

Biology 151

TA Notes – What is Life? Lab 2

BASIC BIOLOGICAL TECHNIQUES



The properties of life depend upon the intricate and rapid interchange of atoms, atom groups and sometimes even parts of atoms (e.g. electrons) among these complicated molecules. Such exchange cannot take place in solid matter because there all molecules are more or less fixed in place and as a result interchanges can take place only slowly or not at all. Such exchanges, similarly, cannot occur in a gaseous state because there, molecules are far apart and interchange cannot be made rapidly enough.

However, in liquid, e.g. water, molecules are close enough together to be in contact, yet they roll and slide over each other freely. In this system, interchange can take place rapidly enough to support the reactions of living tissue.

The two major processes of diffusion across cell membranes are termed dialysis and osmosis. Osmosis is limited to the passage of water across a membrane; dialysis, to passage of substances other than water, e.g. solutes. Diffusion generally refers to movements of solutes in a liquid medium. This lab will allow students to test the properties of diffusion in a model cell system.

This part of the lab is also set up to give them some introduction to developing their own protocols to answer questions. Give them some guidance, for example,:

- a) give them instructions on setting up the system and
- b) let them know that the experimental "cell saps" are combinations of sugar, starch, protein and/or oil
- c) remind them that, as a group, they should work out a method to answer the questions asked and that the reading is designed to give them most of the information they will need to do this. (They'll just need to decide what parts of the reading are important and what parts aren't. In other words, the reading is a small library of information. They will need to decide what parts of this information they need to use to deal with each of the exercises.)

Note: At beginning of lab remind students that Exercises 1 and 4 should be started at the beginning of the lab period.

Before lab:

Check to make sure all supplies and materials are available in lab, both on the front bench and at the student desks. Refer to the note in lab for the materials that should be available.

Announcements to students:

1. Make any announcements needed, e.g.:
 - a) Quiz scheduled for following week. (See attached copy of sample quiz questions which will be available for students in lab.)
 - b) **Have students begin in small groups by working out prelab questions 1 and 2.** Give them about 10 min. Then choose "volunteers" from several groups to do the calculations on the board and discuss how they arrived at their answers (check with rest of class to see if they got the same answers). This will save many later calculation errors.

⇒ **In this lab, many students may encounter "math difficulties".** The lab requires that they calculate percent wet weight, etc. from a subsample of a larger sample.

Having the students do questions 1 and 2 (of Prelab Questions), at the beginning of lab will help overcome some of their problems with the calculations when they come to them in the lab work itself.

STUDENTS HAVE MOST DIFFICULTY WITH CONVERSIONS.

For example, they have an original sample of apple which had a wet weight of 21 g. The dry weight was 3.2 g. They take a 1.8 g sample of the dry material for burning. All but 0.5 g burns away. What was the % inorganic in the original wet weight?

$$0.5/1.8 \times 100 = 27\% = \% \text{ inorganic of dry weight}$$

$$3.2/21 \times 100 = 15\% = \% \text{ of wet weight that was not water or conversely, } 85\% = \% \text{ of original wet weight that is water}$$

$$\% \text{ inorganic of original wet weight} = 27\% \text{ of } 15\% = 4\%$$

⇒ REMIND STUDENTS THAT WHEN THEY CAN SAY OF LIKE THIS IT MEANS MULTIPLY THE NUMBERS TOGETHER.

If 27% of the subsample (1.8 g) is inorganic then the % inorganic of entire dry weight = 27% (They have a hard time with this one sometimes. Remind them that regular coke or 7up has about 10% sugar in it. If they take a subsample of it, e.g. pour 1/4 of the can into a cup - what is the % sugar in the coke in the cup?)

Caution – Some students will argue that water is inorganic and therefore should be included in the % inorganic. You will need to convince them that calculation of organic vs inorganic is done based on dry weight only (see last paragraph of I. A in the Reading for this lab).

DENSITY DETERMINATION --

⇒ When you do the sample quiz question on density determination at the beginning of lab have students brainstorm ideas for how to measure the volume of irregular objects. The lab mentions the water displacement system, but doesn't describe exactly how you'd do this. Have them define what is meant by the water displacement system and have them come up with methods to do this.

After they've done questions 1 and 2 and explained how they arrived at their answers: Let them know:

How this lab will be set up, e.g.

- i. Overhead/board collection of data for Exercises. Sample data charts are available in another file in this TA info folder.
- ii. Questions that will be addressed in end-of-lab discussion.
- iii. All students should start on Exercises 1 and 4 at the beginning of lab.

Indicate where material is located for work with:

- a) density determination
- b) organic vs inorganic and wet vs dry weight
- c) WARNINGS - goggles; crucibles/heat/goggles

Explain a little more about Exercise 1: What types of organic compounds can diffuse through a model cell membrane of dialysis tubing?

Let students know that this exercise is asking them to set up "model unicellular organisms" using mystery "cell saps". Their "organisms" are placed in beakers of distilled water for about 90 min. **Weight needs to be taken every 15 min. for at least 1.5 hours.**

Students test the original cytoplasm for:

carbohydrate (Benedict's test and iodine test)

lipid (paper test)

protein (Biuret's test)

Then students devise a protocol to determine what the membrane (dialysis tubing) is permeable to.

Emphasis:

What characteristics of substances affect osmotic relationships within cells? What effect does differential permeability have on the functioning of the various organelles in the cell?

You will need to demonstrate the technique for filling and tying of the dialysis tubing.

Leakage tends to occur when:

- a) ends are not folded over before tying
- b) ends are twisted too much and tied too tightly
- c) the bag is overfilled and bursts because of increased osmotic pressure as bag picks up water over time

Bags should not be overfilled, but should be flaccid and have air removed prior to tying off. Space for expansion (due to uptake of water) must be available in the bag.

Do a positive Biuret's test on a known protein sample to show them what this should look like. A positive test tends to be purple which is best seen if the tube is held up against white paper.

Instructions for Benedict's and Biuret's tests will be on benches in lab.

NOTE: USE ONLY DISTILLED WATER IN WATER BATHS TO PREVENT LIME BUILDUP.

FOR YOUR INFORMATION ONLY: The dialysis tubing should be permeable to glucose and water (in this system) but not to starch or protein.

Students should put info on board as:

<i>Mystery cytoplasm contents</i>	<i>Permeability of each</i>	<i>Effect on Osmosis</i>

Go over these results as the end of lab and discuss possible reasons for the differences. E.g. starch and protein have little effect on osmosis because they are such large MW molecules. As a result, a 1% solution of glucose have a higher molarity and contain many more molecules of glucose (MW 180) than will a 1% solution of starch (MW 63,000).

In general the smaller the molecules, the greater the number of molecules per gram (e.g. 1 gm /100 ml of solution or a 1% solution). The greater the number of molecules per unit volume, the greater the diffusional gradient or potential. Another way to look at this is that smaller molecules have a greater surface area to volume ratio. The surface area interacts with water molecules forming a ring of hydration. This reduces the relative amount of water (e.g. in Brownian movement) in the area of the solute, leaving a relative higher concentration of water in the area without the solute. Water then moves from the area of higher concentration to lower concentration. Similarly, the solute will tend to diffuse down its concentration gradient (if the dialysis membrane is permeable to it).

Cautions:

ASHING TECHNIQUE (or Don't make an ash of yourself) --

↳ Students appear to have no concept of how hot a bunsen burner can be. The crucibles can get up to 400C. This is much hotter than your oven when you bake. These are **flesh melting** temperatures.

Remind students to: **NEVER TOUCH A CRUCIBLE WITH BARE HANDS** unless they first test it for temperature. This can be done by bringing your hand close to it first.

ALWAYS set HOT crucibles on "asbestos" pads or gloves, NOT ON BENCH TOPS.

SAMPLES FOR ASHING -- Samples are:

apple - fruit	carrot - root
lettuce - leaf	turkey breast - muscle
celery - stem	

Collect data and compare to see if there is any significant difference in dry weight/wet weight and % inorganic among the different types of tissues. Generally there is not much difference except muscle appears to have lower water content -- Remind students the meat was "bled" before sale, therefore, this is probably an artificial factor based on preparation before analysis rather than a real factor/difference.

Biology 151 Questions for Lab Quiz 2

Quiz 2 will consist of questions like those that follow. The total point value of Quiz 2 will be 20 pt. You will be given 20 min for the quiz at the beginning of the next lab period. THIS WILL NOT BE OPEN BOOK. YOU WILL BE ALLOWED TO BRING IN ONE 8.5 by 11 inch sheet of paper with any hand written notes on it you'd like. Your TA may provide additional instructions.

1. A student finds the density of a piece of wood (which floats) to be 1.38 g/cm^3 . Another student tests a different piece of wood and finds its density to be 0.9 g/cm^3 . Which of these results would you be more likely to accept as real and accurate? Explain your answer. (2 pt)

2. A flask containing a piece of metal and water weighs 375 g and has a water level of 110ml. After the metal is removed the weight of the flask plus water is 150 g and the water level initially was 80 ml. Which of the following metals was in the flask? Densities of the various metals are included in parentheses. **Show all work to receive credit.** (6 pt)

Metals:

a. aluminum (2.5g/cc); b. iron (6.3 g/cc); c. copper (7.8 g/cc); d. and alloy of iron and aluminum.

3. Which variable(s) of the equation: $\text{Density} = \text{mass}/\text{volume}$ would be affected by using a specimen that is not completely dry? Explain, how the variable(s) would be affected? (4 pt)

4. The carrying capacity (volume of cargo hold) of your truck is 3000 kg. You are asked to transport 130 plastic boxes of pure distilled water. Each box measures 2 ft X 2ft X 1.5ft. Can you carry all the boxes? If not, how many can you carry? Show how you would determine how many you could carry. (4 pt)

5. A beaker completely filled with water weighs 500 g. A piece of wood weighing 50 g is placed on the surface of the water in the beaker. It displaces some of the water which flows out of the beaker onto the bench. If you now weigh the beaker (containing the remaining water and the floating wood), what will it weigh ($> 500 \text{ g}$, $< 500 \text{ g}$, 500g)? Explain your answer. (4 pt)

Wet vs Dry Weight Organic vs Inorganic Content

6. Modify the following procedure as needed to produce more accurate and representative values of % organic and % inorganic.

- a. weigh an apple
- b. take a 25 g sample of the apple and dry it in a drying oven for several days
- c. determine dry weight
- d. burn a 1 g sample until no weight difference occurs
- e. measure ash weight as final weight of crucible minus initial weight of crucible plus 1 g
- f. calculate inorganics as a % of wet weight by taking the answer in e and dividing by 25 g. (6 pt)

Biochemical Composition

(2 pt) 7. Given the following results of tests conducted outside the dialysis bag (cell model) what can you conclusively say about what was inside the dialysis bag.

Tests performed on water in beaker: Results:

Iodine	-
Benedict's	+
Biuret's	-
Lipid	-

One group recalled that during lab last week their "model cell" (dialysis bag) in distilled water took up water from the beaker. With this in mind, explain what would happen if you used distilled water as a centrifugation medium for cellular organelles. Explain your answer in terms of the osmolarity of a cell vs the osmolarity of distilled water.

What good is knowledge unless you can put it to work?

(4 pt) 8. The alga Chlokybus has 2 types of chloroplasts (photosynthetic organelles in the cytoplasm). The 2 types are the same size, but type 1 contains abundant starch grains and type 2 usually does not. Chloroplasts, if isolated in appropriate buffers and culture media will continue to survive and carry out photosynthesis. You want to study the two types of chloroplasts to determine if and how they are different functionally. What methods would you use to get pure populations of type one and of type two? What methods could you use to determine which type you had and whether or not there was contamination between types in the culture?

(5 pt) 9. The barley used in beer making is first germinated (allowed to sprout roots from the seeds or barley grains). This initiates a series of enzymatic reactions inside the barley grain that convert stored starch into sugar. To brew the best beer, the barley must contain an appropriate combination of stored starch and sugar. Starch gives the beer body and sugar is used by the yeast and ultimately converted to alcohol. Assume you, as the brewer, know what the appropriate starch:sugar ratio is. Given this,

- a) what methods would you need to develop to determine when the barley contained this ratio and
- b) how would you stop the enzymatic digestion in the barley at that time to maintain the ratio?

10. (4 pt) Sherlock Holmes and Dr. Watson are investigating the death of Mrs. Tilie. She appeared to have died of a stroke. Holmes suspects that her nephew, Monroe, poisoned the tea with a rare protein toxin obtained from an African toad. Witnesses however swore that both the nephew and Mrs. Tilie had had tea from the same pot. Mrs. Tilie has hers iced. Monroe preferred his hot, so heated his in a microwave to near boiling before drinking it. If the toxin was in the tea, what tests would you use to find out? If it was in both Monroe's and Mrs. Tilie's teacups, why did only Mrs. Tilie die? Or was it a stroke?

Additional Questions not published to the students:

1. You receive a mylar balloon filled with helium as a present. When you first get it, the balloon floats easily to the ceiling. As it loses some of its helium over time, the balloon loses its ability to float and starts to drag on the floor. Explain. (4 pt)

2. Many ships and ocean liners are made of metal with relative density greater than one. How can they float? Explain. (4 pt.)

33. You measure the fresh weight of a giant puff ball (fungus) as 160 ounces. Upon drying it for 2 days at 85 C you prepare to weigh it again, but now have only a metric balance to use. The dry weight of the organism is measured as 453.6g. What percent of total wet weight is the dry weight? Show all work to receive credit. (4 pt)

4. A student wants to determine the density of a piece of wood. He finds its weight to be 11 g. He then places it in a graduated cylinder and lets it float on the surface where it displaces 9 ml of water. He then calculates the density of the wood as Mass/volume of water displaced or 11 g/9 ml. Will this density be correct or greater or less than it should be? Explain your answer. (4 pt)

5. You are trying to invent new and interesting life jackets. Each jacket will have an overall volume of about 8 liters or 8000 ml. How many pounds can your jacket weigh and still float? Show how you would calculate the answer. You do not need to provide the actual answer, just the method required to obtain the answer. (4 pt)