Laboratory 11

Analysis of Proteins in Different Animal Organs, Part A

I. Introduction

Proteins often comprise more than 50% of the cellular mass. They are found in the plasma membrane, the membranes of various organelles, the cytoskeleton, and the ribosomes. They also are distributed throughout the nucleus and the cytoplasm where they play a variety of physiological or regulatory roles. Many cellular proteins are enzymes and catalyze the various chemical reactions that comprise cellular metabolism. In a multicellular organism like an animal or a plant, the individual cells are often highly differentiated from one another. Each type of cell is defined by the specific set of proteins it contains. In vertebrates, the differentiated cells are organized into four basic types of tissues: epithelial tissue, connective tissue, nervous tissue, and muscular tissue. The various tissue types then are combined into different organs, which are organized into organ systems. The purpose of this three-week project is to use both gel electrophoresis and a specific enzyme assay to compare the proteins found in various animal organs. Each lab group will prepare an extract from one organ; the samples and the resulting class data will then be pooled.

In the first part of the project, you will:

- prepare an extract from one animal organ
- determine the protein concentrations of your extract using a spectrophotometric assay.

In the second part of the project next week, you will use SDS-polyacrylamide gel electrophoresis again to analyze the proteins in the different organ extracts. Then in the third week you will assay your extract for a differentially-expressed enzyme called L-lactate dehydrogenase.

II. Pre-Lab Preparation

Read the Introduction, Background Information, and Experimental Procedures for this experiment. In preparation for this lab, please read the sections of your Cell Biology textbook that deal with proteins, animal tissues, and gel electrophoresis. Because this lab involves a protein assay that is very similar to that done in **Laboratory 3 (Determination of Protein Concentrations by Spectrophotometry)**, review the Background Information and Experimental Procedures for that experiment carefully. After preparing for the lab, you should be able to answer the following questions.

A. What are the major differences between a cell, a tissue, and an organ?

B. What are the four major animal tissues?

C. How will the proteins in different organs be extracted for use in this experiment?

D. Which of the different assay methods will be used to determine the protein concentrations of the organ extracts in this experiment?

E. Why is it necessary to make a new protein standard curve before determining the protein concentration of an organ extract?

F. Why is bovine serum albumin used to make a standard curve if the goal is to measure the protein concentration of a liver or brain extract?

III. Background Information

A. Animal Tissue Organization

In complex multicellular animals, individual cells differentiate during development and become organized into tissues. A tissue is a set of cells with a similar structure or activity, which are held together by cell-cell junctions and an extracellular matrix. The four basic types of animal tissue are epithelial tissue, connective tissue, nervous tissue, and muscle tissue.

Epithelial tissue consists of layers of cells that cover free surfaces, that line body cavities, and that coat tubular structures. The major types of epithelial tissue are simple squamous epithelium, simple columnar epithelium, and simple cuboidal epithelium. Epithelial cells are normally separated from the underlying tissues by a dense basement membrane. Successive layers of epithelial cells may form stratified or transitional epithelial tissues (Figure 11.1)

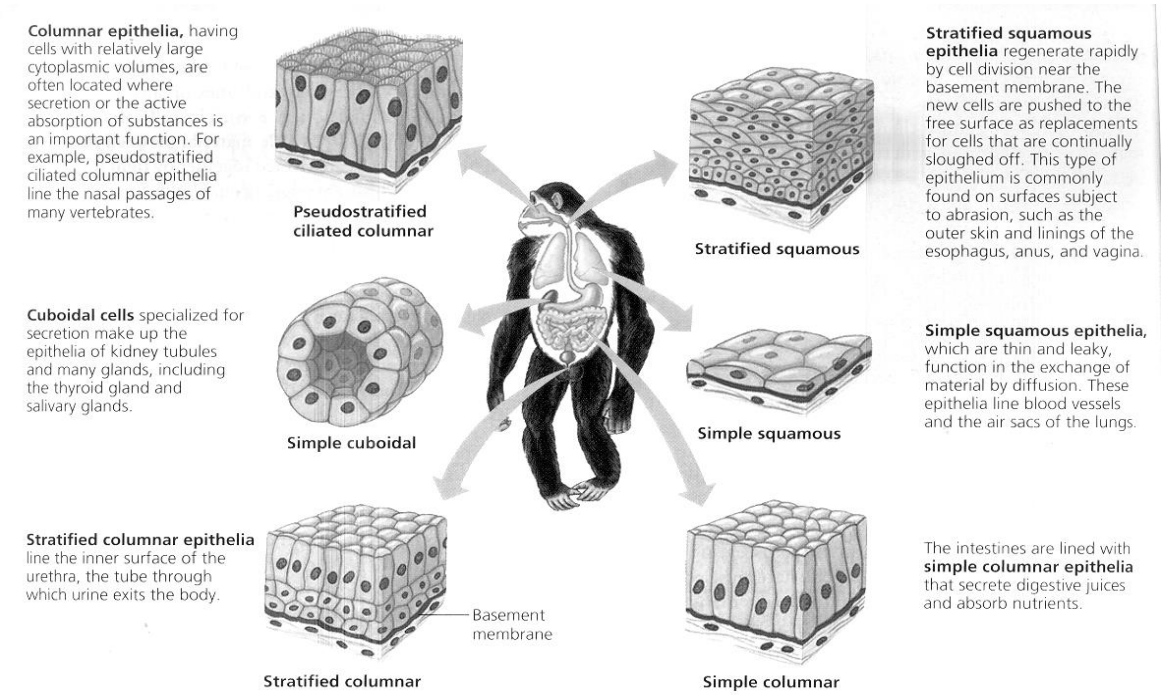

Figure 11.1. The structure and function of epithelial tissues.

Connective tissue consists of diverse groups of cells, which are often encased within a prominent extracellular matrix. The major types of connective tissues are loose connective tissue, adipose tissue, fibrous connective tissue, cartilage, bone, and blood. (Figure 11.2)

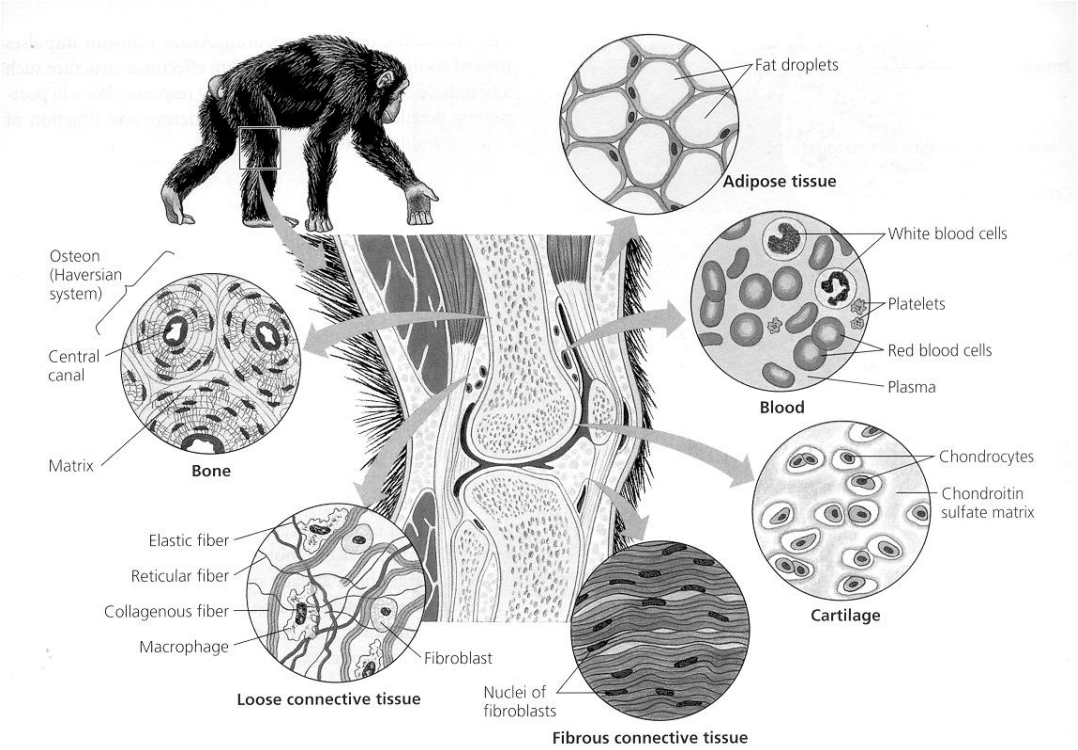

Figure 11.2 Some representative types of connective tissue. The area shown is the region around the knee joint.

Nervous tissue consists of electrically-active cells that can sense and transmit information from both the external environment and the internal environment. Neurons in the nervous tissue are supported by various types of neuroglial cells (Figure 11.3).

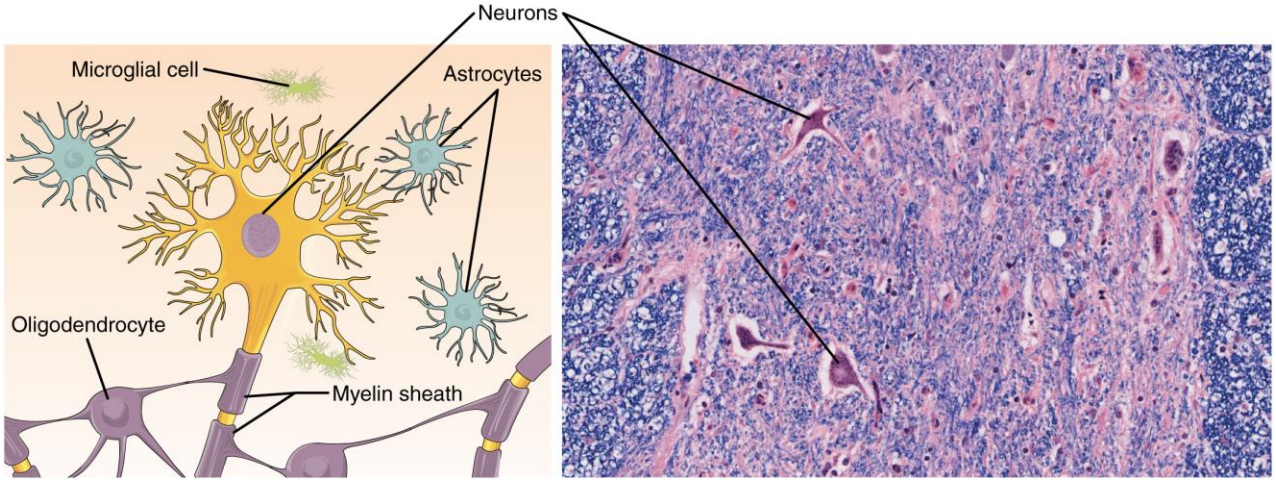

Figure 11.3 Diagram and stained tissue section of neural tissue. Nervous tissue contains electrically-active cells called neurons and supportive neuroglial cells including microglia, astrocytes and oligodendrocytes.

Muscle tissue consists of groups of cells that have the ability to contract. The major types of muscle tissue are smooth muscle, striated muscle, and cardiac muscle. (Figure 11.4)

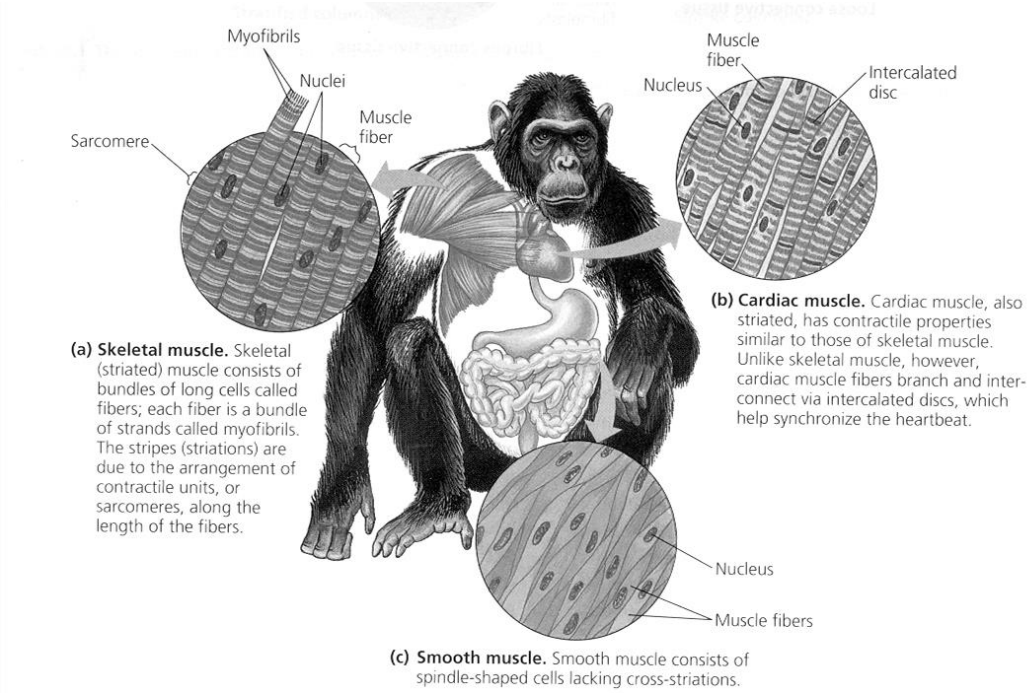

Figure 11.4. Three kinds of vertebrate muscle

In an organ, the four tissue types are combined in varying proportions to make a complex structure. Figure 11.5 shows a section through the stomach as an example.

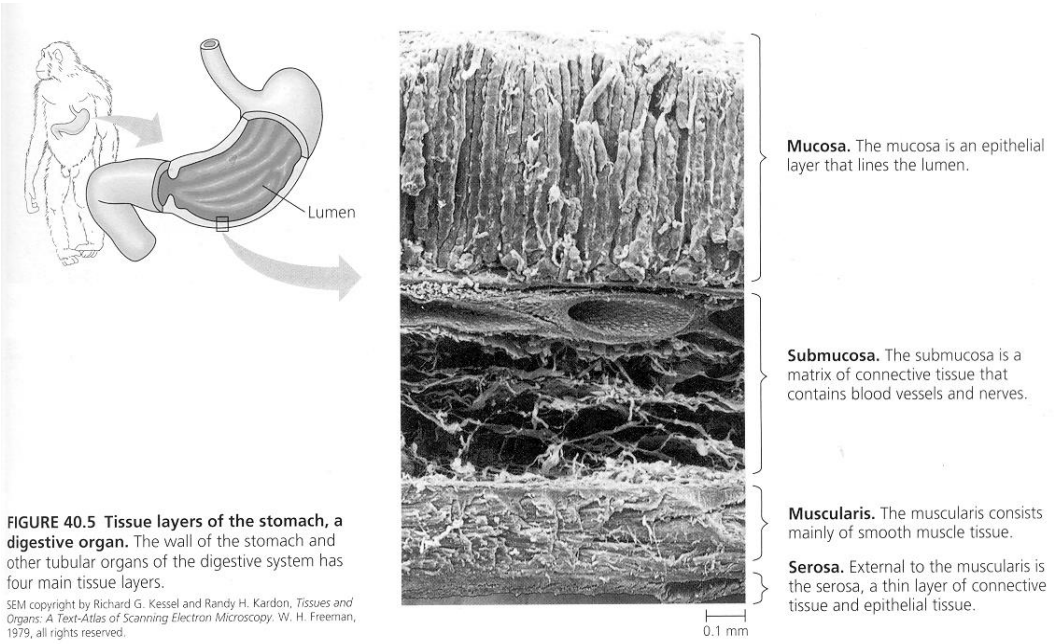

FIGURE 40.5 **Tissue layers of the stomach, a digestive organ.** The wall of the stomach and other tubular organs of the digestive system has four main tissue layers.

Figure 11.5. Tissue layers of the stomach. The wall of the stomach and other tubular organs of the digestive system has four main tissue layers.

B. Protein Samples

In this project, the various lab groups will work together to examine the proteins in different animal organs. Each group will prepare an extract containing the proteins from **one** organ and that extract will be shared with the other groups. In this way, all of the organs can be compared. The source material for this lab will be **acetone powders** of organs including calf liver, chicken breast muscle, bovine (cow) pancreas, porcine (pig) kidney, porcine (pig) brain, and porcine (pig) heart. Acetone powders are prepared by removing the organ from an animal (usually at a slaughter house), homogenizing the tissues to break the cells apart, and then treating the material with acetone (an organic solvent) to remove the lipids from the cellular membranes. The resulting extract is then dried and ground into a fine powder. This powder contains a concentrated mixture of biomolecules, including most of the proteins that were initially part of the tissues of that organ.

C. Determination of Protein Concentrations

Next week we will analyze protein samples using SDS-Polyacrylamide Gel Electrophoresis (PAGE). To do SDS-PAGE effectively, it is necessary to know the protein concentrations of the samples to be loaded onto the gel. If the protein concentration is too high, the gel will be "overloaded" and the bands will merge together. If the protein concentration is too low, nothing may be detected.

As indicated in **Laboratory 4 (Determination of Protein Concentrations by Spectrophotometry)**, there are several spectrophotometric assays available that can be used to determine the protein content of an extract or solution: 1) the absorbance at 280 nm method; 2) the Lowry method; 3) the Bradford or Coomassie blue R-250 dye binding method; and 4) the bicinchoninic acid (BCA) method. In measuring the "protein content" of an extract by any of these methods, it is important to remember that what is actually measured is the total concentration of all extracted proteins. This content does not reflect any particular type of protein but rather represents the cumulative amounts of all of the different cellular proteins in their varying proportions. As in other spectrophotometric assays, it is important to create a standard curve relating the amount of absorbance to the amount of protein. While any protein can be used as the standard, the most commonly-used protein is bovine serum albumin (BSA), which is readily available in a purified form. When the absorbance of an extract is converted to micrograms of protein using a BSA standard curve, it only means that the proteins in the extract reacted as much as a certain number of micrograms of BSA. In this experiment, you will use a version of the Bradford Reagent made by the Pierce Chemical Company called Coomassie Plus, which is designed to be compatible with the extraction buffer used with the tissue homogenates and acetone powders of the animal organs.

IV. Experimental Procedures

The following is a flow chart for this laboratory session:

Preparation of Acetone Powder Suspensions
(Section IVA)
↓
↓
Protein Concentrations of Acetone Powder Suspensions
(Section IVB)

A. Preparation of Acetone Powder Suspensions

The objective of this part of the experiment is to prepare extracts of different animal organs for protein analysis by SDS-PAGE and for enzyme assays, starting with acetone powders. **Each group will work with one of the acetone powders.** The class samples will be exchanged and data eventually will be pooled.

1. The following samples will be available. Use the designated letter for each sample along with the abbreviation listed here:

   PK: porcine kidney
   CB: chicken breast muscle
   BP: bovine pancreas
   CL: calf liver
   PH: porcine heart
   PB: porcine brain

2. Use two of the ~50 mg samples provided of your assigned acetone powder. They will be in 1.5 mL tubes, Add 200 μL of the T-PER reagent to each tube and homogenize the suspension with a small pestle for five minutes. Add another 800 μL of the T-PER reagent to each tube and homogenize for another 5 minutes.

3. Centrifuge the samples in a microcentrifuge for 5 minutes at 10,000 rpm.

4. Carefully remove the supernatant liquid and transfer it to one clean two mL tube mix it thoroughly then place half of it into each of two clean 1.5 mL centrifuge tubes. Label them with your initials and the organ letter and type. Keep the tubes on ice, both will be saved but make sure that you give one to your instructor right away so it can be frozen for use in the LDH assay. (Label it LDH in addition to the organ type and group initials)

B. Protein Concentrations of the Acetone Powder Suspensions

The objective of this part of the experiment is to determine the protein concentrations of the acetone powder suspensions. These concentrations will be used in the next two weeks both to determine how much of each solution should be loaded onto another SDS-polyacrylamide gel and then to measure the specific activity of L-lactate dehydrogenase. This part of the experiment is basically the same as **Laboratory 4 (Determination of Protein Concentrations by Spectrophotometry)**, so review that experiment carefully.

1. Obtain a tube of bovine serum albumin (BSA) at a concentration of 1.0 mg/mL (1.0 μg/μL) to use as a protein standard.

2. Prepare a BSA standard curve as you did in Laboratory 4 (Determination of Protein Concentrations by Spectrophotometry). Use the space below and on the next page to describe how the standard curve was prepared. **Note: Use <u>3.0 ml</u> of the Coomassie Plus Reagent per tube.** Plot the standard curve on a piece of linear graph paper and **insert this graph in your lab manual**.

Use the space on this page to record notes on standard curve preparation.

3. Discuss the graph with the instructor. If it looks good, you can proceed to the next part of the experiment. If some of the points deviate badly from the straight line, set up new tubes for those amounts of protein and repeat the assay.

4. Once you get a good standard curve, make up a conversion factor relating the absorbance at 595 nm to the amount of protein (_____ A_{595}/µg). This conversion factor is the same as the slope of the line. You can also make a graph in Excel.

5. Now set up a new assay for your extract of the acetone powder suspension. Since you do not know the protein concentration of this suspension, you will need to test the original suspension, a 1/10 dilution, and a 1/100 dilution of it. Refer back to **Laboratory 4 (Determination of Protein Concentrations by Spectrophotometry)** if necessary. To make the dilutions, add 900 µl of water to a two separate 1.5 ml microcentrifuge tube with a P-1000 micropipetter. Label the tubes 10^{-1} and 10^{-2}. Then use a P-100 micropipetter to add 100 µl of the acetone powder suspension to the first dilution tube. Mix the solution carefully by inversion. Then, using a new tip, transfer 100 µl of the 10^{-1} dilution to the next dilution tube.

6. Arrange a series of 13 new 13 x 100 mm test tubes in a test tube rack and number the tubes 1 through 13. Add the volumes of water and the protein solutions shown in the following table. As before, use micropipetters and fresh plastic tips to add the samples to the tubes. Add the water to the tubes first and then add the stock or diluted extract of the acetone powder suspension.

7. When all of the tubes have been set up, add **3.0 ml** of the Coomassie Plus Reagent to each tube with the Repipetter. Mix each tube by inversion using a piece of Parafilm and allow the tubes to sit for about 5 minutes at room temperature.

8. Read the absorbance of each tube at 595 nm using tube # 1 as the reference solution. You should use a separate cuvette for each dilution again starting with low concentration and working to high concentration. **Record your results in the table on the next page**. **Save your tubes until the instructor has checked your results.**

Table _____ Protein Assay of Acetone Powder Suspension

Tube	Solution	Water (µL)	Extract (µL)	Coomassie Plus Reagent (mL)	A_{595}	µg
1	---	50	0	3		
2	Stock Solution	45	5	3		
3		40	10	3		
4		30	20	3		
5		0	50	3		
6	1/10 Dilution	45	5	3		
7		40	10	3		
8		30	20	3		
9		0	50	3		
10	1/100 Dilution	45	5	3		
11		40	10	3		
12		30	20	3		
13		0	50	3		

9. Use the conversion factor derived from your standard curve to determine the number of μg of protein in all of the usable samples (that is, those that give absorbance values within the range of your standard curve). **Show your calculations here and add these values to the table on the previous page.**

10. Determine the concentration in **mg/ml** for these samples by dividing the amount of protein by the volume included in the assay. Multiply by 10 if necessary for the 1/10 dilution or by 100 if necessary for the 1/100 dilution**. Average the values to get a single protein concentration for the original suspension in mg/ml. Include those calculations here.**

11. When you are satisfied with your readings, discard the liquid in the designated container and discard the tubes. (**have your instructor check your numbers before you do this**)

12. Place the remaining acetone powder suspension with your other tube (the LDH assay one) in the designated box for storage until the next laboratory session.