PROTEIN LAB

BASED ON THE RESEARCH OF DR. RICHARD LONDRAVILLE

LAB OUTLINE-

WEEK ONE

- **PROCCESS WORMS FOR ANALYSIS**
- **SET UP A STANDARD CURVE WITH KNOWN CONCENTRATIONS**
- **PERFORM PROTEIN ASSAYS ON WORM SECTION**
- **INTERPRET RESULTS USING EXCEL SPREADSHEET**
- **COMPLETE THE PROPOSAL WORKSHEET THAT OUTLINES YOUR EXPERIMENT**

WHAT YOU HAND IN FOR WEEK ONE

- **PRELAB QUIZ**
- **PROPOSAL WORKSHEET FOR PROTEIN LAB**

WEEK TWO

- **COMPLETE YOUR EXPERIMENT AND ANALYZE RESULTS**
- **SUMMARIZE THE INTERPRETATION OF YOUR RESULTS**
- **GENERATE NEW HYPOTHESIS BASED UPON YOUR RESULTS**

WHAT YOU HAND IN FOR WEEK TWO

- **POSTLAB QUIZ**
- **RESULTS OF YOUR GROUPS EXPERIMENT**
- **ANSWER QUESTIONS AS A GROUP IN REGARD TO INTERPRETATION OF NEW DATA**

Points

PRELAB QUIZ (INDIVIDUAL)	10POINTS
PROPOSAL WORKSHEET (GROUP GRADE)	35 POINTS
RESULTS (GROUP GRADE)	35 POINTS
DATA INTERPRETATION (GROUP GRADE)	20 POINTS
	100 POINTS

<u>Proteins</u>

NOTE: YOU WILL BE **QUIZZED** ON THIS BACKGROUND READING**. IN ADDITION TO THIS SHEET, READ PG 36-45 OF YOUR TEXTBOOK**. PAY PARTICULAR ATTENTION TO THE CONCEPTS IN **BOLDFACE.**

Proteins are the workhorses of the cell. For almost anything you can think of that cells do, proteins are involved. When you see, think, grow, run, eat, develop, age, etc., you do so because of proteins. In fact, virtually everything you look at when you look in the mirror is made of protein. Because of their importance, most modern cell biology research is directly or indirectly concerned with the function of protein.

Proteins are **polymers** of **amino acids**. For most organisms, there are 20 different types of amino acids that differ in their properties (**nonpolar, aromatic, polar uncharged, charged**). The tens of thousands of different proteins found in an organism differ from each other in the number and arrangement of these 20 amino acids. The order, or **primary sequence** of the amino acids in a protein determine its shape, and its shape determines its function.

When an organism is presented with a physiological challenge, it will typically respond by modifying its body. Some examples of this include a rabbit increasing the density of its fur in the winter, a person with high blood pressure developing an enlarged heart, and a migrating salmon losing muscle mass along its migration route. Many of these responses involve changes in how much, and which types of proteins are **expressed** (cell biologists typically refer to a protein being **expressed** instead of being *synthesized*). We can measure, or **assay** how much protein is present in an organism with a relatively simple protein assay.

Protein assays are based on a simple idea. A dye, called **Coomassie Blue**, is added to a sample of protein (the dye is in a solution called **Bradford Reagent**). This dye binds to aromatic amino acids (e.g. phenylalanine, tryptophan, tyrosine). Most proteins will have some of these amino acids in them, so the more protein you have, the more the dye will bind to your sample. You can measure how much dye binds to the sample by putting the sample in a **spectrophotometer**. This is a device that shines light through a sample, and measures how much light passes through the sample. The more dye in the sample, the harder it is for light to get through it (thus more of the light is absorbed by the sample). How much light is absorbed is called **absorbance**, and **absorbance** is directly proportional to **protein** concentration (e.g. twice as much **protein** leads to twice as much **absorbance**). By putting in standardized amounts of **protein** and measuring the **absorbance** associated with each standard, you can plot a line that describes the relationship of **protein** to **absorbance** (the relationship is called a **standard curve**). Then you can put dye in a sample where you don't know what the **protein** concentration is, measure the **absorbance**, and calculate the concentration from the **standard curve**.

In this lab you will present some worms with a physiological challenge and predict what will happen to their total protein concentration. You will then test your prediction by homogenizing worm sections and measuring their total protein concentration.

Proteins-Procedures-Week One

1. This week your group needs to know:
   - -how to use a pipettor
   - -how to do a protein assay
   - -how to determine how much protein is in an unknown sample
   - -what is your experiment for next week (and any setup needed)

2. Your T.A. will give you a demonstration on the pipettors. These little sticks of plastic are about $200 each-so be careful with them. If you ever do any molecular/cell/microbiology research you will use these a lot, so it pays to get good at it. Things to remember:

-**Pipettors come in different sizes** -make sure you have the right one.
-**Think in microliters (μl).**

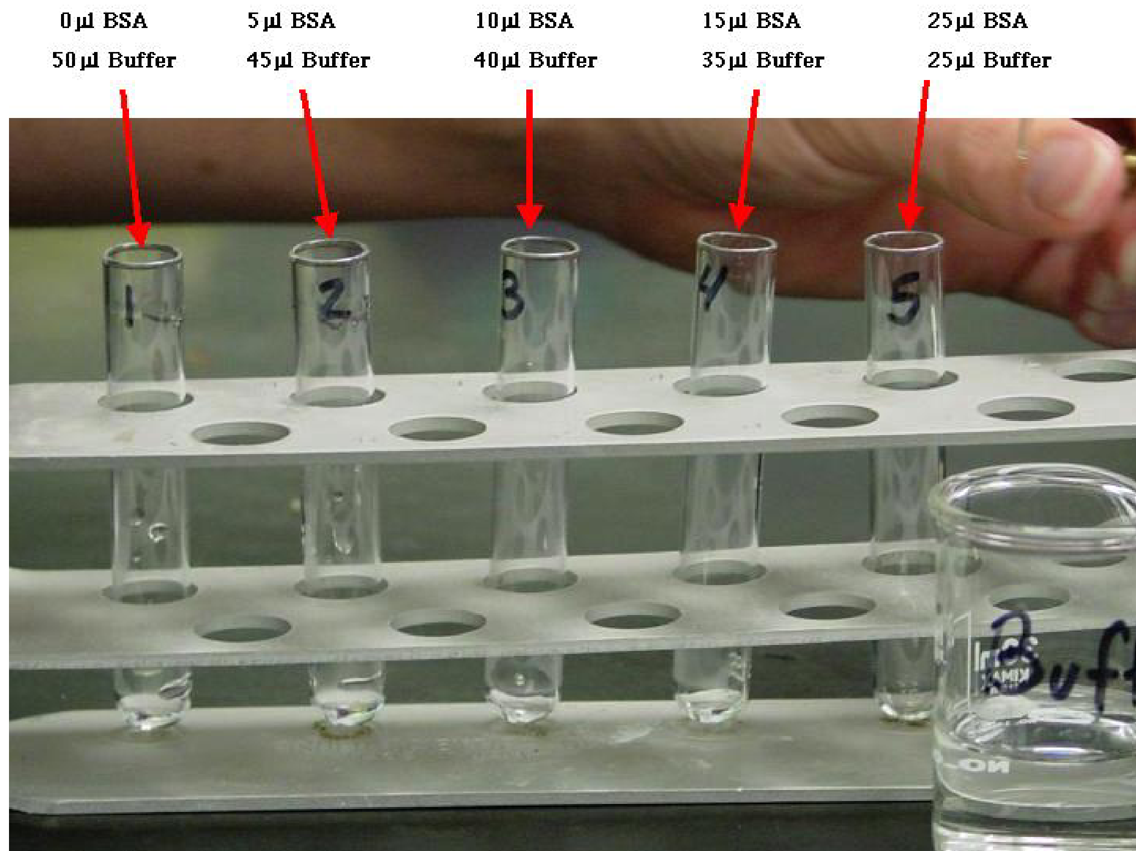
1 μl is 1/1000 milliliter, or 1/1,000,000 liter.
Useful conversions 1ml=1000 μl , or 1 μl = .001 ml

-**Always** keep the pointy end pointed toward the floor (otherwise you will gunk up the inside of the pipettor)
-**Always** make sure the pipettor has a tip on it, and **use a new tip for every new sample**.
-**There are two "stops" on a pipettor.** The first stop is where you suck up and deliver your sample. The second stop is only to blow out the last itty-bit of your sample. If you get all macho and go all the way to the second stop to suck up your sample, you will get about twice as much as you need, and your assay won't work.
-**Pipet slowly**. Slow up, slow down-this prevents spatter and gives the most accurate delivery.
-**Watch for bubbles.** Bubbles in your tip will mess up your pipetting.

3. Do a practice protein assay.
Set up a standard curve. This is where you add a known amount of protein to your tube, add the dye, and see what the absorbance is. We will use **bovine serum albumin (BSA)** as our standard protein. This is a protein found in cow's blood that binds fatty acids. It's easy to purify and cheap (that's why we use it). Your T.A. has provided you with a small tube of BSA- it contains 1 milligram (mg)/1 milliliter(ml) concentration. You will do dilutions of this sample to create your standard curve. We suggest the following dilutions:

Tube #	μl BSA	μl BUFFER
1	0	50
2	5	45
3	10	40
4	15	35
5	25	25

Notice every tube has the same amount of liquid in it- 50 µl



4. Place a worm on a plastic weigh boat and set on a block of dry ice. For rapid and complete freezing, you may need to manipulate the worm in order for contact between the worm and weigh boat to occur. After freezing is complete, cut the worm into 0.5 cm sections. Place a weigh boat on the balance and press the tare button. Weigh the section of worm on the balance and record.

NOTE: the weight of the worm should be approximately 0.1 grams. Not less than 0.05 grams and not exceeding 0.15 grams.

5. Add 3 ml of buffer and the section of worm to a large test tube. Insert the homogenizer into the test tube containing the worm/buffer and turn it on until the worm is totally homogenized (about 1 minute).

Then take some of this **homogenate** (we suggest 10 µl and add it to a test tube, then add 40 µl of buffer = total 50 µl).

Question-the protein concentration will obviously change depending on how much buffer you add. How will you standardize this, so that you can see if protein concentration changes, regardless of the size of the worm section?

Question-are you absolutely sure that measuring the protein concentration of the worm section just once will give you an accurate answer? How will you deal with this?

6. **PUT ON GLOVES AND KEEP THEM ON WHEN YOU HANDLE BRADFORD SOLUTION (CONTAINS COOMASSIE DYE AND AN ACID)**. To every test tube, **add 3 ml of Bradford solution** (named after the guy who invented this assay). Flick the tubes to shake them. Incubate them at room temperature for 5 minutes.

7. Measure absorbance.

Do your standard curve first (lowest protein to highest), then your unknowns. If you measure in this order, you don't have to change or rinse your cuvettes.

Put the solution from the test tubes (one by one) into a **cuvette**. Place the cuvette in the colorimeter. Be sure that the clear(no lines) sides of the cuvette are facing left and right, not towards the top and bottom of the colorimeter. Dial the colorimeter to 565 nm wavelength (green). Record the **absorbance** on your computer. Hit **COLLECT** to record the data, then hit **KEEP** to actually keep your data. The program will ask you for a value-enter the value of the amount of protein you are adding (example-enter '10' for

the 10 μg BSA sample). Remove the cuvette, pour out your sample into the sink, rinse and dry the cuvette, and repeat until you have measured the absorbance for all of your samples (standard curve and worm section). Do your standard curve first, then your unknowns.

8. Plot a linear regression for the **standard curve only** (not your worm section). μg BSA is the same as μl BSA in #3 above. Plot a linear regression (click on Analyze, Linear Fit). This draws the best straight line through your data. The equation it gives you is for that line (y=mx + b), where y is absorbance, m is the slope, x is μg protein and b is the point where the line crosses the y axis. "Cor" is the correlation coefficient -the closer you are to "1"-the better. The program gives you **m** and **b**, you must solve for **x**. **Print this graph-write the names of your group members and lab section on it.**

You can now plug in the absorbance for the worm, and solve for x. This will tell you the protein concentration of the worm sample (which is why you did all of this). If you took 25 μl of sample and there is 200 total, multiply your concentration X 8 to get the amount of protein in the whole bug (200 ÷ 25 = 8). Open up EXCEL, and open a spreadsheet called "Protein". If you plug in your values here, the spreadsheet will compute your protein concentrations.

9. Devise an experiment where you would expect the protein concentration of worms to change. If you need your TA to do something over the next week-make sure you work that out with them. You can't work your TA to death-but you can make a reasonable request. Write down your complete experimental design and methods, so that you are ready to go for next week.

Protein Lab-Proposal Worksheet

State your hypothesis, as specifically as possible:

Does your experiment involve a treatment to the pillbugs? If so, what is it?

What is the control in your experiment?

What is your sample size? (Number of pillbugs in each treatment group, number of assay tubes)

How will you be convinced that your hypothesis is supported or not supported? (How

will you analyze your data?)

How will you present your data?

Proteins-Procedures-Week Two

1. Complete your experiment and analyze your results. Don't hesitate to ask for help.
2. Print off your raw data-make sure everything is labeled and has the names of all group members.
3. Reduce your data somehow (graph it).
4. Do a statistical test to see if your data support your hypothesis.
5. Write a short (one paragraph) summary of how your group interprets these results. Start with a statement on whether the results do or do not support your hypothesis.
6. Generate a new hypothesis based on your data (write it out).

#2-5 REQUIRE THAT YOU HAND SOMETHING IN TO YOUR T.A. FOR EACH ITEM.

Proteins-Interpretation of New Data

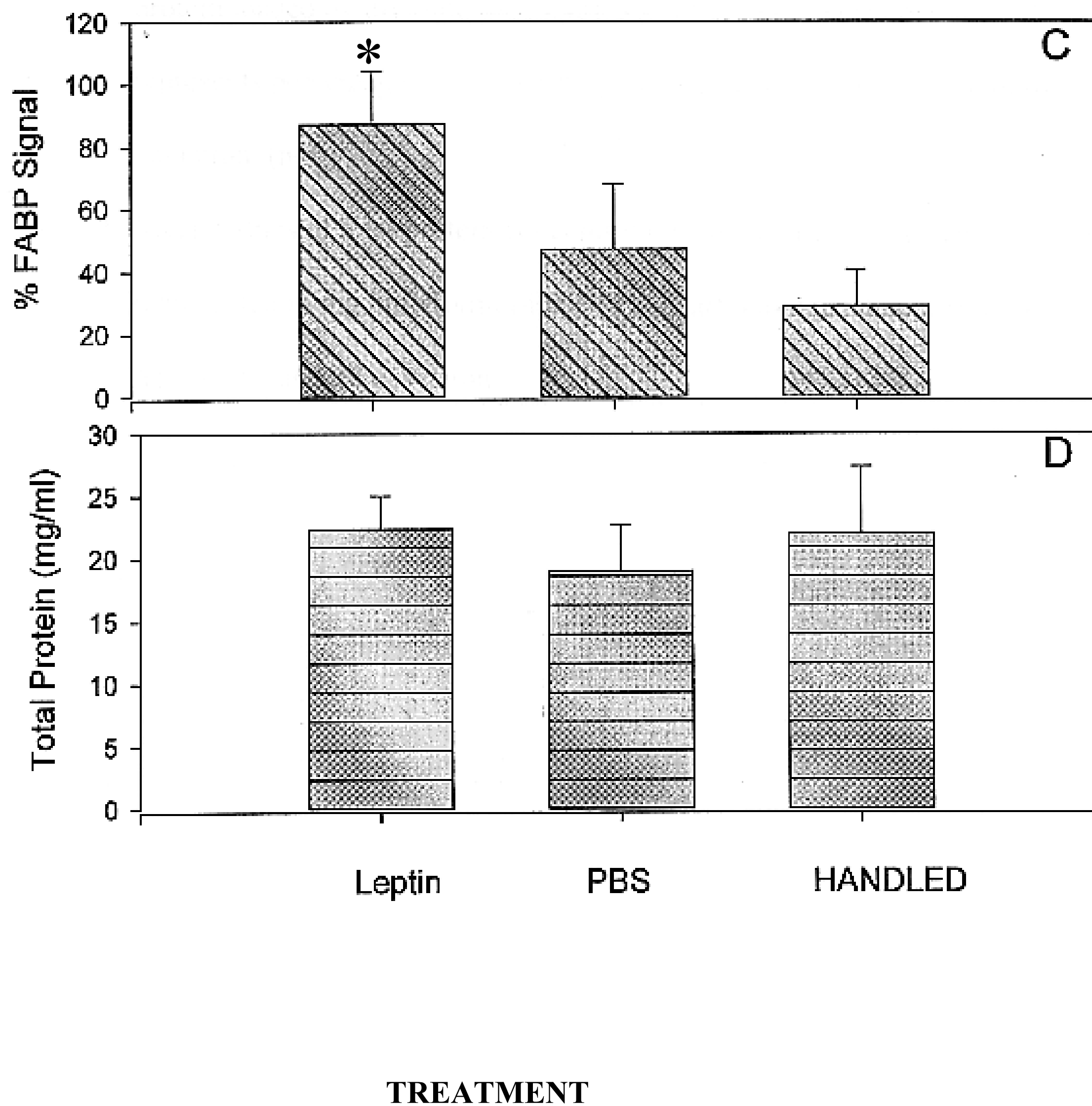
Below are data from Dr. Londraville's lab. These data were collected by Shawn DuVall, a Master's student in Biology. Dr. Londraville's lab studies the effects of a hormone called leptin. This hormone is made by fat tissue in mammals. When leptin is injected into a mouse or rat, it causes the animal to lose weight by breaking down its fat tissue. Shawn and Dr. Londraville wanted to see if it had the same effect in fish. They injected a group of fish with leptin (from mice) over a two-week period. For controls, they injected another group of fish with PBS (a buffer), and a third group got handled, but no injections. At the end of the experiment, they measured the concentration of a particular protein that carries fat in the cell (FABP). They also measured the concentration of all proteins in the cell (just like you did with the worms). In these experiments, they only looked at hearts (vs. the whole animal). There was no significant difference in total heart protein among the 3 groups, but there was a statistically significant increase in FABP in the leptin group (indicated by a *). The error bars are standard errors. Look at the data below, discuss them with your group, and try to answer the following questions.

1) Do you think leptin has any effect on fish? Why, or why not?

2) How can FABP increase, when total protein does not?

3) How do your data for worms compare with these data from fish heart?

Want to learn more? Ask your T.A., then ask Dr. Londraville (londraville@ uakron.edu) or Dr. Niewiarowski (phn@uakron.edu).



TREATMENT