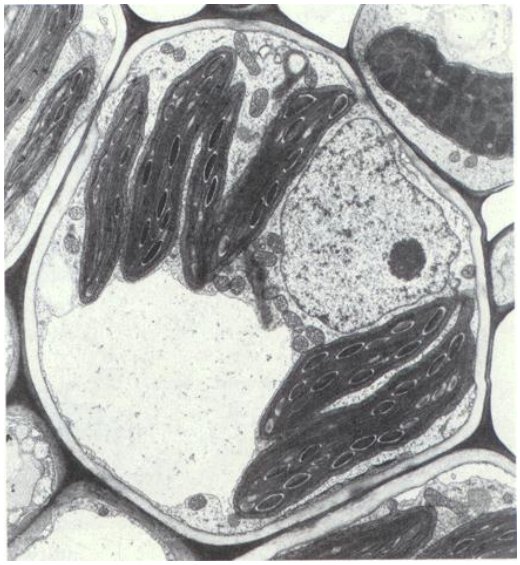
e) Measure the size of the drawing. Convert to *u*m.

f) Calculate the magnification of Anne's drawing.

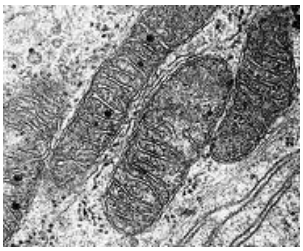
Electron microscopy

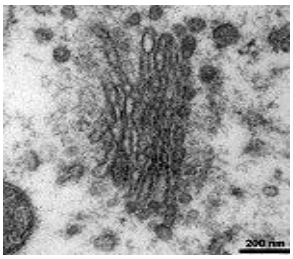
There are copies of an **electron micrograph (EM)** of liver tissue available. Get one of the EMs and work with a partner to answer the following questions.

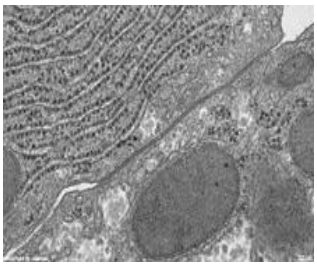
(1)Try to identify as many cellular organelles as you can.
(2) Does this EM show a whole cell?



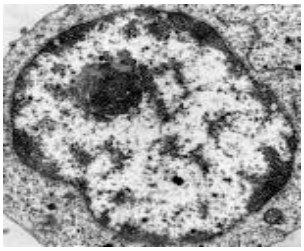
(3) Identify the following major structures.

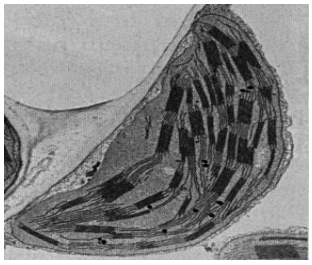
(a) 

(b) 

(c) 

_____ _____ _____

(e) 

(f) 

_____ _____

(5) What differences are there between looking at a slide on the light microscope and looking at an EM?

Experiment 6- A Look at Plant and Animal Cells

Aim: To use a microscope as well as prepared and wet mount slides to view cells, to estimate the size of cells, and to prepare formal biological drawings of cells.

Materials:

- Compound microscope
- Microscope slides
- Cover slips
- Eyedropper
- Water
- Small knife or scalpel
- Forceps
- Toothpick
- Onion bulb
- Stain – Iodine or Methylene Blue
- Prepared slides of Animal cells

Part A – Procedure:

The following lab assumes that you have already calculated the total magnification and the field of view for low, medium and high power on your microscope. You will be asked to use these numbers in the following procedures. If you have not done this or if you are using a different microscope you need to calculate and record these numbers.

1. Set up microscope and gather necessary materials.

2. Obtain a small piece of onion.

3. Use the scalpel and forceps to gently remove a small piece of the thin "skin" from the concave side of the piece of onion. This thin skin is epidermal tissue which makes up the epithelium if the onion.

4. Place the onion skin on a clean glass slide. Use a toothpick to remove any wrinkles from the onion epithelium.

5. Using an eyedropper, add a few drops of water to the slide and then place a cover slip over the onion and water.

6. Place the slide on the stage of your microscope. Using low power focus on the cells.

Q.1. Describe the general shape and structure of the epidermal cells as seen on low power.

7. Switch to the medium power objective lens and use the fine adjustment knob to focus on the cells.

Q.2. Describe the general shape and structure of the epidermal cells as seen on medium power.

8. Switch to the high power objective lens and use the fine adjustment knob to focus on the cells. (if you can not get a clear image on high power, use medium power for the following calculations and drawing.)

Q.3. Describe the general shape and structure of the epidermal cells as seen on high power.

9. Remove the slide from the stage. Stain the onion cell with a small drop of iodine or methylene blue. The following link describes the correct procedure for staining a slide. Staining a Slide

10. Place the stained onion cells back on the microscope stage and use high power to focus on the cells.

Q. 4. Describe how the stain has changed the appearance of the cells?

Q. 5 Record the field of view (in micrometers) of your microscope for the power you are using.

11. You can calculate the approximate size of one cell based on the field of view and the number of cells that you estimate will fit across the field of view.

Q. 6. What is the size of one onion epidermal cell. (Show you work)

12. Prepare a formal biological drawing of 3-4 onion cells as viewed under high power.

Label the cell wall, cell membrane, nucleus and cytoplasm on one cell. Be sure to use the proper components and format outlined on the page on Biological Drawings. See "Biological Drawings" if you need additional directions.

Q. 7. Calculate the magnification of your drawing. (Show your work and then record the answer on your drawing.)

Part B - Procedure:

1. Obtain a prepared slide of an animal cell. (the slide may be labeled typical animal cells, animal liver cells etc.)

2. Place the slide on the stage of your microscope and use the low power to focus on the cells.

Q. 8. Describe the shape and structure of the animal cells as viewed on low power.

3. Switch to the medium power objective lens and focus on the cells.

Q. 9. Describe the shape and structure of the animal cells as viewed on medium power.

4. Switch to the high power objective lens and focus on the cells using the fine focus only.

Q. 10. Describe the shape and structure of the animal cells as viewed on high power.

Q. 11. Describe the differences in appearance between the animal cells and the onion (plant) cells as viewed on high power. (Focus on the structure and shape. Remember that the color of the cells is a result of the stain used.)

Q. 12. a. Calculate the approximate size of one animal cell. (show your work).

b. How does the size of one animal cell compare to the size of the onion (plant) cell.

5. Prepare a formal biological drawing of 3-4 animal cells. Label the cell membrane, cytoplasm and nucleus on one cell.

Q. 13. Calculate the magnification of your drawing. (Show your work and record your answer in your drawing)

Note: Before submitting this assignment for evaluation, check that all questions are properly labeled and answered. Formulas and calculations must be shown for all questions where applicable.

Lab due on _____.

Experiment 7 - Tissues
Title: Structure of roots
Aim: To make a plan drawing of the transverse section of a dicotyledonous root from a pre-prepared slide.

Tissues may also be classified as:

1. simple if composed of only one cell type performing a single function

2. complex if composed of several cell types performing more than one function.

Simply tissues include the fundamental tissues such as parenchyma, and collenchyma while the complex tissues are schlerenchyma and the vascular tissues, xylem and phloem.

Materials:

Compound microscope

Prepared slides of: 1. x-s of *Ranunculus* young root

Procedure

1. Examine the slide of the cross section of *Ranunculus* young root the phloem, and xylem. Shift to HPO and take a close look of the shape and arrangement of the cells making up these tissues.
2. Make a plan drawing of the cross section and identify a few of the following cells in the drawing.

epidermis, cortex, pith, phloem, vascular cambium, xylem

Results

Drawings: Fig 5.2 x-s of the *Ranunculus* young root and label parts enumerate in procedure no. 2

END OF LAB.

Lab due on _____.

Experiment 8 – Water Potential

Aim: To determine water potential of potato tuber cells using the weighing method

Background:

If a plant cell is in equilibrium with an external solution of such a concentration that there is not net loss or gain of water, the water potential of the external solution will be equal to the water potential of the cell.

Use of this fact can be made in estimating the water potential of a plant tissue. Samples of tissue are immersed in a range of external solutions of different strengths. The solution that induces neither an increase nor a decrease in the volume or mass of the tissue has the same water potential as that of the cells in the tissue.

The cells to be investigated in this experiment are those of the potato tuber. Changes in mass will be used as an indication of whether the cells are taking up or losing water.

Materials/ Appartus:

PROCEDURE

1 Label six specimen tubes: distilled water, 0.1, 0.2, 0.3, 0.4, 0.5 mol dm^{-3}. Place approximately one third of a tube of distilled water in the first, and an equal volume of each of a series of sucrose solutions of different strengths and (molarities) in the remainder. Each tube should be firmly stoppered.

2 Using a cork borer and a razor blade, prepare six solid cylinders of potato. Each cylinder should be approximately 10mm in diameter and 12mm long. Slice up each cylinder into six discs of approximately equal thickness. Place each group of discs on a separate piece of filter paper.

3 Weigh each group of discs. (In each case weigh them on the piece of filter paper alone, and subtract the one from the other to get the mass of the discs). Record the mass of each group.

4 Put one group of discs into each of the labelled tubes and as you add each group, record its mass. Stopper the latter firmly and leave for not less than 24 hours.

5 After about 24 hours remove the discs from each tube. Remove any surplus fluid from them quickly and gently with filter paper, using the standardised procedure for all of them. Then re-weigh them. Record the new mass of each group of discs.

6 Graph you r results by plotting the percentage change in mass (change in mass multiplied by 100 divided by original mass) against the molarity of the sucrose solutions. The latter, being the independent variable, should be on the horizontal axis; the former on the vertical axis.

7 Calculate the water potential of the potato cells as follows. Find the point on your graph corresponding to a percentage mass change of zero. The molarity of sucrose corresponding to this zero mass change can now be read from the horizontal axis. From table 4.1 find the water

potential of a sucrose solution of that molarity. That is the water potential of your sample of potato cells. Express your result in a kPa.

Results: Data table, Graph which was plotted and calculations made

Discussion:
Your discussion should consist of the following:

1 A Background to enlighten the reader about the theory of water potential, tissues and how the lab will allow the investigation of these to occur.
2 Explain your results thoroughly. Highlight any trends shown. Use the theory to assist with explanations
3 Criticise this method of finding the water potential of plant cells. How might it be improved?
4 What was the reason for dividing each cylinder into six discs, and why was it necessary to standardise the procedure for drying the discs?
5 How does the value of the water potential differ from the osmotic potential of the solution in the vacuole?

Limitations, Precautions, Sources of Error:
Give atleast two Limitations and a source of error or precaution for the lab.

Conclusion: Answer the aim.

Table 4.1 Relationship between molarity and osmotic potential of sucrose solutions

Molarity /mol dm^{-3}	Osmotic potential of sucrose solutions /kPa
0.05	-130
0.10	-260
0.15	-410
0.20	-540
0.25	-680
0.30	-860
0.35	-970
0.40	-1120
0.45	-1280
0.50	-1450
0.55	-1620
0.60	-1800
0.65	-1980
0.70	-2180
0.75	-2370
0.80	-2580
0.85	-2790
0.90	-3000
0.95	-3250
1.00	-3500

Experiment 9 - Membrane Transport
Aim: To make a drawing of plasmolysed Rhoeo discolor cells

Prior knowledge Use of the microscope, plant cell structure, the principles of osmosis, the meaning of 'epidermis' and 'cortex'.

Apparatus-*per group*

scalpel or razor blade
fine forceps
microscope slide
cover slip
microscope with x 100 magnification
dropping pipette
container for sucrose solution
container for water
4 strips of blotting paper

Method:
(a) Use a scalpel or razor blade to make a shallow transverse cut in the red epidermis of the piece of rhubarb stalk.

(b) With a pair of fine forceps lift up a strip of the epidermis at one side of the cut. Lift only the epidermis and not the underlying cortex. Having freed a narrow band of epidermis, pull it off with the forceps (Fig. 1) and press it flat on a slide with the outermost surface upwards.

(c) Use the scalpel or razor blade to cut about 10 mm of this strip from the thinnest and reddest portion (Fig. 2) and, using a dropping pipette, cover this with 3 drops of water.

(d) Use the forceps to lower a cover slip carefully on to the water drops, avoiding trapping air bubbles (Fig. 3), and examine the epidermis under the microscope using the x10 objective.

(e) Move the slide about to find a group of clearly defined cells near the edge, with red cell sap, and make a drawing in your notebook to show one of these cells. Draw the cell at least 50 mm long, representing the outline accurately and shading the area filled with cell sap. Clip the slide securely to the microscope stage and leave it in this position for the rest of the experiment.

(f) Use the pipette to place 2 drops of sucrose solution on the left-hand side of the slide, just touching the edge of the cover slip.

(g) Draw all this solution under the cover slip by applying a strip of blotting paper to the right- hand edge of the cover slip. Try not to move the slide, the cover slip or the epidermis.

(h) Examine the cells again and watch for about 2 minutes. If nothing happens, draw through some more sucrose solution.

(i) When a significant change has occurred in the cells, draw the same cell as before to show the

cell wall and the cell sap. The cell is plasmolysed.

(j) Use the pipette to place 3 drops of water on the left hand' side of the slide and draw it through under the cover slip as before. Do this twice to flush out all the sucrose solution.

(k) Study the cells again for about 2 minutes repeating operation (j) if nothing happens in this time.

Results: Drawings of plasmolysed cells

Discussion
Your discussion should consist of the following:

1. A Background to enlighten the reader about the theory of membranes, osmosis and plasmolysis.It should include how the lab will allow the investigation of these to occur.

2. When the rhubarb cells were exposed to sucrose solution what change did you observe in (a) the shape of the vacuole and (b) the colour of the cell sap? What change, if any, took place in the shape of the cell?

3. Bearing in mind the fact that liquids cannot be compressed, what must have happened to the cell sap to account for (a) the change in volume and (b) the change in colour?

4. After exposure to the sucrose solution, what do you suppose occupied the space between the vacuole and the cell wall in the plasmolysed cells?

5. Why did the cell sap not mix with the liquid in this space?

6. Which part of the cell must be 'selectively-permeable' in order to explain these results?

7. How do the results of this experiment lead to the conclusion that the cell wall is permeable not only to water but also to dissolved sucrose?

8. What effect would it have on the tissues of the whole plant if all the cells were plasmolysed?

9. (a) What changes took place in the cells when the sucrose was replaced by water? (b) How can you explain these changes in terms of osmosis?

10. How would this change, if it applied to all the cells, affect the tissues of the plant as a whole?

Limitations, Precautions, Sources of Error:
Give atleast two Limitations and a source of error or precaution for the lab.

Conclusion: Answer the aim.

Lab due on _____.

Experiment 10 – Enzymes 1

Introduction

Hydrogen peroxide (H_2O_2) is a by-product of **respiration** and is made in all living cells. Hydrogen peroxide is harmful and must be removed as soon as it is produced in the cell. Cells make the enzyme **catalase** to remove hydrogen peroxide. This investigation looks at the rate of oxygen production by the catalase in **pureed potato** as the **concentration** of hydrogen peroxide varies. The oxygen produced in 30 seconds is collected over water. Then the **rate of reaction** is calculated.

Aim: To investigate the effect of substrate concentration on the activity of catalase

Procedure

SAFETY:

Wear eye protection and protect clothing from hydrogen peroxide. Rinse splashes of peroxide and pureed potato off the skin as quickly as possible...

Apparatus And Chemicals

For the class – set up by technician/ teacher:

Hydrogen peroxide, range of concentrations, 10 vol, 15 vol, 20 vol, 25 vol, and 30 vol, 2 cm^3 per group of each concentration (see note 1)

Pureed potato, fresh, in beaker with syringe to measure at least 20 cm^3, 20 cm^3 per group per concentration of peroxide investigated (see note 2)

Rubber bung, 2-holed, to fit 100 cm^3 conical flasks – delivery tube in one hole (connected to 50 cm rubber tubing)

For each group of students:

Pneumatic trough/ plastic bowl/ access to suitable sink of water

Conical flask, 100 cm^3, 2

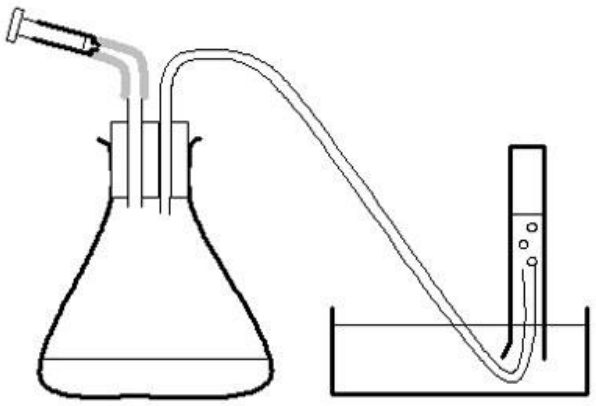
Syringe (2 cm^3) to fit the second hole of the rubber bung, 1

Measuring cylinder, 100 cm^3, 1

Measuring cylinder, 50 cm^3, 1

Clamp stand, boss and clamp, 2

Stopclock/ stopwatch



Investigation

a Use the large syringe to measure 20 cm^3 pureed potato into the conical flask.
b Put the bung securely in the flask – twist and push carefully.
c Half-fill the trough, bowl or sink with water.
d Fill the 50 cm^3 measuring cylinder with water. Invert it over the trough of water, with the open end under the surface of the water in the bowl and with the end of the rubber tubing in the measuring cylinder. Clamp in place.
e Measure 2 cm^3 of hydrogen peroxide into the 2 cm^3 syringe. Put the syringe in place in the bung of the flask but do not push the plunger straight away.
f Check the rubber tube is safely in the measuring cylinder. Push the plunger on the syringe and immediately start the stopclock.
g After 30 seconds, note the volume of oxygen in the measuring cylinder in a suitable table of results.
h Empty and rinse the conical flask and measure another 20 cm^3 pureed potato into it. Reassemble the apparatus, refill the measuring cylinder, and repeat from **d** to **g** with another concentration of hydrogen peroxide. Use a 100 cm^3 measuring cylinder for concentrations of hydrogen peroxide over 20 vol.
i Calculate the rate of oxygen production in cm^3/ s.
j Plot a graph of rate of oxygen production against concentration of hydrogen peroxide.

Discussion:
USUAL FORMAT

Background, Explanation of results with supporting data and theory

1 Apart from oxygen, what product is made when hydrogen peroxide breaks down?

2 Identify any anomalies or inconsistencies in your results.

3 Describe the shape of the graph.

4 Explain the shape of the graph in relevant biological terms.

5 Describe any technical difficulties you had with this apparatus and explain how these could be overcome.

6 Describe how you would extend this investigation to provide more evidence/ data to support your understanding of enzyme-controlled reactions.

Limitations, Precautions, Sources of Error:
Give atleast two Limitations and a source of error or precaution for the lab.

Conclusion: Answer the aim.

Lab due on _____.

Experiment 11: Enzymes and Temperature

Abstract

Enzymes speed up chemical reactions by factors of at least a million. Now that's acceleration! This project investigates some of the factors that affect how fast enzymatic reactions occur.

Objective

The goal of this project is to investigate enzyme kinetics, using catalase enzyme extracted from potatoes. Enzyme activity will be measured as a function of temperature. A protocol for measuring activity as a function of enzyme concentration is also provided for those that have access to a 1 mL adjustable pipettor.

Introduction

One of the by-products of many cellular reactions is hydrogen peroxide (H_2O_2). It is extremely toxic to living cells. All aerobic organisms use oxygen for respiration or oxidation of nutrients. During reduction of molecular oxygen to water, hydrogen peroxide is generated. Two examples of reactions that produce H_2O_2 are conversions of amino acids into "fuel" molecules and conversion of lipids to carbohydrates. It can damage DNA, protein and lipid membranes and may even be a causative factor in cancer. There are some human immune system cells that actually use H_2O_2 to kill foreign invaders. The catalase enzyme is specific for the hydrolysis of H_2O_2:

$$2H_2O_2 \xrightleftharpoons{\text{catalase}} 2H_2O + O_2$$

Catalase is found in animal and plant tissues, and is especially abundant in plant storage organs such as potato tubers, corms, and in the fleshy parts of fruits. You will use catalase isolated from potato tubers and measure its rate of activity under different conditions.

Like other enzymes, catalase is a protein. Enzymes speed up chemical reactions by reducing the activation energy required to convert substrate(s) into product(s). Enzymes have specialized binding sites to do this.

Because enzymes are proteins, they are somewhat fragile. They can be denatured by heat, and can easily be broken down by proteases when cells are homogenized. To preserve activity of proteins in solution, it is important to keep the solutions on ice until you are ready to use them. Denaturing conditions, such as boiling, can also be used as evidence to show that an enzyme-based reaction is protein-dependent.

In the experimental protocol described here a filter paper disk will be immersed in a solution of the enzyme, then placed in the hydrogen peroxide. The oxygen produced from the subsequent reaction becomes trapped in the disc and will give it buoyancy. The time measured from the moment the disc touches the bottom of the container substrate to the time it reaches the surface of the solution is an indirect, but easily quantifiable measure of the rate of the enzyme activity.

Terms, Concepts, and Questions to Start Background Research

To do this project, you should do research that enables you to understand the following terms and concepts:

- enzyme,
- active site,
- substrate,
- catalase,
- anti-oxidants.
- How big is the variation between and within groups?
- How big is the variation within and between treatments?
- How does reaction rate vary with temperature? What causes this?
- How does enzyme activity vary with enzyme concentration? What causes this?

Materials and Equipment

To do this experiment you will need the following materials and equipment:

- potatoes,
- gram balance,
- blender,
- ice,
- insulated ice bucket or cooler,
- water baths at 0, 10, 30, and 40°C,
- 500 mL 1% H_2O_2,
- 1 L distilled water,
- 1 mL adjustable pipettor (e.g., Gilson PipetMan) and tips,
- filter paper disks,
- stop watch or watch with second hand,
- forceps,
- 5 50 mL beakers,
- 100 mL graduated cylinder,
- thermometer,
- 1.5 mL plastic microcentrifuge tubes.

Experimental Procedure

Extraction of Catalase

1. Peel a fresh potato tuber and cut the tissue into small cubes. Weigh out 50 g of potato cubes.
2. Place the potato cubes, 50 mL of cold distilled water, and a small amount of crushed ice in a blender.
3. Homogenize for 30 seconds at high speed. From this point on, the enzyme preparation must be carried out in an ice bath.
4. Filter the potato extract, then pour the filtrate into a 100 mL graduated cylinder.
5. Add ice-cold distilled water to bring up the final volume to 100 mL. Mix well. This extract is the 100% enzyme solution.
6. **Note:** This rough 100% enzyme solution should work OK although it is worth testing it before proceeding with the experiment. At room temperature (approx. 20°C) in a 1% H_2O_2 solution it would be sensible if the disk took about 20 seconds to rise in the beaker you are using. If it is faster than this, dilute the enzyme and use that as the 100% solution. If it is slower, prepare the extract again, starting with an increased amount of potato cubes. If the filter rises too quickly at room temperature, then the reaction at higher temperatures will be too quick to measure. If the filter rises too slowly, then the lower temperatures will take forever.
7. Keep your catalase preparation on ice.

The Effect of Temperature on Enzyme Activity

1. Label five 50 mL beakers with the temperature for testing (0, 10, 20, 30, and 40°C).
2. Add 40 mL of 1% hydrogen peroxide solution taken from the appropriate temperature water bath to each beaker.
3. Put the beakers in the appropriate water bath.
4. Using forceps, immerse a filter paper disk into the catalase solution you have prepared.
5. Allow the disc to absorb the enzyme solution for 5 seconds, then remove it and drain off the excess enzyme solution by touching the filter paper to the edge of the beaker.
6. Drop the disc into the first substrate solution.
7. The oxygen produced from the breakdown of the hydrogen peroxide by catalase becomes trapped in the fibres of the disc causing the disc to float to the surface of the solution.
8. The time (t) in seconds, from the second the disc touches the solution to the time it again reaches the surface is an indirect measure of enzyme activity.
9. Remove the disk from the beaker once it reaches the surface and dispose of it.
10. Record the time taken in a table in your notebook.
11. Clean the beaker and repeat the procedure until you have 5 replicates at the first temperature.

12. Repeat for each temperature so you have data for 0, 10, 20, 30, and 40°C.
13. Calculate mean and standard deviation for each temperature.
14. Construct and label a graph of your results.

Results: Graph of Rate of reaction versus temperature.

Data Table

Discussion: WRITE YOUR OWN DISCUSSION BY NOW YOU SHOULD KNOW WHAT IS EXPECTED

Conclusion: Answer the Aim.

Another alternative:

INVESTIGATE THE EFFECT OF TEMPERATURE ON THE RATE OF CATALASE ACTIVITY

Materials/Equipment

Enzyme source e.g. potatoes,	Knife
Hydrogen peroxide (20% or less)	Chopping board
Buffer solution (pH 9)	Electronic balance
Washing-up liquid	Weighing dishes
Boiling tubes	Dropper
Syringe	Test-tube holder
Graduated cylinders (100 cm3)	Disposable gloves
Water baths (0 °C – 60 °C)	Timer
Thermometer	Test-tube rack

Procedure

1. Familiarise yourself with all procedures before starting.
2. Add 20 cm3 of the buffer to the graduated cylinder.
3. Using the dropper, add one drop of washing-up liquid.
4. Add 5 g of finely chopped radish to the cylinder.
5. Add 2 cm3 of hydrogen peroxide to the boiling tube.
6. Stand the cylinder and the boiling tube in an ice-cold water bath until the desired temperature (0 °C) is reached.

7. Pour the hydrogen peroxide into the cylinder.

8. Note the volume in the cylinder immediately and record.

9. Read the volume again after a measured amount of time, e.g. 2 minutes, and record.

10. Subtract the initial volume from the final volume to get the volume of foam and record.

11. Repeat the procedure from step 3 for at least four more temperatures, to include a sample in the 50 °C – 60 °C range.

12. A graph should be drawn of enzyme activity (volume of foam after 2 minutes) against temperature. Put temperature on the horizontal axis.

Result: Create a table with the following heading:

Temperature (°C), Initial volume (cm^3), Final volume (cm^3), Volume of foam produced (cm^3)

Plot a graph of Enzyme activity versus Temperature

Discussion: WRITE YOUR OWN DISCUSSION BY NOW YOU SHOULD KNOW WHAT IS EXPECTED

Conclusion: Answer the Aim.

Lab due on _____.

MODULE # 2- Heredity and Variation

Experiment 12 - Mitosis

Growth is a result of 1) cell division, which increases the number of cells that generally brings about an increase in the size of the organism and 2) cell elongation. Cell division makes possible tissue specialization for the different structural and physiological activities including absorption, conduction, reproduction, photosynthesis, and support. It is centered about the nucleus but the cytoplasm also undergoes changes during the entire process. Plants have their greatest activities in cell division, in spore production, in stem and root and in the meristems of the cambium, cork cambium, and pericyle.

In the laboratory, in order to see the progressive steps which occur during mitosis, the tissues of the root tip (especially of the *Allium cepa*) are cut by means of a microtome to a thinness of 10 to 15 microns and stained with variety of dyes to achieve contrast in the cellular organelles. For convenience in discussing the entire process of cell division, it is generally thought of as having five phases but one must bear in mind that mitosis is a continuous process.

Aims:

To be able to:

1. construct a diagram that depicts the stages of plant mitosis
2. name the principal events that characterize each period and phase
3. identify and explain the significance of each of the stages of mitosis

Materials:

Microscope, Prepared slides of *Allium cepa* mitosis

Procedure:

1. Examine a section of the *Allium cepa* root tip under the LPO. Then shift to HPO for a detailed study of the different stages.
2. Look for the cell in the process of cell division; identify the stages and observe the following: spindle fibers, chromosomes, cell plate, equator, and poles.
3. After drawing each of the different stages, summarize that occur in each.

Results**:**

1. Make at least one drawing of each of the following stages as observed under the microscope:
    a. Interphase or resting cell
    b. prophase
    c. metaphase
    d. anaphase

e. telophase

Discussion questions:

1. What is mitosis?

2. What is its significance?

3. Suppose a nucleus contains 4 chromosomes, how many chromatids would each possess at prophase?

4. How many chromosomes would be distinguishable at anaphase? Why?

5. What marks the initiation of cytokinesis in plant mitosis?

6. What is the direction of the division of the cytoplasm?

Lab due on _____.

Experiment 13 - Meiosis

Aim: To construct models to demonstrate chromosome behavior in meiosis.

Background: The body cells of plant and animals are diploid. A **diploid** (2n) cell has two sets of chromosomes in its nucleus. A cell with only one set of chromosomes in its nucleus is termed **haploid (n)**. **Gametes**, egg and sperm, are examples of haploid cells. When gametes fuse at fertilization, a diploid **zygote** is formed. The zygote contains one set of chromosomes from each parent.

Meiosis is a process that produces haploid cells, such as gametes from diploid cells. Before meiosis begins, DNA replication occurs. Following replication, each chromosome consists of two chromatids that are joined by a centromere. Meiosis involves two successive division of the nucleus.

Meiosis 1 (the first stage): homologous chromosomes (chromosomes that carry the same genes and that are similar in size and shape) Ex. #11 carries hemoglobin gene at the tip. One from mom and one from dad. SO everyone has two copies of the gene.

a. a. homologous chromosomes pair up and then separate.
b. b. The nuclei that result from meiosis 1 contain only one set of chromosomes. (one chromosome from each pair)
c. c. Law of independent assortment: tetrads line up at the equator independent of their neighbors.

Meiosis 2: the chromatids of a homologue (member of a homologous pair) may exchange parts.

a. a. Crossing over
b. b. Crossing over as well as the fusion of gametes in sexual reproduction is a type of genetic recombination.

VARIETY LEADS TO MORE FIT INDIVIDUALS

Procedure:

1. Using 1 m piece of string, make a circle on your desk to represent the cell membrane. Using a 40 mm piece of string, make another circle inside the cell for the nuclear membrane.

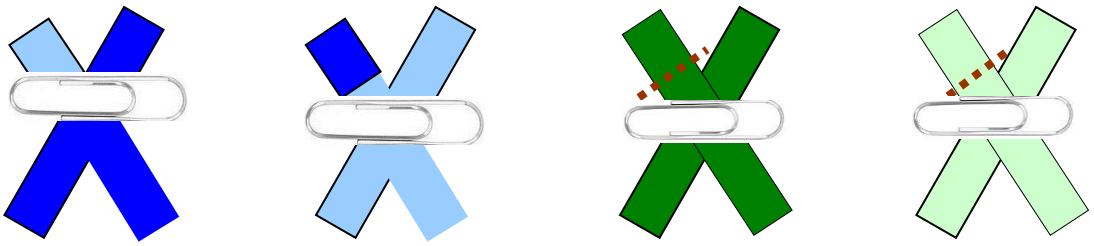
2. Fold each of the four strips of paper (one light blue, one dark blue, one light green, and one dark green) in half, lengthwise. Then place one strip of each color inside the nucleus to represent a chromosome before replication. The light and dark strips of the same color represent homologous chromosomes. At one end of the dark blue strip make a Large FR (no freckles) on the light blue make a lower case fr (freckles). On the dark green at a tip write T (for tall) and on the light green write a lower case t (short)

3. **INTERPHASE**: to represent DNA replication, unfold each paper strip and cut in half lengthwise. The two pieces that result from cutting each homologue represent the chromatids. Attach the two identical chromatid strips at the center with a paperclip so an X is formed. Each paper clip represents a **centromere**

Q1. What process did you model when you cut the strips in half?

Q2. What is the function of the centromere?

4. **PROPHASE 1**: remove the nuclear membrane. Place the blue chromosomes side by side. Two XX's in a row. Repeat for the green pairs. Simulate crossing over by measuring and cutting a 2 cm tip for a light blue strip. Do the same with a dark blue strip. Tape the light blue tip to the dark blue strip and repeat for the dark tip onto a light blue strip. Repeat for green.



Dark Blue Light Blue Dark Green Light Green

Q3. What is the purpose of placing the light and dark strips of the same color side by side?

5. **METAPHASE 1:** Place four 10 cm strings inside the cell, so that two strings extend from one side into the center of the cell and two strings extend from the opposite side into the center of the cell. The string represents the spindle fibers. Attach a string to the centromere of each chromosome (chromatid pair) with tape. Move the chromosomes to the center of the cell. NOTE: Make sure that the strings attached to similar colors come from opposite sides of the cell. TETRADES are now at the equator.

6. **ANAPHASE 1**: Gather the loose ends of the strings on both sides of the cell, and pull the strings in opposite directions.

7. **TELOPHASE 1:** un-tape the string from each centromere. Place a 40 cm piece of string around each group of chromatids, forming two nuclei. Place a 1 m piece of string around each cell, forming two membranes.

Q4. How many chromosomes are in each cell? Describe what each part represents.

MEIOSIS 2

8. **PROPHASE 2**: Remove the strings that represent the nuclear membrane and the cell membranes in both cells. Attach a 10 cm piece of string to each chromatid.

Q5. What must happen before the chromatids to separate?

9. **METAPHASE 2:** Move the chromosomes to the center of each cell. Make sure the strings attached to the two strips in each chromosome come from opposite sides of the cell.

10. **ANAPHASE 2:** Gather the strings on both sides of each cell, and pull the strings in opposite directions, separating the paper strips. Note: Only one strip in each pair will have a paper clip attached.

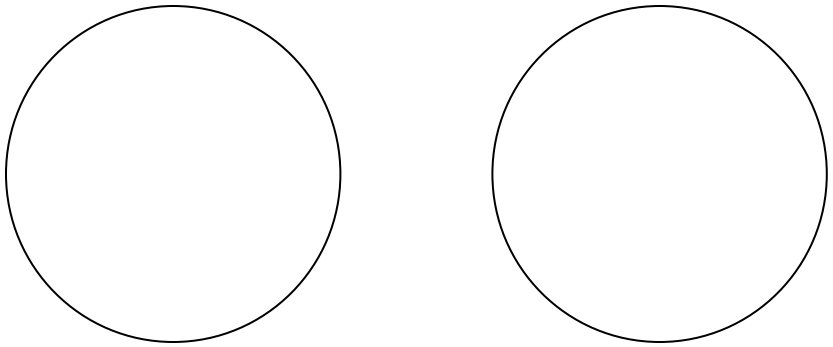
11. **TELOPHASE 2**: Untape the strings. Remove the strings and paper clips. Each strip of paper now represents a chromosome. Place a 40-cm. piece of string around each group of chromosomes, forming four nuclei. Place a 1m string around each cell, forming four membranes.

    Q6: How many cells did you make? How many chromosomes are in each cell? Are the cells haploid or diploid in number?

Analysis:

Q7. What is the diploid chromosome number of the original cell in your model? How many homologous pairs does this represent?

Q8. If a cell with a diploid number of 6 chromosomes undergoes meiosis, what will the cell look like after Telophase 1? Draw the result below:



Q9. Give two reasons why meiosis is important in sexual reproduction.

Q10. How might crossing over affect the rate of evolution?

Q**11**. Use your model to show what would happen if homologous chromosomes did not pair in Prophase 1. Predict the outcome.

Lab due on _____.

Experiment 14 – Lab exercises involving inheritance

Study Of Genetic Corn & Parakeets

Chi Square Test For Dihybrid Cross

1. Introduction

Introduction: This laboratory investigates a dihybrid cross as shown in the above photo of an ear of corn. The four different genes and grain types are identified in the following photo, and the complete cross is shown in Table 1 below.

Dominant Genes	Recessive Genes
P = Purple	p= Yellow
S= Smooth	s = Shrunken

There are four grain phenotypes in the above ear of genetic corn: **Purple & Smooth (A)**, **Purple & Shrunken (B)**, **Yellow & Smooth (C)** and **Yellow & Shrunken (D)**. These four grain phenotypes are produced by the following two pairs of heterozygous genes (**P & p** and **S & s**) located on two pairs of homologous chromosomes (each gene on a separate chromosome).

The following Table 1 shows a dihybrid cross between two heterozygous parents (**PpSs X PpSs**). The four gametes of each parent are shown along the top and left sides of the table. This cross produced the ear of genetic corn shown at the top of this page. Table I is essentially a genetic checkboard called a Punnett square after R.C Punnett, a colleague of William Bateson who devised this method. In 1900, English Geneticist William Bateson had Gregor Mendel's original 1865 paper on the genetics of garden peas translated into English and published. Thus Mendel became known to the entire scientific world. Bateson is also credited with the discovery of gene linkage in 1905.

Gametes	PS	Ps	pS	ps
PS	PPSS	PPSs	PpSS	PpSs
Ps	PPSs	PPss	PpSs	Ppss
pS	PpSS	PpSs	ppSS	ppSs
ps	PpSs	Ppss	ppSs	ppss

Table 1. This table shows four different phenotypes with the following fractional ratios: 9/16 Purple & Smooth (blue), 3/16 Purple & Shrunken (red), 3/16 Yellow & Smooth (green), and 1/16 yellow and shrunken (pink). There are nine different genotypes in the table: PPSS (1), PPSs (2), PpSS (2), PpSs (4), PPss (1), Ppss (2), ppSS (1), ppSs (2) and ppss (1). You can easily calculate the number of different phenotypes and genotypes in a dihybrid cross using the following formulae:

Number of phenotypes: $2^2 = 4$ Number of genotypes: $3^2 = 9$

---

2. Sample Chi Square Problem

Chi Square Problem: An ear of corn has a total of 381 grains, including 216 Purple & Smooth, 79 Purple & Shrunken, 65 Yellow & Smooth, and 21 Yellow & Shrunken. These phenotypes and numbers are entered in Columns 1 and 2 of the following Table 2.

Your Tentative Hypothesis: This ear of corn was produced by a dihybrid cross (PpSs x PpSs) involving two pairs of heterozygous genes resulting in a theoretical (expected) ratio of 9:3:3:1. See dihybrid cross in Table 1.

Objective: Test your hypothesis using **chi square** and **probability** values. In order to test your hypothesis you must fill in the columns in the following Table 2.

1. For the observed number (Column 2), enter the number of each grain phenotype counted on the ear of corn.

2. To calculate the observed ratio (Column 3), divide the number of each grain phenotype by 21 (the grain phenotype with the lowest number of grains).

3. For the expected ratio (Column 4), use 9:3:3:1, the theoretical ratio for a dihybrid cross. The fractional ratios for these four phenotypes are 9/16, 3/16, 3/16 and 1/16.

4. To calculate the expected number (Column 5), multiply the number of each grain phenotype by the expected fractional ratio for that grain phenotype.

5. In the last column (Column 6), for each grain phenotype take the observed number of grains (Column 2) and subtract the expected number (Column 5), square this difference, and then divide by the expected number (Column 5). Round off to three decimal places.

6. To calculate the chi square value, add up the four decimal values in the last column (Column 6).

Grain Phenotype (Column 1)	Observed Number (Column 2)	Observed Ratio (Column 3)	Expected Ratio (Column 4)	Expected Number (Column 5)	[Obs No. - Exp No.]^2 ÷ Expected No. (Column 6)
Purple & Smooth	216	10.3	9	381 x 9/16 = 214	4 ÷ 214 = 0.019
Purple & Shrunken	79	3.8	3	381 x 3/16 = 71	64/71 = 0.901
Yellow & Smooth	65	3.1	3	381 x 3/16 = 71	36/71 = 0.507
Yellow & Shrunken	21	1.0	1	381 x 1/16 = 24	9/24 = 0.375
Total Number:	381	----------	----------	Chi Square Value:	1.80

Table 2. Chi Square Data

7. **Degrees Of Freedom:** Number of phenotypes - 1. In this problem the number of phenotypes is four; therefore, the degrees of freedom (df) is three (4 - 1 = 3). In the following Table 3 you need to locate the number in row three that is nearest to your chi square value of 1.80.

8. **Probability Value:** In the following Table 3, find the number in row three that is closest to your chi square value of 1.80. In this table 1.85 (shaded in yellow) is the closest number. Then go to the top of the column and locate your probability value. In this case the probability value that lines up with 1.85 is .60 (shaded in yellow). This number means that the probability that your hypothesis is correct is 0.60 or 60 percent. The probability that your hypothesis is incorrect is 0.40 or 40 percent.

Good Fit Between Ear & Data							Poor Fit	
df	.90	.70	.60	.50	.20	.10	.05	.01
1	.02	.15	.31	.46	1.64	2.71	3.85	6.64
2	.21	.71	1.05	1.39	3.22	4.60	5.99	9.21
3	.58	1.42	1.85	2.37	4.64	6.25	7.82	11.34
4	1.06	2.20	2.78	3.36	5.99	7.78	9.49	13.28

Table 3. Probability Values

3. A Chi Square Problem For Credit

Chi Square Problem: A large ear of corn has a total of 433 grains, including 271 Purple & Smooth, 73 Purple & Shrunken, 63 Yellow & Smooth, and 26 Yellow & Shrunken. These numbers are entered in Columns 1 and 2 of the following Table 4.

Your Tentative Hypothesis: This ear of corn was produced by a dihybrid cross (PpSs x PpSs) involving two pairs of heterozygous genes resulting in a theoretical (expected) ratio of 9:3:3:1. See dihybrid cross in Table 1.

Objective: Test your hypothesis using **chi square** and **probability** values. In order to test your hypothesis you must fill in the columns in the following Table 4.

1. For the observed number (Column 2), enter the number of each grain phenotype counted on the ear of corn. [Note: These numbers are already entered in Table 4.]

2. To calculate the observed ratio (Column 3), divide the number of each grain phenotype by 26 (the grain phenotype with the lowest number of grains).

3. For the expected ratio (Column 4), use 9:3:3:1, the theoretical ratio for a dihybrid cross.

4. To calculate the expected number (Column 5), multiply the number of each grain type by the expected fractional ratio for that grain phenotype. The fractional ratios for these four phenotypes are 9/16, 3/16, 3/16 and 1/16.

5. In the last column (Column 6), for each grain phenotype take the observed number of grains (Column 2) and subtract the expected number (Column 5), square this difference, and then divide by the expected number (Column 5). Round off to three decimal places.

6. To calculate the chi square value, add up the four decimal values in the last column (Column 6).

Grain Phenotype (Column 1)	Observed Number (Column 2)	Observed Ratio (Column 3)	Expected Ratio (Column 4)	Expected Number (Column 5)	$[\text{Obs No.} - \text{Exp No.}]^2 \div \text{Expected No.}$ (Column 6)
Purple & Smooth	**271**	**?**	**9/16**	**433 x 9/16 =**	**?**
Purple & Shrunken	**73**	**?**	**3/16**	**433 x 3/16 =**	**?**
Yellow & Smooth	**63**	**?**	**3/16**	**433 x 3/16 =**	**?**
Yellow & Shrunken	**26**	**1.0**	**1/16**	**433 x 1/16 =**	**?**
Total Number:	**433**	----------	----------	**Chi Square Value:**	**?**

Table 4. Chi Square Data

7. **Degrees Of Freedom:** Number of phenotypes - 1. In this problem the number of phenotypes is four; therefore, the degrees of freedom (df) is three (4 - 1 = 3). In the following Table 5 you need to

locate the number in row three that is nearest to your chi square value.

8. **Probability Value:** In the following Table 5, find the number in row three that is closest to your chi square value. For an explanation of how to find and interpret the probability value, go back to the previous example.

4. Chi Square Table Of Probabilities

Good Fit Between Ear & Data							Poor Fit	
df	**.90**	**.70**	**.60**	**.50**	**.20**	**.10**	**.05**	**.01**
1	**.02**	**.15**	**.31**	**.46**	**1.64**	**2.71**	**3.85**	**6.64**
2	**.21**	**.71**	**1.05**	**1.39**	**3.22**	**4.60**	**5.99**	**9.21**
3	**.58**	**1.42**	**1.85**	**2.37**	**4.64**	**6.25**	**7.82**	**11.34**
4	**1.06**	**2.20**	**2.78**	**3.36**	**5.99**	**7.78**	**9.49**	**13.28**

Table 5. Probability Values

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5. Chi Square Quiz # 1 Scantron Questions

1. What is the **chi square** value? [Use Chi Square Choices]

2. What is the **probability** value? [Use Probability Decimal Choices]

3. Is There a **GOOD** or **POOR** fit between your hypothesis and your data? I.e. is the probability value within acceptable limits?

(a) Good Fit (b) Poor Fit

4. What is the **percent probability** that your hypothesis is correct? I.e. the observed ratio of grains in

the ear of corn represents a dihybrid cross involving two pairs of heterozygous genes (PpSs X PpSs). [Use The Percent Probability Choices]

5. What is the **percent probability** that the observed ratio of grains in the ear of corn deviates from the expected 9:3:3:1 due to an incorrect hypothesis? I.e. your ear of corn does NOT represent a dihybrid cross involving two pairs of heterozygous genes (PpSs X PpSs). [Use The Percent Probability Choices]

6. The following question refers to a cross involving linkage, where the genes P & s are linked to the same chromosome, and the genes p & S are linked to the homologous chromosome. Refer to Section 7 below. What percent of the grains from this cross will be purple and smooth? [Use The Percent Probability Choices]

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6. Chi Square Quiz # 1 Scantron Choices

Chi Square Choices (Choose Number Closest To You Chi Square Value)			
(a) 0.58	**(a) 2.20**	**(a) 6.65**	**(a) 11.34**
(b) 1.01	**(b) 2.37**	**(b) 7.78**	**(b) 11.79**
(c) 1.06	**(c) 3.36**	**(c) 7.82**	**(c) 12.26**
(d) 1.42	**(d) 4.64**	**(d) 9.49**	**(d) 13.28**
(e) 1.80	**(e) 5.99**	**(e) 9.98**	**(e) 14.13**

Probability Value Decimal Choices

(a) < .01	(a) 0.05	(a) 0.50	(a) 1.00
(b) 0.01	(b) 0.10	(b) 0.60	(b) 1.10
(c) 0.02	(c) 0.20	(c) 0.70	(c) 1.20
(d) 0.03	(d) 0.30	(d) 0.80	(d) 1.30
(e) 0.04	(e) 0.40	(e) 0.90	(e) 1.40

Percent Probability Choices			
(a) 1%	(a) 7%	(a) 50%	(a) 92%
(b) 2%	(b) 10%	(b) 60%	(b) 93%
(c) 3%	(c) 15%	(c) 70%	(c) 94%
(d) 4%	(d) 20%	(d) 80%	(d) 95%
(e) 5%	(e) 35%	(e) 90%	(e) 98%

7. Possible Reasons For Incorrect Hypothesis

Reasons For Incorrect Hypothesis: If your probability value is .05 (5%) or less, then your ear of corn deviates significantly from the theoretical (expected) ratio of 9:3:3:1 for a dihybrid cross. A probability value of 5% or less is considered to be a poor fit. One possible reason for a poor fit is that your original ear of corn was not produced by a dihybrid cross (PpSs X PpSs). The original parents

may have had different genotypes, such as PpSS or PPSs. These genotypes when crossed together will not produce a 9:3:3:1 ratio typical of a true dihybrid cross. Another reason for an incorrect hypothesis might be due to linkage (autosomal linkage), where more than one gene is linked to the same chromosome. For example, what if the genes P & s are linked to a maternal chromosome and the genes p & S are linked to the homologous paternal chromosome. Since they occur on the same chromosomes, these linked genes will also appear together in the same gametes. They will not be assorted independently as in dihybrid cross shown in Table 1 above. The following Table 7 shows a genetic corn cross involving linkage:

Gametes	Ps	pS
Ps	PPss	PpSs
pS	PpSs	ppSS

Table 7. A Genetic Corn Cross Involving Linkage.

There are three different phenotypes in the offspring from this cross: 1/4 Purple & Shrunken (blue), 2/4 Purple & Smooth (red) and 1/4 Yellow & Smooth (green). There are also three different genotypes: 1/4 PPss (blue), 2/4 PpSs (red) and 1/4 ppSS (green). Compare the phenotypes and genotypes in this table with the original 9:3:3:1 dihybrid cross shown above in Table 1.

When Gregor Mendel completed his research on genetic crosses with garden peas in 1865, he assumed that the individual traits were assorted independently of each other. One of his hypotheses became known as the Law of Independent Assortment. Today we can explain this law because the traits Mendel studied just happened to occur on separate chromosomes. This law also explains the assortment of the four different gametes from each heterozygous parent (PpSs) in the 9:3:3:1 dihyrid cross shown in Table 1: Ps, Ps, pS and ps. Linkage of P & s and p & S on one pair of homologous chromosomes would give only two gametes from a hterozygous parent (PpSs): Ps and pS. Additional gametes (pS and ps) could be produced by crossing over between the homologous chromosomes during synapsis of meiosis, but the

resulting four gametes would not be in equal proportions as in a cross in which all four genes occur on separate chromosomes. So the neat ratios of offspring described in Mendel's monohybrid (3:1) and dihybrid (9:3:3:1) crosses only occur when all the genes occur on separate chromosomes and are segregated and assorted independently of each other. Whether Mendel encountered linkage in his experiments has been debated by geneticists for decades, but one thing for sure, he primarily concentrated on traits that just happened to be on the seven pairs of separate chromosomes in the garden pea (**Pisum sativum**).

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9. Dihybrid Cross Between Two Green Parakeets

Gametes	BC	Bc	bC	bc
BC	BBCC	BBCc	BbCC	BbCc
Bc	BBCc	BBcc	BbCc	Bbcc
bC	BbCC	BbCc	bbCC	bbCc
bc	BbCc	Bbcc	bbCc	bbcc

Table 8. A dihybrid cross between two green parakeets (BbCc X BbCc). This cross involves codominance and gene interaction resulting in a 9:3:3:1 phenotypic ratio of offspring. Codominant alleles B & C together = Green (neither gene is completely dominant over the other). Homozygous or heterozygous dominant B alleles with recessive c alleles = Blue. Homozygous or heterozygous dominant C alleles with recessive b alleles = yellow. All recessive alleles (bbcc) = white.

Lab due on _____.

MODULE # 3- Reproduction

Introduction All animal species reproduce sexually. However, some animal species also have the ability to reproduce non-sexually at times. Non-sexual reproduction can be a very efficient way of producing large numbers of individuals for dispersal or making large numbers of individuals with successful genotypes during times of favorable environmental conditions when genetic variability is less important. However, all of these species also practice sexual reproduction to increase the genetic variability in the species.

Experiment 15 - Plant Reproductive Organs 1 (Anther)

The internal structure of stamen and carpels can be seen most clearly in cross- sections of unopened flower buds. The stamens produce pollen grains, the male spores, in pollen sacs in the anthers. The carpels ultimately contain egg cells, the female gametes, produce inside the ovules within the ovaries.

Aim: To make annotated drawings from prepared slides of transverse section of anthers
Apparatus/ Materials: microscope slide of Lilium sp.(T.S), microscope

Method:

1. Examine, under low power a cross section of the bud of lily and identify the anthers
2. Examine the anther at high power. Notice the four pollen sacs and examine their contents.
3. Make a drawing of the anther, under high power and be sure to annotate the key structures.

Results: Annotated drawing of Anther.

NO DISCUSSION

Lab due on _____.

Experiment 15 - Plant Reproductive Organs 2 (Ovary)

Aim: To make annotated drawings of transverse section of Lilium ovary and an embryo sac from prepared slides

Apparatus/ Materials: microscope slide of Lilium sp.(T.S), microscope

Method:

1. Examine, under low power a cross section of the bud of lily and identify the ovary. Look for within.
2. Examine the ovules at high power and observe the structures labeled in the figure seen below.
3. Make a plan drawing of the ovary under high power and be sure to annotate the key structures. Annotate the key structures.
4. Make a drawing of one of the embryo sacs as seen under high power.

Results: Annotated plan drawing of ovary and drawing of an embryo sac.

NO DISCUSSION

Lab due on _____.

Experiment 16- Animal Reproductive Organs 1 (Ovary)

Each is a follicle composed of an oocyte (a diploid cell which will produce an egg) surrounded by one or more layers of epithelial cells. The cells in the follicle will go through a sequence of changes called the ovarian cycle. During the ovarian cycle the epithelial cells multiply and secrete increasing amounts of oestrogens (including estradiol) and progesterone, the female sex hormones. These hormone coordinate changes in the uterus which prepare it to receive and nourish the 62 embryo (the uterine cycle). At the same time, the oocyte undergoes Oogenesis to produce the egg (ovum). The diploid oocyte divides by meiosis to produce one large haploid egg cell and three small haploid polar bodies which have very little cytoplasm. As the follicles mature they grow larger and the number of epithelial layers increases. You will see follicles in different stages of maturity. Somewhere on the slide you should see one or more large fluid-filled follicles containing a large egg ready for release from the ovary (ovulation). This is a mature follicle, a Graafian (or Vesicular) follicle. The egg will enter the oviducts and be carried down to the uterus.

Title: Animal Reproductive Organs 1
Aim: To make annotated drawings from prepared slides of transverse section of human ovary
Apparatus/ Materials: microscope slide of human ovary (T.S), microscope

Method:
Examine the microscope slide of a mammalian ovary under low power.
Observe the outer layer of the ovary. Note the numerous small circular structures. Use the wall charts or models that depict the Ovarian Cycle in humans to better understand these events.

1. Examine the ovary at high power and observe the structures labeled in the figure seen below.
2. Make a plan drawing of the ovary under high power and be sure to annotate the key structures.

Results: Annotated drawing of Transverse section of Human Ovary as seen under high power.
NO DISCUSSION NECESSARY

Lab due on _____.

Experiment 17- Animal Reproductive Organs 2 (Testes)

Each tube is called a seminiferous tubule. It is in the seminiferous tubules where sperm are produced by meiosis. Spermatogenesis produces four haploid (1N) sperm from each diploid mother cell. You should be able to see the hair-like tails (flagella) of many sperm in the lumen of each tubule. Are you able to see the heads of the sperm under high power? Thin layers of connective tissue bind the tubules together. Observe the dark-staining cells scattered between the seminiferous tubules. These are interstitial cells. The interstitial cells produce androgens, the male sex hormones. Testosterone is the principle androgen produced. Observe the demo slides of Sperm from a variety of species. A sperm is composed of a head (location of the DNA), midpiece (which contains mitochondria), and flagellum (for movement). Note their small size and that the appearance of sperm varies in different taxa.

Title: Animal Reproductive Organs 2
Aim: To make annotated drawings from prepared slides of transverse section of human testes.
Apparatus/ Materials: microscope slide of human testis (T.S), microscope

Method:

1. Examine the microscope slide of a Mammalian Testis (XS) under low power. Note that the slide shows numerous tubes that are cut in cross-section.
2. Make a plan drawing of the Testis under high power and be sure to annotate the key structures.

Results: Annotated drawing of Transverse section of Human Testis as seen under high power.

NO DISCUSSION NECESSARY

Lab due on _____.