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Perspectives on Writing Lab Manuals

Elizabeth L. Cantwell, Brian W. Lipscomb, and Laurel S. Rodgers

Shenandoah University, Biology Department, 1460 University Dr, Winchester VA 22601 USA
(**ecantwel@su.edu**; **blipscom@su.edu**; **lrodgers2@su.edu**)

Writing a lab manual well requires planning, time and follow-through. The best lab manuals are those that enhance students' lecture experience and, for this reason, making deliberate choices and providing clear learning objectives are imperative. Good planning is obviously necessary, and there are many options available for those who wish to construct their own manual, each with its own pros and cons. We present two differing perspectives on constructing a whole lab manual, as well as an example of a lab exercise written to complement the digestive system lecture in Human Anatomy & Physiology. Participants who would like to write their own manual are encouraged to bring their lab materials with them to this workshop for the discussion. By using participant examples, we will explore the different ways that writing a lab manual can be approached, and we will expand upon the benefits and drawbacks of these differing methods.

Keywords: lab manual, digestive enzymes, chemical digestion, digestive system

Introduction

Writing a Lab Manual

Writing a lab manual is a time-consuming undertaking. Collectively, the authors have written two: one for a one semester general biology course and the other for a two-semester anatomy and physiology series. Different choices were made in the construction of these manuals, from the computer programs used, to the sources utilized, to the timeline followed, to the method of dissemination to students. Each choice made has its own benefits and downsides, and manual writers should consider the needs of their particular course in deciding how to approach this potentially overwhelming task.

Some Reasons for Writing a Lab Manual

In General Biology I, the authors previously used a combination of labs written by faculty, as well as labs from a published manual. Those from the published lab manual had to be frequently modified in order to address the learning objectives. The assignments associated with the published labs were also cumbersome to grade by instructors who had multiple lab sections and thus a large number of assignments to grade. Finally, the authors wanted to reduce the cost of the lab manual for students. By constructing a lab manual and printing it from the campus copy center, students pay between ten and fifteen dollars for the manual each semester.

In Human Anatomy & Physiology (A&P), the decision to write a lab manual was precipitated by a general lack of enthusiasm for the mass-produced manual used previously. First, students expressed frustration regarding the number of supplemental handouts they had to print on their own. Second, students regularly indicated that they would like to see lecture and lab be better synchronized. Third, lab instructors were dissatisfied with how long it was taking them to grade each week. Finally, the need to significantly modify lab exercises and the addition of supplemental materials left students confused about what they would be tested on. In determining how to best solve these issues, the authors concluded that they should write their own manual.

Establishing Priorities and Overall Goals

One of the most important things that should be considered when planning a lab manual is how it will benefit the course. In General Biology I, the authors wanted to focus on the scientific method. By writing labs, they were able to provide students with opportunities to learn new lab techniques and design their own experiments nearly every week. Instructors are also now able to switch out labs from one semester to another in order to align the lab topics with the lecture topics and prevent students who are repeating the course from performing the same labs each semester.

In A&P, there is less focus on inquiry, but a significant amount of interaction with and identification of anatomical structures. Physiological concepts are addressed less frequently, and their coverage is intended to reinforce concepts introduced in lecture. Therefore, the goals that drove the construction of this manual were quite different. The authors wanted to craft lab activities that: 1) presented the material that students had to know and would

be tested on clearly, 2) followed the lecture schedule more closely, 3) were more straightforward to assess and 4) were thoughtfully constructed to best complement material addressed in lecture.

Priorities and goals will vary from course to course. However, it is these very priorities and goals that should influence how a manual is constructed.

Identifying Source Material for Lab Exercises

In constructing a lab manual, information can be collected in a variety of ways. Lab activities can be adapted from other sources, or they can be written from scratch. The General Biology I manual author took advantage of published labs available through ABLE's *Tested Studies for Laboratory Teaching* archives. More than half of the General Biology I labs are based on labs found in the *Tested Studies for Laboratory Teaching* archives that have then been modified to meet course needs.

On the other hand, the A&P manual was written from scratch. A significant challenge in the construction of this manual involved decisions regarding what information should be included and what had to be left out due to time constraints. In order to make these decisions, the authors had to take careful inventory of the models and resources available. Over time, activities have been added and adjusted as new resources have become available.

One of the most difficult elements to deal with in the development of a manual is figures. For example, in General Biology I, the authors decided to use open source figures or draw their own figures. On the other hand, in A&P, the authors used open source figures as much as possible, but ultimately found that in some cases it was best to adapt figures from the textbook and make the manual available electronically to students free of charge on a password protected learning management system to avoid copyright infringement. The authors of these two manuals did not consider working with a publisher at any stage, which provided a certain amount of flexibility in this aspect of manual preparation.

Practical Strategies for Writing a Lab Manual

There are many practical elements involved in writing a manual that need to be decided ahead of time if the authors hope to provide a consistently formatted and organized manual. For example, there are many publisher, slideshow and word processing programs available for manual preparation. Which an author chooses should depend in part upon how they want to make the manual available to students. The authors of the A&P manual considered making the manual available for sale as an iBook, so they opted to use iBooks Author to generate that manual. This platform is now used to generate a PDF that is made available to students through a password-protected course management system. The author of the General Biology I manual, on the other hand, has the manual printed by the campus copy center, and therefore opted to use Word. No matter what program is used, it is necessary to make formatting decisions early in order to maintain cohesion through the manual.

It is also necessary to decide whether the goal is to write the entire lab manual at once or to change a smaller number of labs at one time. In General Biology I, the author wrote one or two labs a year until she had produced an acceptable lab manual. The advantage of this strategy is that the process was not squeezed into one semester or one summer, and the author was able to carefully think through how to format each lab. Occasionally there was even time to have upperclassmen test a lab, which allowed for the opportunity to improve the quality of instruction. The disadvantage of this timeline is that it took about four years to complete a lab manual that suited the needs of the course. Additionally, for about half of the lab topics, the authors wrote two different labs so that the fall semester and the spring semester General Biology 1 lab would not be exactly the same. Since a fair number of students repeat the course from fall to spring, the goal was to ensure those students do not perform the exact same labs each time. Changing out labs also prevents current students from copying materials of students who recently took the course.

In A&P, the authors made the decision to write two manuals in their entirety in seven months. The A&P I manual was launched in a fall semester, and then A&P II manual was introduced the following spring. Therefore, one was complete before the other was undertaken. The authors planned to use the summer leading up to implementation working on the A&P I manual, and then to spend the fall semester writing the A&P II manual. Since there were two authors writing, the work was divided, with each individual writing five labs per manual. The upside to this strategy was that the manual was immediately cohesive, and included only the information that the instructors intended to address. The biggest downside to writing two manuals in rapid succession was that the authors found the task took longer than anticipated. Writing original text, with figures and guidelines on what students should be doing in lab, required working and reworking even in the production of the first edition. Because the authors did not hold fast to the planned deadlines, they ultimately ended up working on the manuals as the courses were in session, sometimes a week ahead of implementation. Therefore, the first edition of the manual wasn't as polished as would have been preferred. Regardless, at the end of a year the authors had two manuals that presented an intentional and cohesive lab experience, which was the goal.

Ensuring that Implementation is Intentional

In cases where the author of the manual teaches all sections of a lab, implementation is not an issue because that person retains control of information delivery.

However, lab courses such as General Biology I and A&P are frequently too large to be taught by a single individual. Therefore, it becomes important to be sure that expectations are conveyed effectively to instructors so that the manual is implemented as the author intended. For example, in General Biology I, the intent is to encourage students to think more about executing their experiments as the semester progresses; therefore, details such as how much solution to make are left out as the semester progresses. It is essential that all lab instructors know that this "missing" information is intentional so they do not provide that information too early. In A&P, is it important that concepts addressed in lecture are reinforced by lab, which means that terminology used and the depth of coverage must be coordinated. There are also concepts that are addressed in lab that are not addressed in lecture, and so that material requires more complete coverage. Currently in both courses, these instructions are provided orally in weekly meetings, however it is the authors' intention to eventually provide instructors with manuals for these courses.

Summary

There are a wide variety of benefits and downsides to writing a manual.

Table 1. Pros and cons of writing a lab manual.

Pros: • Forced contemplation about the point of each activity • Ability to edit at will and swap out labs each semester • Allows better alignment with lecture and lab • Enhanced control over how material is presented • Ease of writing pre- and post-lab assignments that specifically address learning objectives
Cons: • Difficulty prioritizing concepts • More continuous maintenance needed • Introduction of issues with copyright • Responsibility for clear and correct presentation of material lies with author • Responsibility for editing lies with the author

No matter which method is chosen for writing a lab manual, an author must have the flexibility to adjust labs from semester to semester in order to accommodate the needs of their students. The following exercise is one that was adjusted multiple times in A&P before it satisfactorily served its purpose in lab. The course for which this lab was developed is 200-level, and labs meet for 2 hours 45 minutes each week. The students who enroll in this course come from a wide variety of majors, including Biology, Chemistry, Exercise Science, Nursing, and Psychology.

Chemical Digestion

Chemical digestion is a difficult concept for students to master, particularly when their exposure is limited to lecture. This activity demonstrates one example of how we have tailored our Human Anatomy & Physiology II lab manual to provide our students with practical experience, helping to enhance their understanding of challenging physiological concepts. In particular, this activity highlights how digestive enzymes function and how changing environmental conditions can alter their activity. Student groups are assigned to test digestion of one macromolecule type and complete a worksheet. Following the digestion lab, a write-up is posted on Canvas, the course management system used at Shenandoah University, that explains in detail the purpose and expected results of these experiments. Students must read that write-up prior to the next week's lab. At the beginning of that lab, instructors lead a classroom discussion regarding chemical digestion of all three major macromolecules.

Prior to the digestion lab, students are required to read the lab activity, aware that they may be given a pre-lab quiz when they arrive to lab. Additionally, this concept has already been introduced in lecture by the time this lab takes place. At the outset of lab, instructors give students a basic review of enzyme activity, including the definitions of reactants and products. We discuss the roles of digestive enzymes in the digestive process, indicating that it is the products of these enzymatic reactions that are absorbed in the small intestine. The three macromolecules that will be digested in lab are introduced, and their breakdown by specific enzymes is explained. Finally, we review the methods that students will be using in this lab, taking care to address the importance of controls, how changing environmental conditions can affect enzyme activity, and the colorimetric assays that are used to test for reactants and products in these experiments. Students, working in groups, are then required to complete the chemical digestion of one macromolecule type—carbohydrates, fats or proteins—and collect data in photographic and chart form on the macromolecule-specific worksheet provided to each student.

The following week, it is expected that students will have read the instructor write-up of this activity, which may be represented on a pre-lab quiz. Instructors then spend some time ensuring that students understand the write-up, especially the tests that were run and the

appropriate analysis of those results. In particular, instructors discuss the importance of controls and how they can help to interpret experimental results. As this discussion occurs, students complete questions on that week's lab worksheet, and are ultimately tested on this material on a practical exam.

Student Outline
Human Anatomy & Physiology II Lab Manual

Students are given the following information in the manual introduction:

Each activity will have several learning objectives. For each objective you will be expected to know terms that occur in **bold** in the text.

There will often also be specific instructions about what you are expected to learn for the practical or do in lab. These instructions will be in blue text.

Critical points related to safety or steps that are crucial to an activity will be found in sentences printed in red text.

Lab 7: Digestive System
Activity 3: Simulating Chemical Digestion

An Introduction to Chemical Digestion

There are four major types of biological molecules, three of which we will focus upon in this activity: carbohydrates, proteins and fats.

Enzymes are molecules (usually proteins) that facilitate chemical reactions. **Digestive enzymes** are enzymes that **catabolize**, or breakdown, large food molecules (**substrates**) into smaller molecules (**products**) that can be absorbed in the small intestine. For example, if you eat a protein-rich meal, that protein substrate will be chemically digested in the stomach and small intestine, releasing amino acid products that can be absorbed and used as either fuel molecules or as structural building blocks within your body.

Digestive enzymes come in different varieties, and each specializes in the catabolism of a specific type of fuel molecule. Each enzyme functions best at optimal temperatures and within a favorable pH range.

Human digestive enzymes function most efficiently at body temperature (37°C), and are affected by temperature fluctuations in a predictable way: lowering temperature decreases enzymatic activity, causing them to be less effective, and raising temperature increases enzymatic activity, thus speeding the reactions they catalyze. However, increasing the temperature to 41°C causes these protein enzymes to **denature**, and they lose their shape and hence their function. That's a fever of about 106°F!

Changes in pH have differential effects on digestive enzyme activity. Enzymes that are most active in the stomach function best at a highly acidic pH. On the other hand, digestive enzymes of the small intestine function better at a less acidic pH, and those that function in the mouth are most active at a slightly alkaline pH.

Digestive enzymes may also function outside the body, as long as experimental conditions are favorable to their action. Conveniently, we can alter variables such as temperature and pH in order to determine how they affect chemical digestion of nutrients.

Review: The Scientific Method

In this activity, you will be expected to use the scientific method. This method of inquiry begins with a **question**. For example: Does fertilizer enhance plant growth. A **hypothesis** is a potential explanation to your scientific question, and describes what we would view as the most likely outcome given what we already know. A good hypothesis has two features: 1) it can be tested and 2) it can be proven false. Therefore, a suitable hypothesis in our hypothetical example would be: Fertilizer will enhance plant growth.

This hypothesis can be tested. In order to properly test a hypothesis, it is important to have at least one **scientific control** (or simply, **control)**, which provides a basis for comparison. A good scientific control is different from the experimental condition in only one aspect. For example, in testing our fertilizer hypothesis, best practice would be to grow plants in the same exact conditions (lighting, soil, water, plant type, etc.), but to water some with fertilizer and some without. Comparing the results between fertilized and non-fertilized plants would allow you to state with confidence whether you have supported your hypothesis.

This leads us to another important element of the scientific method: a hypothesis is never proven true. A hypothesis can be proven false, because we can clearly determine when something has not worked as we expected. However, in science, one must always be open to the idea that at another time, or using another method, the hypothesis might be proven false. Therefore, when we get positive results from experimentation, we say that we have supported the hypothesis. Frequently, scientific results lead to additional questions, and so experimentation begins anew.

When a hypothesis has been tested in many ways and has been supported by an abundance of data, it is called a **theory**.

Working in groups of two or three, you will set up and complete one of the following three experiments. Groups from each section will pool their data together, and it will be shared with the class as a whole. You will be asked to identify and use controls to analyze your results in the following experiments.

You will be responsible for understanding each of the three experiments and their results, even though you will only complete one of them. A handout explaining the expected results for each experiment will be posted to Canvas at the end of this week. Your lab instructor will review that information with you next week in lab.

Special Instructions

- You will set up these experiments at the beginning of lab so that you have plenty of time to complete them.
- If you are working with a boiling water bath, be sure to use the test tube clamp provided to carefully transfer tubes in and out of the water.
- Follow all instructions provided in the activities.
- If you spill anything, notify your instructor immediately. Be very careful with the acid and base solutions.
- All reagents may be disposed of in the waste bottle provided. If you are instructed to do so at the station, be sure to dispose of your used test tubes in the glass waste container.
- At the station for your experiment, you will find additional information and questions that you will answer on your worksheet.

A. Be Able to Describe the Digestion of Carbohydrates

Carbohydrates are catabolized by enzymes called **amylases**, which are present in saliva and in the pancreatic juices that are released into the small intestine. Therefore, carbohydrate digestion begins in the mouth and is completed in the small intestine.

Today, we will observe the breakdown of the substrate **starch**, a complex polysaccharide composed of covalently bound sugars. This large molecule is catabolized by amylase, producing **maltose**, a smaller disaccharide. Therefore, as amylase activity increases, so does starch breakdown and maltose formation.

In this experiment, several tubes will be prepared in order to test the effects of changing conditions on amylase activity. In order to test for the presence of starch (the substrate) in each tube, we will use Lugol's reagent. In order to test for the presence of maltose (the product), we will use Benedict's reagent. These reagents cause color changes that can be interpreted to determine the level of amylase activity.

Procedure

1. Be sure to read through these instructions carefully before you get started.
2. Obtain five test tubes and use a Sharpie to label each with one group member's initials and the tube number.
3. Fill the tubes up to the first line with the following:
   Tube 1: water
   Tube 2: amylase
   Tube 3: amylase + 5 drops 1N HCl
   Tube 4: amylase
   Tube 5: amylase
4. Boil Tube 4 ONLY for five minutes in the boiling water bath provided. Keep in mind that water boils at 100°C.
5. Fill Tubes 1-4 to the second line with starch solution. Fill Tube 5 to the second line with water.
6. Put the tubes in the 37°C incubator for one hour.
7. Obtain four additional test tubes, and label them according to the instructions in Step 1.
8. Using a new plastic pipette for each, mix the contents of your experimental tubes and reduce the volume down to the first line. Put the contents of each pipette into the empty labeled tube of the same number.
9. Organize your tubes into two sets: the original set will be used for one test and the new set will be used for the other test.
10. Using one set of tubes, fill each tube to the second line with Benedict's reagent, swirl the contents, and place them in the boiling water bath for 90 seconds.

    Benedict's reagent tests for the presence of reducing sugars such as maltose. Use the guide provided at your station to interpret your results.
11. In the other set of tubes, add 1 drop of Lugol's solution to each tube and swirl the contents.

Lugol's solution indicates the presence of starch. Use the guide provided at your station to interpret your results.

12. Take a photo of each set of tubes at the photo station, email both photos as instructed, and complete the worksheet at your station.

B. Be Able to Describe the Digestion of Proteins

Proteins are catabolized by a family of enzymes called proteases. Several proteases are produced by the pancreas, and active in the small intestine, but protein digestion begins in the stomach, where the digestive enzyme **pepsin** is produced.

Proteins are polymers that consist of many amino acids held together by covalent peptide bonds. Pepsin breaks those peptide bonds, breaking long amino acid chains called polypeptides into smaller amino acid chains called peptides, and finally releasing free amino acids, which can then be absorbed in the small intestine. Therefore, the presence of peptides and free amino acids in a solution indicates pepsin activity, and presence of proteins indicates lower pepsin activity.

In this experiment, several tubes will be prepared in order to test the effects of changing conditions on pepsin activity. In order to test for the breakdown of protein into peptides and/or amino acids, we will use Biuret reagent, which causes color changes that can be interpreted to determine the level of pepsin activity.

Procedure

1. Be sure to read through these instructions carefully before you get started.
2. Obtain six test tubes and use a Sharpie to label each with one group member's initials and the tube number.
3. Fill the tubes up to the first line with the following:
   - Tube 1: water
   - Tube 2: pepsin
   - Tube 3: pepsin + 5 drops of 1N HCl
   - Tube 4: pepsin + 5 drops of 1N HCl
   - Tube 5: pepsin + 5 drops of 1N HCl
   - Tube 6: pepsin
4. Cut five chunks of egg white small enough to fit in a test tube (about 5 mm^3). Use the scale provided to assure that they are all roughly the same weight. Then, cut up one of the chunks into smaller pieces.
5. Place the four large chunks of egg into Tubes 1-4. Place the smaller pieces of egg white into Tube 5. You may have to invert the test tubes to get the egg into the solution. Use a new square of Parafilm® to cover each test tube opening. There should be no egg in Tube 6.
6. Place Tubes 1, 2, 3, 5 and 6 in the 37°C incubator. Place Tube 4 on ice in the container provided. Leave these tubes to incubate for one hour.
7. Remove the tubes from the incubator/ice and perform the Biuret test. Put 5 drops of copper sulfate and 10 drops of potassium hydroxide into each test tube. Using a new square of Parafilm® for each tube, invert the test tubes to mix.

   Biuret reagent tests for the presence of proteins, peptides and amino acids. Use the guide provided at your station to interpret your results.

8. Take a photo of your tubes at the photo station, email the photo as instructed, and complete the worksheet at your station.

C. Be Able to Describe the Digestion of Fats

Fats, and lipids in general, are catabolized by a family of enzymes called lipases. Lipases are produced in the salivary glands, stomach and pancreas. However, the lingual lipase found in the saliva is not activated until it reaches an acidic environment, so fat digestion begins in the stomach. We will use lipase that is produced in the pancreas, which is found in pancreatin, and is active in the small intestine.

Fats consist of one glycerol molecule covalently bound to three fatty acids. When they are catabolized, the bonds between these four components are broken. The fatty acids that have been freed increase the acidity of the solution. Therefore, effective catabolism of fats by pancreatin will lower the pH of a solution.

Because fats are hydrophobic, enzymes in aqueous solution have trouble interacting with them. **Bile salts** (found in bile) help by **emulsifying** the fats and the enzyme solutions, allowing them to mix. Therefore, the presence of bile salts makes digestion of fats by pancreatin more effective because they allow the enzyme to physically interact with the fats.

The heavy cream used in this experiment has an important additive. Bromothymol blue is a color indicator that is blue at alkaline pH, green at neutral pH and yellow at acidic pH. Therefore, this color indicator will allow us to determine the relative pH of each solution, and therefore determine the effectiveness of pancreatin in each experimental tube.

Procedure

1. Be sure to read through these instructions carefully before you get started.
2. Obtain 3 test tubes and use a Sharpie to label each with one group member's initials and the tube number.
3. Fill the tubes up to the first line with the following:
   Tube 1: Water
   Tube 2: Pancreatin + Bile salts (see step 4)
   Tube 3: Pancreatin
4. Using the spatula provided, add a pinch of bile salts to Tube 2 only (something similar in size to the tip of a sharpened pencil).
5. Add indicator cream up to the second line. Indicator cream is light cream (source of fat) with pH indicator dissolved into it.
6. Using a new square of Parafilm® for each, cover the test tube openings and shake each tube.
7. Take a photo of your tubes at the photo station and put them into the 37°C incubator.
8. Set a timer reminding you to take a picture of your tubes 10, 20, 30 and 40 minutes after incubation begins. You should remove them from the incubator and take your photos at the photo station.
9. Because the indicator in the cream demonstrates any pH changes that occur, no additional testing is necessary.

   The bromothymol blue pH indicator indicates the relative pH of a solution. Use the guide provided at your station to interpret your results using the pictures you took.

10. Email all of your photos to your lab instructor and complete the worksheet at your station.

Sample Prelab Quiz Questions (Students answer these questions after reading the lab activity above.)

1. Of the four major types of biological molecule, we will chemically digest three in lab today. Name one of those three.
2. Name one environmental condition that can affect enzyme activity.
3. What is the importance of a scientific control?
4. Name one digestive enzyme and the biological molecule it is involved in breaking down.
5. What is the function of a digestive enzyme?
6. What are the two features of a good hypothesis?

Activity 3A Station Information Sheet: Chemical Digestion of Carbohydrates

A Few Additional Instructions

- If you accidentally fill just past one of the lines on the tube, it will not negatively affect the experiment. You can use the tube as-is.
- If you accidentally fill way past one of the lines on the tube, just dispose of it and remake that tube. **Never take solutions out of a test tube and put them back into the stock bottle.**
- Fill Tube 4 first and go ahead and get it boiling first thing to avoid sitting and waiting.
- When you move test tubes into or out of the boiling water bath, be sure to use the tube clamps provided.

Reading Benedict's Test Results

In the absence of sugars (in this case, maltose), the solution will remain blue. In the presence of sugars, the solution turns an orange/brown color, which gets progressively more saturated as the amount of sugar increases. In this particular set of reactions, the color is quite brown. It's not unusual for it to be more orange.

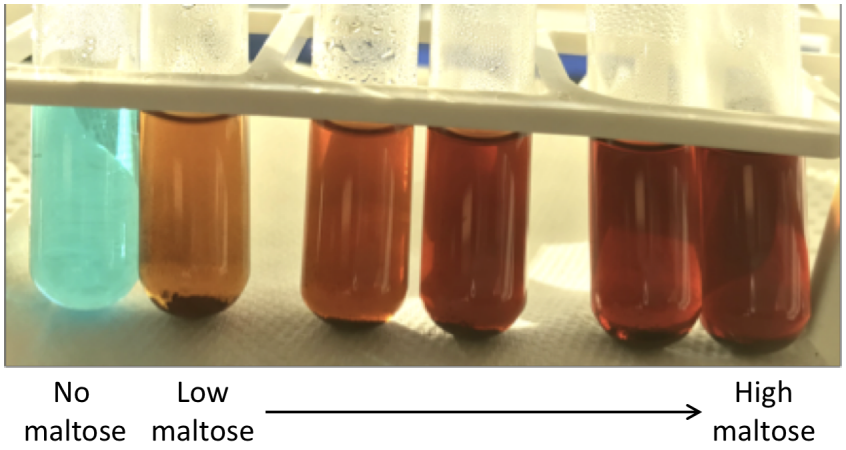

Fig. 1. These tubes demonstrate the representative results of Benedict's test.

Reading Lugol's Test Results

Lugol's contains iodine, so it turns a starch-free solution yellow. In the presence of low starch, the solution turns brown, which gets darker and darker until the solution turns a blue/black color in the presence of high starch.

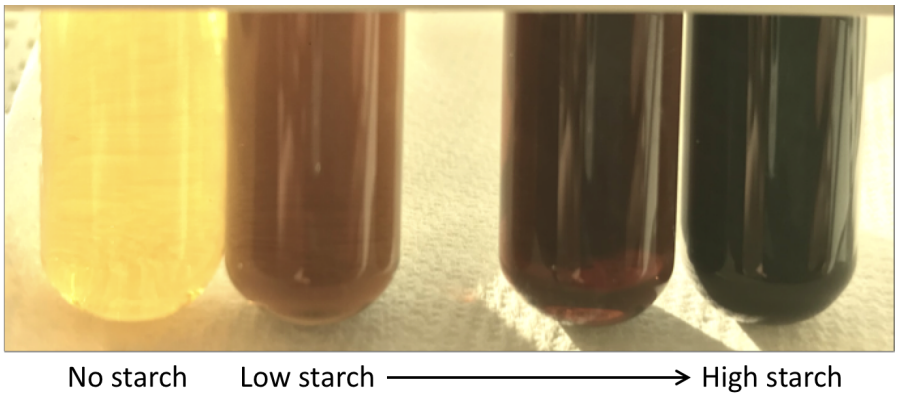

Fig. 2. These tubes demonstrate the representative results of Lugol's test.

Lab 7 Activity 3A Student Worksheet: Carbohydrate Digestion

The purpose of this experiment is to understand how chemical digestion of carbohydrates in the GI tract is affected by amylase and how different factors affect change amylase activity. As your lab manual states, when starch breakdown is catalyzed by amylase, the disaccharide maltose is produced. When amylase is very active, you should see more maltose and less starch in solution. When amylase is less active, you should see less maltose and more starch.

Table 2. Carbohydrate digestion results.

Solution	Lugol's Test Results	Benedict's Test Results
Tube 1: water + starch		
Tube 2: amylase + starch		
Tube 3: amylase + HCl + starch		
Tube 4: boiled amylase + starch		
Tube 5: amylase + water		

Referring to the Activity 3A Station Information Sheet, fill out Table 2 (2 pts):

- For Lugol's Test, indicate the quantity of starch present.
- For Benedict's test, indicate the amount of maltose present.

Email the photos you took to your lab instructor. Be sure to include the names of everyone in your group! (1pt)

Consider what you added to each tube and then answer the following questions:

1) Should Tube 1 with water and starch (but no amylase) test positive for the presence of maltose? (1pt)

2) Should Tube 5 with amylase and water (but no starch) test positive for the presence of maltose? (1pt)

3) Boiling a protein enzyme such as amylase denatures it. Based upon your results, does denaturation increase enzyme activity or decrease it? (1pt)

4) Hypothesis: The presence of HCl will increase the activity of amylase in this experiment.

   Questions:
   a. Which two tubes should you compare to each other to test this hypothesis? (1pt)

   b. Did you support the hypothesis or prove it false? Explain, citing your results in your answer. (1pt)

Activity 3B Station Information Sheet: Chemical Digestion of Proteins

A Few Additional Instructions

- While one person completes Steps 2 and 3, other group member(s) should move on to Step 4 and start preparing chunks of egg white of similar weight to minimize prep time.
- If you accidentally fill just past one of the lines on the tube, it will not negatively affect the experiment. You can use the tube as-is.
- If you accidentally fill way past one of the lines on the tube, just dispose of it and remake that tube. Never take solutions out of a test tube and put them back into the stock bottle.
- Throw away used Parafilm® in the trash can.

Reading Biuret Test Results

Blue indicates a lack of reaction, meaning there are no proteins, peptides or amino acids present. Purple indicates proteins, pink indicates peptides and amino acids and intermediate results (purple/pink) indicate all three.

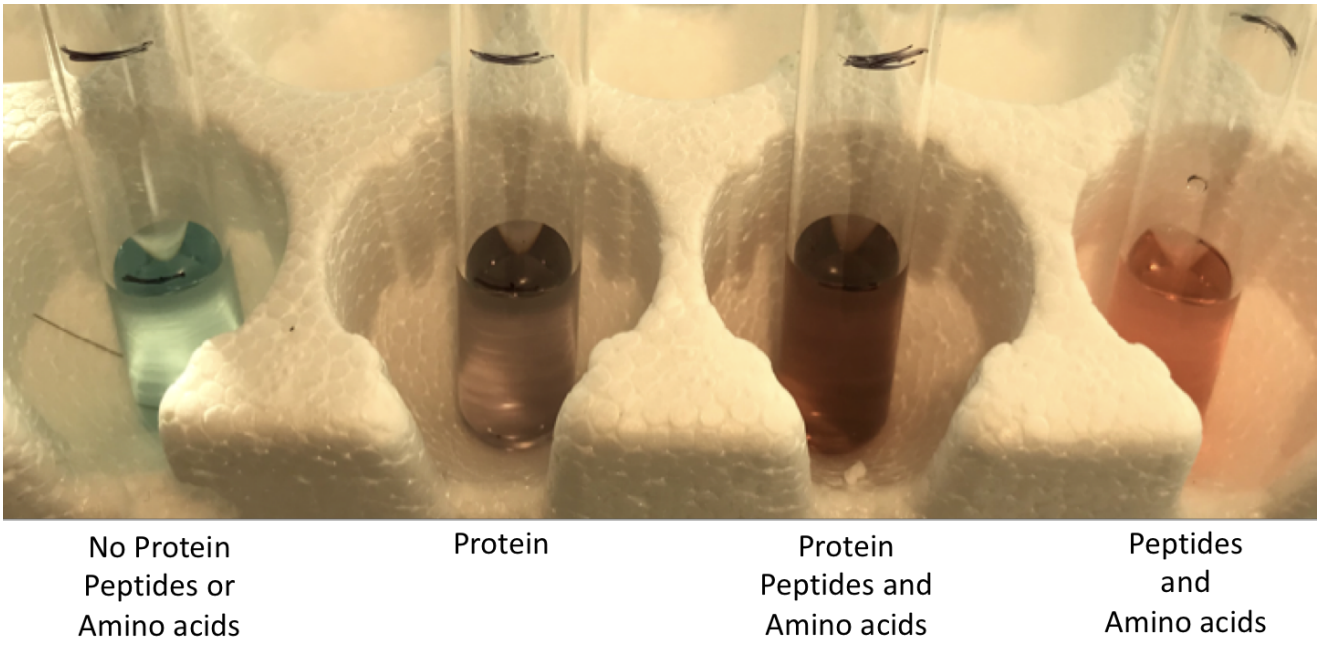

Fig. 3. These tubes demonstrate the representative results of the Biuret test.

Lab 7 Activity 3B Student Worksheet: Protein Digestion

The purpose of this experiment is to understand how chemical digestion of proteins in the GI tract is affected by pepsin and how different environments can affect pepsin activity. The digestion of proteins begins in the stomach, where pepsin is produced. Proteins are polymers that consist of many amino acids held together by covalent peptide bonds. In this lab, pepsin catalyzes the breakage of these peptide bonds, releasing proteins from the egg white, and then proteins are further catabolized, releasing peptides and free amino acids.

Table 3. Protein digestion results.

Solution	Biuret Test Results
Tube 1: water (egg chunk)	
Tube 2: pepsin (egg chunk)	
Tube 3: pepsin + HCl (egg chunk)	
Tube 4: pepsin + HCl (egg chunk on ice)	
Tube 5: pepsin + HCl (chopped egg)	
Tube 6: pepsin + water (no egg)	

Referring to the Activity 3B Station Information Sheet, fill out Table 3 (2 pts):

- Indicate the relative quantity of proteins and peptides/amino acids.

Email the photos you took to your lab instructor. Be sure to include the names of everyone in your group! (1pt)

Consider what you have put into each tube and then answer the following questions:

1) Should Tube 1 with water and egg (but no pepsin) test positive for protein breakdown with the Biuret test? (1pt)

2) Should Tube 6 with pepsin and water (but no egg) test positive for protein breakdown with the Biuret test? (1pt)

3) Explain why your result for Tube 5 was different than the result for Tube 3. Hint: there is no difference in enzyme activity between the two… think about what cutting up the egg accomplished. If you are confused by your results, talk to your instructor! (1pt)

4) Hypothesis: Decreasing incubation temperature will increase pepsin activity.

   Questions:
   a. Which two tubes should you compare to each other to test this hypothesis? (1pt)

   b. Did you support the hypothesis or prove it false? Explain, citing your results in your answer. (1pt)

Activity 3C Station Information Sheet: Chemical Digestion of Lipids

A Few Additional Instructions

- You should take a picture of your tubes every 10 minutes. If after 40 minutes Tubes 2 and 3 are not the same color, then return the tubes to the incubator and continue taking pictures every 10 minutes until they are the same color (up to 60 minutes).
- If you accidentally fill just past one of the lines on the tube, it will not negatively affect the experiment. You can use the tube as is.
- If you accidentally fill way past one of the lines on the tube, just dispose of it and remake that tube. Never take solutions out of a test tube and put them back into the stock bottle.
- Throw away used Parafilm® in the trash can.

Reading Bromothymol Blue Results

This is a pH indicator, meaning it changes color depending upon the pH of the solution it is mixed in. You should record general pH levels, and not worry about specific numbers. You will see a continuum of colors ranging from blue (basic) to green (neutral) to yellow (acidic).

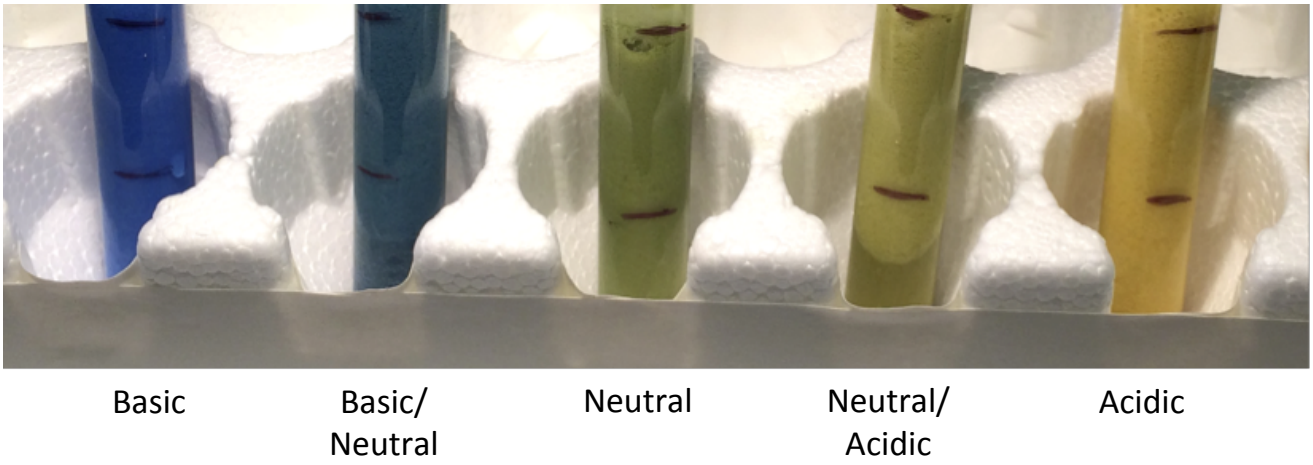

Fig. 4. These tubes demonstrate the representative results obtained using bromothymol blue pH indicator.

Lab 7 Activity 3C Student Worksheet: Lipid Digestion

The purpose of this experiment is to understand how chemical digestion of lipids in the GI tract is affected by lipase and how the presence of bile salts affects fat digestion. In this lab, pancreatin, a combination of pancreatic enzymes that includes lipase, is used. The optimal pH for pancreatin function is around 8, which is basic. This is why, when we prepared light cream with pH indicator, we added base until it was blue. As you observe your samples, you will notice them transitioning from blue to green to yellow. This color change indicates that the pH becomes more acidic over time. This is because, as pancreatin breaks down the fats in the cream, fatty acids are released into the solution. They are called fatty acids because they are acidic in solution!

Indicate the relative pH of each tube at each time point in Table 5 below. Refer to the information sheet at your station for guidance in recording your results.

Table 4. Lipid digestion results.

Solution	0 min	10 min	20 min	30 min	40 min
Tube 1: Water + Indicator cream					
Tube 2: Pancreatin + Bile Salts + Indicator cream					
Tube 3: Pancreatin + Indicator cream					

Referring to the Activity 3C Station Information Sheet, fill out Table 4 (2 pts):

- Indicate the relative acidity of the solution in each tube.

Email the photos you took to your lab instructor. Be sure to include the names of everyone in your group! (1pt)

Consider what you have put into each tube and then answer the following questions:

1) Does high acidity in a tube after 40 minutes indicate there was increased or decreased enzyme activity? (1pt)

2) Should Tube 1 with water and indicator cream (but no pancreatin) change color as quickly as the other two? (1pt)

3) How do you think the results would have varied if we had started with acidic indicator cream? Be sure to mention specific color changes! (1pt)

4) Hypothesis: The presence of bile salts will enhance pancreatin function in this experiment.

   Questions:

   a. Which two tubes should you compare to each other to test this hypothesis? (1pt)

   b. Did you support the hypothesis or prove it false? Explain, citing your results in your answer. (1pts)

Lab 7 Activity 3: Chemical Digestion Lab Expected Results

You are responsible for reading this report before Lab 8. If there is a prelab quiz, this information will be on that quiz. You will answer some worksheet questions following class discussion.

Carbohydrate Digestion

The purpose of this experiment is to understand how chemical digestion of carbohydrates in the GI tract is affected by amylase and how different factors affect change amylase activity. As your lab manual states, when starch breakdown is catalyzed by amylase, the disaccharide maltose is produced. The breakdown of carbohydrates first occurs in the mouth and is completed in the small intestine.

After incubating the correct solutions for the correct period of time, each solution was split in half between two test tubes. One set of solutions were tested with Lugol's solution to determine the presence of starch, while the other set of solutions were tested with Benedict's reagent to determine the presence of maltose.

Table 5. Expected results of carbohydrate digestion.

Solution	Lugol's Test Results	Benedict's Test Results
Tube 1: water + starch	Starch	No maltose
Tube 2: amylase + starch	Little starch	More maltose
Tube 3: 5 drops 1N HCl + amylase + starch	Starch	Less maltose
Tube 4: boiled amylase + starch	Some starch	More maltose
Tube 5: amylase + water	No starch	More maltose

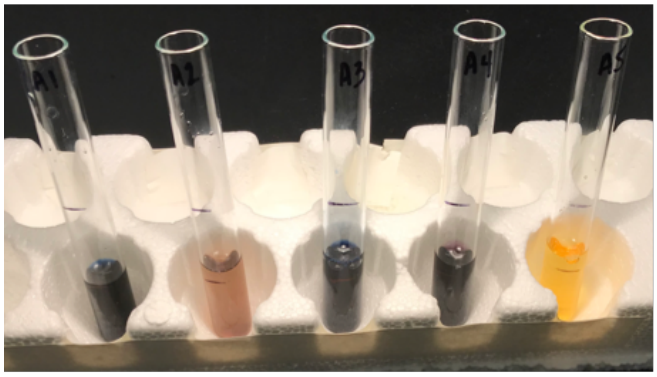

Fig 5. Lugol's test for starch: Tubes 1-5, left to right.

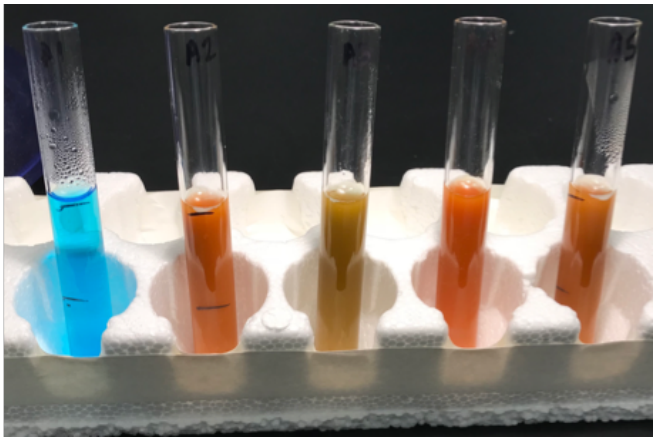

Fig 6. Benedict's test for maltose: Tubes 1-5, left to right.

Explanation of Expected Results: Carbohydrate Digestion

Tube 1 contained water and starch; it tested positive for starch and negative for maltose. This is because there was no amylase to catalyze the breakdown of starch. You may have seen a very small amount of orange precipitate at the bottom of this tube, indicating that the starch was not 100% pure (there was just a little maltose in the starch). This is expected because starch will spontaneously break down into maltose, just much less quickly.

Tube 2 contained amylase and starch; it tested negative for starch and positive for maltose. This is a great example of the starch breakdown that begins in the mouth. In the presence of amylase at neutral pH, the starch was fully catabolized to produce maltose.

Tube 3 contained amylase, five drops of 1N HCl, and starch; it tested positive for both starch and positive for maltose. This is a good example of how starch breakdown is affected by the acidic pH found in the stomach. In the acidic solution, amylase activity was slowed, and it was not able to fully catalyze the breakdown of the starch. There was, however, enough starch breakdown to produce maltose. This explains why the breakdown of starch is completed in the small intestines, where the pH is more neutral and not the stomach, where the pH is more acidic.

Tube 4 contained amylase and starch solution, but the enzyme was boiled for five minutes prior to the incubation period; it tested somewhat positive for starch and positive for maltose. This demonstrates how temperature affects an enzyme. Boiling partially denatured the enzyme, decreasing its ability to catalyze the breakdown of starch.

Tube 5 contained amylase and water, but no starch; it tested negative for starch and positive for maltose. On the face of it, these results make no sense. There was no starch added to the tube, so the Lugol's results make sense because there was no starch in the tube at any point. However, since there was no starch to break down, there should also have been no maltose present. These results would indicate that there is likely some sort of contamination of our enzyme. After doing some research, we (your instructors) discovered that this amylase was collected from cow's milk, which contains lactose, another sugar that tests positive with Benedict's. You may have noticed that Tube 5 comes out a bit browner compared to the orange of the other tubes that test positive. This is a feature of lactose compared to maltose. As we will discuss, the fact that amylase tests positive for maltose will make it difficult to interpret some of our results.

Protein Digestion

The purpose of this experiment is to understand how chemical digestion of proteins in the GI tract is affected by pepsin and how different environments can affect pepsin activity. The digestion of proteins begins in the stomach, where pepsin is produced. Proteins are polymers that consist of many amino acids held together by covalent peptide bonds. In this lab, pepsin catalyzes the breakage of these peptide bonds, releasing proteins from the egg white, and then proteins are further catabolized, releasing peptides and free amino acids. Following the incubation of the correct solutions with the appropriate substrates (egg) for 90 minutes, Biuret reagent (two parts potassium chloride and one part cupric sulfate) was used to test for the presence of proteins and peptides/amino acids.

Table 6. Expected results of protein digestion.

Solution	Biuret Test Results
Tube 1: water	No protein or peptides (blue)
Tube 2: pepsin	Protein present (purple)
Tube 3: 5 drops of 1N HCl + pepsin	Proteins and Peptides/amino acids present (more purple-less pink)
Tube 4: 5 drops of 1N HCl + pepsin	Protein present (purple)
Tube 5: 5 drops of 1N HCl + pepsin	Proteins and peptides/amino acids present (more pink-less purple)
Tube 6: pepsin + water (no egg)	Protein present (purple)

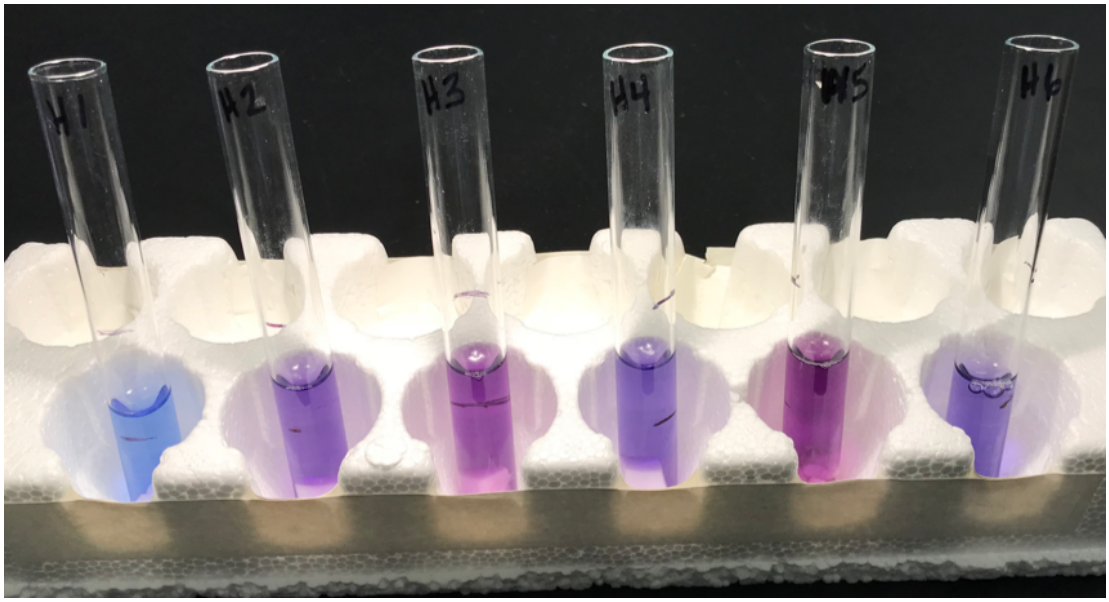

Fig. 7. Biuret test results for protein digestion: Tubes 1-6 from left to right.

Explanation of Expected Results: Protein Digestion

Tube 1 contained an egg chunk and water; it tested negative for the presence of proteins and peptides/amino acids. This is because no pepsin was present to digest the egg. You may have noticed the egg turned purple as it absorbed the Biuret reagent!

Tube 2 contained an egg chunk and pepsin; it tested positive for the presence of proteins. This is because pepsin is was present to begin digesting the egg. However, enzyme activity was clearly low because there was no significant release of peptides and amino acids.

Tube 3 contained an egg chunk, 5 drops of 1N HCl, and pepsin; it was an intermediate purple/pink color, indicating the presence of proteins and peptides/amino acids. This is because pepsin is optimally functional at acidic pH. Remember that pepsin is active in the stomach, where the environment is extremely acidic. Therefore, making the solution more acidic increased pepsin activity.

Tube 4 contained an egg chunk, 5 drops of 1N HCl, and pepsin. This tube was not placed in an incubator, rather it was placed in ice for the duration of the test; it tested positive for proteins, and had a coloration similar to Tube 2, which had a more neutral pH. Although it contained the same components as Tube 3, which was placed in the incubator, it lacked the pink coloration, indicating pepsin activity was slowed by low temperature. Despite the decreased environmental temperature, pepsin was still able to function, breaking down the egg and releasing proteins, just not as efficiently, and not as completely.

Tube 5 contained small chopped up egg pieces, 5 drops of 1N HCl and pepsin; it tested positive for proteins and peptides/amino acids. The pink is more pronounced in this tube, indicating increased protein digestion. This is a great illustration of how breaking food into smaller pieces enhances digestion. Because the pieces are smaller, pepsin is better able to access the egg (due to its increased surface area) and cleave the peptide bonds of the protein to release peptides free amino acids into the solution. As a practical application, this is why mechanical breakdown in the mouth and the stomach is particularly helpful to the process of chemical digestion.

Tube 6 contained no egg, pepsin and water; it tested positive for protein. If you think about it, this makes sense, because the enzyme pepsin is a protein. Because the enzyme itself produces a positive result, when the color reaction on a tube is purple, it will be difficult to interpret the results.

Lipid Digestion

The purpose of this experiment is to understand how chemical digestion of lipids in the GI tract is affected by lipase and how the presence of bile salts affects fat digestion. As the lab manual indicates, lingual lipase (found in saliva) is not active until it has reached an acidic environment, and so lipid digestion does not start until the stomach. In this lab, pancreatin, a combination of pancreatic enzymes that includes lipase, is used. The optimal pH for pancreatin function is around 8, which is basic. This is why, when we prepared light cream with pH indicator, we added base until it was blue. In the digestive system, the pancreas releases sodium bicarbonate-rich fluid that makes the environment basic. Tubes containing the solutions in the table were placed in the incubator and checked every 10 minutes for a total of 40 minutes.

Table 7. Expected results of lipid digestion**.**

Solution	0 min	10 min	20 min	30 min	40 min
Tube 1: Water + Indicator cream	Basic	Basic	Basic	Basic	Basic
Tube 2: Pancreatin + Bile Salts + Indicator cream	Basic	Acidic	Acidic	Acidic	Acidic
Tube 3: Pancreatin + Indicator cream	Basic	Neutral	Neutral to Acidic	Mostly acidic	Acidic

-Continued on next page-

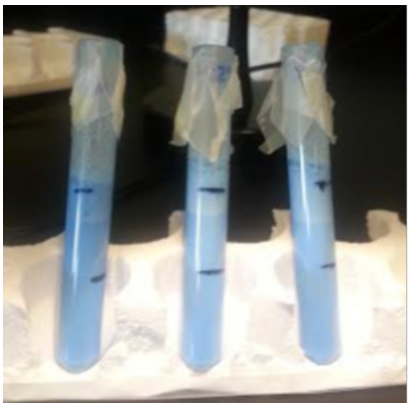

Fig. 8. Lipid digestion: 0 min.

Fig 9. Lipid digestion: After 10 min.

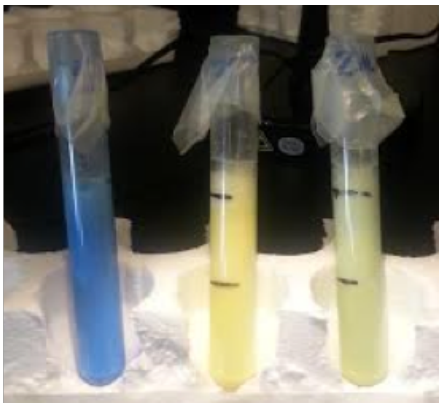

Fig. 10. Lipid digestion: After 20 min**.**

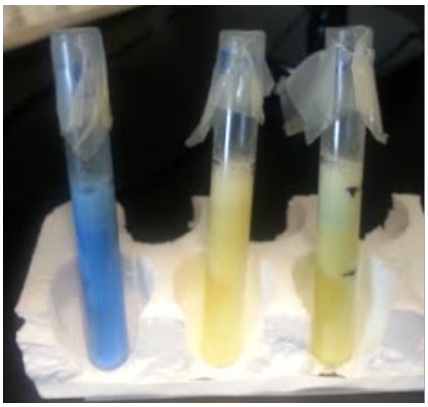

Fig. 11. Lipid digestion: After 30 min.

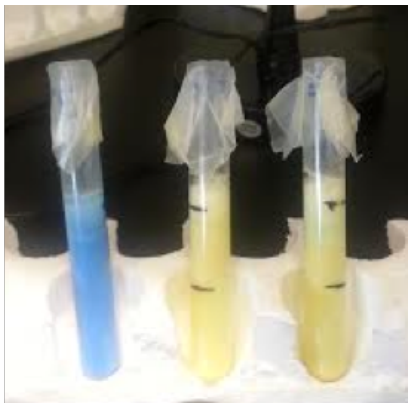

Fig. 12. Lipid digestion: After 40 min.

Explanation of Expected Results: Lipid Digestion

Tube 1 contained water and indicator cream, and after 40 minutes the color was blue, indicating it was basic, and therefore no free fatty acids were catabolized from the fats in the cream. This makes sense because the indicator cream was blue and water does not have any enzymatic properties.

Tube 2 contained pancreatin, bile salts, and indicator cream. After 10 minutes, the color was yellow, meaning it was acidic (caused by the release of fatty acids from the fat). Of the three solutions, this started to change color and pH first. This was expected because bile salts help emulsify the lipids and enzyme solutions, allowing them to combine, giving the enzyme better access to the fats. Therefore, it was expected that tube 2 would begin digestion first.

Tube 3 contained pancreatin and indicator cream; after 40 minutes the color was yellow, indicating it was acidic; however, it retained some green coloration through 30 minutes, indicating residual neutrality, because the fats were not digested as rapidly as Tube 2, and fatty acids accumulated more slowly. The lack of bile salts inhibited the ability of pancreatin to access fats.

Lab 7 Activity 3 Follow-up Worksheet (Week of Lab 8)

The purpose of this worksheet is to ensure that you have spent time considering each of the three chemical digestion experiments from last week, and to apply the knowledge you have gained to a new scenario.

1. Use your Activity 3 worksheet from last week's lab to consider Question 1, where you were asked a yes or no question regarding Tube 1 in your experiment. Give the correct answer to that question and then explain your answer clearly.

Using the handout that was posted to Canvas entitled *Lab 7, Activity 3: Chemical Digestion Lab Expected Results,* answer the following questions.

2. You test the hypothesis that pepsin activity is higher in acidic conditions than it is in neutral conditions. Which two tubes will you compare to each other and why? Do the expected results support the hypothesis or prove it false?

3. You test the hypothesis that fat catabolism does not occur in the absence of pancreatin. Which tubes will you compare and why? Which of these tubes is the control? Do the expected results support the hypothesis or prove it false?

4. You test the hypothesis that denaturation by boiling completely eliminates amylase activity. Which tubes will you compare and why? Do the expected results support the hypothesis or prove it false? Explain.

For the remaining questions, consider the following:

As with proteins, carbohydrates and fats, nucleic acids are broken down by digestive enzymes called nucleases into their component nucleotides and, when nuclease activity is high, into bases, sugars and phosphates, which are absorbed in the small intestine for use in the body in energy production and as structural building blocks for functional nucleotides and nucleic acids.

You prepare the following:
Tube 1: DNA + water
Tube 2: DNA + nuclease
Tube 3: DNA + nuclease + 5 drops hydrochloric acid
Tube 4: DNA + nuclease + 5 drops sodium hydroxide (a base)

5. Working with a group, but without looking at anything other than this side of this piece of paper, write two hypotheses that can be tested in the experiment outlined above.

Using a complex set of tests to identify reactant and products (which explains why we don't actually do this in lab), we obtain the following results (record the results given to you by your instructor):

Solution	**Test Result***
Tube 1: DNA + water	*Abundant DNA, No nucleotides, bases, sugars or phosphates*
Tube 2: DNA + nuclease	*Some DNA, Some nucleotides, Some bases/sugars/phosphates*
Tube 3: DNA + nuclease + HCl	*Some DNA, Some nucleotides, Minimal bases/sugars/phosphates*
Tube 4: DNA + nuclease + NaOH	*Minimal DNA, Some nucleotides, Abundant bases/sugars/phosphates*

6. For each of your hypotheses, indicate whether you support it or prove it false and explain completely.

7. Based upon the results presented, where are nucleases likely the most active: the mouth, stomach or small intestine? Explain.

* *The italicized information in the chart is not included on the worksheet given to students, rather it is provided to them after they have completed question 5.*

Answers to Questions

Sample Prelab Quiz Questions

1. Carbohydrates, Proteins, Lipids
2. Temperature, pH (also must accept salt)
3. Controls give us a basis for comparison to help us interpret our experimental results.
4. Amylase – carbohydrates; Pancreatin – lipids/fats; Pepsin – proteins
5. To catalyze the breakdown of a large food molecule in the GI tract
6. They are testable and they can be proven false

Lab 7 Activity 3A Student Worksheet: Carbohydrate Digestion

1. No
2. No
3. Decreases it (If a student's results were unusual, 'Increases it' may be acceptable.)

3a. Tubes 2 and 3

3b. This hypothesis is false. There was maltose in each tube, so clearly the enzyme was functional; however, there was hardly any starch is tube 2 and there was abundant starch in tube 3, which indicates that enzyme activity was decreased in the presence of HCl. (If a student's results were unusual, this answer may vary.)

Lab 7 Activity 3B Student Worksheet: Protein Digestion

1. No
2. No
3. Cutting up the egg left more surface area available for the enzyme to break it down; therefore, more proteins and peptides/amino acids were released. (If a student's results were unusual, this answer may vary.)

3a. Tubes 3 and 4

3b. This hypothesis is false. There was more protein breakdown in tube 3, which was in the oven, releasing proteins, peptides and amino acids. In tube 4, which was on ice, there were only protein present, but no peptides/amino acids, indicating enzyme activity was decreased at the lower temperature. (If a student's results were unusual, this answer may vary.)

Lab 7 Activity 3C Student Worksheet: Lipid Digestion

1. Increased
2. No
3. Starting with acidic indicator cream would not have given us clear results, because the breakdown of fat, releasing fatty acids, makes the cream more acidic. So, if we had started with yellow, the cream would have become more intensely yellow over time. It would be very hard to interpret results.

3a. Tubes 2 and 3

3b. This hypothesis is supported. The cream in tube 2 turned yellow much more quickly than the cream in tube 3, demonstrating that the release of fatty acids was increased in the presence of bile salts. (If a student's results were unusual, this answer may vary.)

Lab 7 Activity 3 Follow-up Worksheet

1. In all cases, the answer is the same: No. Tube 1 had water in place of enzyme, making it a control to be sure the enzyme was responsible for breakdown of the nutrient. One would not expect abundant product formation in the absence of enzyme.
2. Compare tubes 2 and 3, because they both contain reactant and enzyme, and the only difference between them is the presence or absence of HCl. Tubes 4 and 5 also contain reactant, enzyme and HCl, but each has an additional difference (temperature and egg preparation). We support this hypothesis.
3. Compare tubes 1 and 3, because they both contain reactant, and the only difference is that tube 3 contains enzyme and tube 1 does not. Tube 3 is the control, since the experiment is testing whether lack of enzyme affects fat catabolism. We support the hypothesis.
4. Compare tubes 2 and 4 because they both contain reactant and enzyme, and the only difference is that the amylase in tube 4 has been boiled. Tube 3 is not suitable for comparison because the amylase is not boiled and it contains HCl. The hypothesis is proven false. While some starch remains in the tube, some has been broken down, indicating that enzyme activity has been decreased, but not completely eliminated.
5. There are many possible hypotheses. Here are the ones we expect in some form or another:
    a. DNA breakdown is more efficient in the presence of nuclease.
    b. Nuclease activity is higher in neutral conditions.
    c. Nuclease activity is higher in acidic conditions.
    d. Nuclease activity is higher in basic conditions.
6. Responses are provided for each of the anticipated hypotheses:
    a. We support this hypothesis. In the presence of nuclease, we find reduced levels of reactant, and increased amounts of product, indicating more DNA breakdown.
    b. We prove this hypothesis false.
    c. We prove this hypothesis false.
    d. We support this hypothesis.

    The explanation for b-d is the same. Given neutral, acidic and basic conditions, we found the lowest levels of reactant and the highest amounts of product in the basic conditions, indicating that nuclease activity is higher in basic conditions.
7. Small intestine. Nuclease activity was highest in basic conditions, which is what we find in the small intestine. Therefore, it makes sense that we would find high nuclease activity in the small intestine, which is made alkaline by the pancreatic juice.

Materials

Lab groups should contain 2-3 students. Prepare for six groups in each section (two for each station).

All setup is for the week of Lab 7. No setup is necessary for discussion the following week.

Activity 3A

A rack of 50 mL conical tubes containing:
- 1% Amylase in water (13 mL/group)
- 1N HCl
- Water
- Lugol's solution
- Benedict's reagent
- 1% starch

Test tubes (10/group; see preparation notes)
Permanent maker
2 Test tube racks
Boiling water bath (hot plate, beaker and water)
Plastic transfer pipettes
2 Test tube clamps, Stoddard style
Information Sheet

Activity 3B

A rack of 50 mL conical tubes containing:
- 2% Pepsin in water (15 mL/group)
- 1N HCl
- Water
- 10% Potassium hydroxide in water
- 3% Copper sulfate in water

Egg white (one egg per lab section)
Ice in bucket
Test tubes (6/group; see preparation notes)
Permanent marker
2 Scales
Weigh boats
2 Kitchen knives
Plastic transfer pipettes
2 Test tube racks
Parafilm® squares (10/group)
Information Sheet

Activity 3C

A rack of 50 mL conical tubes containing:
- 1% Pancreatin in water (8 mL/group)
- Bile salts
- Water
- Indicator cream (see preparation notes)

Test tubes (3/group; see preparation notes)
Permanent marker
Metal spatula (for bile salts)
Plastic transfer pipettes
2 Test tube racks
Parafilm® squares (3/group)
Information Sheet

Common Use Station

Incubation oven large enough to hold all tubes at 37°C
Modified racks to hold test tubes for photos
Desk lamp

Preparation Notes

Stations should be set up as far apart as possible so that students do not mix up supplies between stations.

Disposable borosilicate test tubes (13 x 100mm) should be labeled with permanent marker. Start by making a guide tube: put 2.5 mL of water into the tube and mark the meniscus with a straight line, then put an additional 2.5 mL of water (5 mL total) into the tube and mark the meniscus with a straight line. Empty the water from this tube and use it as a template to mark enough tubes for students to use in lab. Tubes may be washed and reused following lab; however, make extra tubes because they do not all come clean easily.

Enzyme solutions should be stirred for at least ten minutes upon production. They will appear a bit cloudy. They can be stored for a day in the refrigerator without losing their activity, and do not have to be stirred again upon removal from the refrigerator.

The egg white should be separated from the yolk and rinsed prior to lab.

100 mL of the following solutions should be prepared and kept available to refill tubes as needed.
- 1% Starch
- 1N HCl
- 10% Potassium hydroxide
- 3% Copper sulfate

The following items should be purchased and refilled as necessary:
Lugol's solution
Benedict's reagent
Bile salts

To make indictor cream, add 200 mg bromothymol blue and 3 ml 10% potassium hydroxide to 100 mL light cream and mix well. It should be a deep blue.

Notes for the Instructor

Week 1

The purpose of this activity is to enhance students understanding of how digestive enzymes
breakdown larger food molecules so that they can be absorbed in the small intestine.

Following the prelab quiz, if there is one, there are some concepts that instructors should address with the class before getting started:

Define *enzyme, reactant, and product*. High enzyme activity results in the conversion of reactant to product. Low enzyme activity results in less product formation.

Chemical digestion occurs in multiple locations in the GI tract, particularly the mouth, the stomach and the small intestine, and that the environmental conditions vary in these locations.

As proteins, digestive enzymes are susceptible to changes in environmental conditions, which may increase or decrease their activity.

Students will consider a hypothesis during their experiment. A good hypothesis can be tested and can be proven false. We can support a hypothesis with positive data, but we never prove a hypothesis true!

Scientific controls give us a way to analyze our experimental results. For example, if we hypothesize that fertilizer enhances plant growth, we cannot know for certain that is the case unless we also grow some plants without fertilizer. Scientific controls give us a basis for comparison, and without them, we cannot be certain that we're seeing what we think we are.

Next, cover some procedural issues. Let students know that, although they will only work on one nutrient today, they will be responsible for understanding all three experiments. Assure them that there will be further discussion at the beginning of lab next week.

Address the special instructions from the lab manual:

- If you are working with a boiling water bath, be sure to use the test tube clamp provided to carefully transfer tubes in and out of the water.
- Follow all instructions provided in the activities.
- If you spill anything, notify your instructor immediately. Be very careful with the acid and base solutions.
- All reagents may be disposed of in the waste bottle provided. If you are instructed to do so at the station, be sure to dispose of your used test tubes in the glass waste container.
- At the station for your experiment, you will find additional information and questions that you will answer on your worksheet.

Encourage students to read through the lab exercise they are assigned and the information sheet at their station **BEFORE** they get started. They are not long, and it is best to have an idea of what they are doing before they get going.

Although this is Activity 3, students should start this activity before undertaking Activities 1 and 2 to ensure they have time to complete it. Students should be put into groups of 2-3 people (no more than six groups per lab section), and each group should be assigned a nutrient. Split them up evenly to ensure there is enough material for everyone.

Make sure students are wearing gloves and goggles at all times when working with chemicals.

As groups put their tubes into the oven, please stress to them that they must take photos in the white racks at the photo station provided. If they attempt to take photos in a wire tube rack, the photos will come out too dark and it will be difficult to see their results. A lamp is provided. If their results are not clearly visible, they will not get credit for their photos!

Students can work on Activities 1 and 2 while their tubes are incubating. Please remind them to set timers and take pictures at the appropriate intervals.

The contents of all tubes may be collected in the same bottle for pickup by a chemical waste disposal company. If tubes can be cleaned, leave them in the bucket of water provided in the sink by the computer. If any tubes cannot be satisfactorily cleaned, they should be disposed of in glass waste. Before reusing tubes, be sure the two marks on the outside are clearly visible.

Be sure to make yourself available for questions at all stages of this activity. Some students may get odd results, and will need reassurance. Others will simply need confirmation that their results are the expected ones or that they've recorded their results properly. Encourage students to look at their pictures while recording their results on their worksheets, particularly the fat digestion groups.

As students turn in their worksheets to you, ask them whether they have emailed their photos and whether they included their group members' names in the email.

Week 2

The purpose of this week's activity is to ensure that students get exposure to all three chemical digestion experiments that were carried out and that they understand what these experiments told us about digestive enzyme function. Students will also get the opportunity to develop their own hypothesis in response to a novel situation and, using hypothetical data, determine whether they have supported it or proven it false.

Before beginning your review, hand back the graded lab worksheets from the previous week. Then, have students complete the front page of the follow-up worksheet. They will need access to the handout entitled *Lab 7, Activity 3: Chemical Digestion Lab Expected Results.* Students may work in groups. Encourage them to

work with students who worked on different nutrient digestions.

Discuss the worksheet questions (1-4) that have been addressed this far. Project the Expected Results document on the board and ask pointed questions about why different components were put into different tubes. For example:

- What was the role of Tube 1 (no enzyme) in each experiment?
- What was the role of carbohydrate Tube 6 and protein Tube 5?
- What effect did cutting up the egg have on chemical digestion in Tube 5 of the protein experiment? Why is this information relevant to our lives?
- Did bile salts increase pancreatin activity in the fat experiment?
- Does the presence of maltose in an experimental tube always mean that amylase activity is high? (Why did we test both starch and maltose?)

Upon asking these questions, encourage students to discuss the answers with each other and then report back to the class.

Be sure to address carbohydrate tube 5 and protein tube 6. These demonstrate flaws in the experiment. Sometimes those exist… that's why controls are important!

Carbohydrate tube 5 demonstrates that the amylase is contaminated with lactose. Therefore, you can't come to any conclusions by observing the Benedict's tubes only… you must analyze it together with the results of Lugol's test.

Protein tube 6 demonstrates that the pepsin itself is sufficiently concentrated to bring back a "protein present" result. Therefore, in Tubes 2, 4 and 6, where they are rather equally purple, it's difficult to interpret the results.

Next, allow students to complete question 5 on the back page of the follow-up worksheet.

Once students have had the opportunity to develop their hypotheses, poll the class and write the most popular ones up on the board.

Next, provide students with the hypothetical experimental results and have them complete questions 6 and 7.

Once students have had the opportunity to answer these questions, lead a brief class discussion and address the hypotheses that you wrote on the board. Be sure to address which tubes they compared and which tubes served as controls.

Finally, have students turn in this worksheet. It will be graded based on completion.

About the Authors

Dr. Beth Cantwell is an Assistant Professor and Chair of Biology at Shenandoah University. She has a B.A. in Biology from the University of Virginia, and earned her Ph.D. in Zoology at Texas A&M University, where she studied the neuroanatomy and physiology of the biological clock in the domestic chicken, and taught anatomy & physiology lecture and labs. She completed post-doctoral studies on the light input pathway of the mammalian biological clock and lectured introductory biology at the University of Tennessee-Knoxville. In her current position, she teaches anatomy & physiology and related upper-level electives, and mentors undergraduates in the continuation of her postdoctoral research.

Dr. Brian Lipscomb is an Assistant Professor of Biology at Shenandoah University. He has a B.A. in Biochemistry from the University of Kansas and earned his Ph.D. in Neuroscience at Yale University with an emphasis on the development and anatomy of the olfactory system. He completed post-doctoral studies at the University of Arizona and the University of California-Berkeley. In his current position, he teaches anatomy & physiology, as well as upper-level neuroscience courses.

Dr. Laurel Rodgers is an Associate Professor of Biology at Shenandoah University. She has a B.S. in Biology from Sweet Briar College and a Ph.D. In Cell Biology and Anatomy from the University of Arizona where she studied coronary vessel development. As a post-doctoral fellow, she completed a SPIRE fellowship in teaching undergraduate science at the University of North Carolina-Chapel Hill where she also studied the protein structure and function of ZO-1 in tight junctions of epithelial cells. At Shenandoah University, Dr. Rodgers teaches General Biology, non-majors Biology, Cell Biology, and Developmental Biology, and continues her undergraduate research project studying the microbiome of the American chestnut tree.

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Appendix
Verbal Feedback Has Driven Changes to the Lab

In its initial inception, *Digestive Activity 3* was conducted more simply than it is now. First, the lab manual introduction to the activity contained significantly less information about enzymes in general, and no information about the conditions that affect digestive enzyme function. Our thought was that this information would be learned while completing the activity, and so we didn't want to give too much away. In addition, the section titled *Review: The Scientific Method* was not present. Since the prerequisite for this course is General Biology I, our expectation was that students should already have practice formulating hypotheses and drawing conclusions based upon data. Students were expected to complete the lab activity, each group working on the chemical digestion of one nutrient, and answer the lab worksheet questions shown below. Second, in order to ensure that students had the opportunity to review the experimental outcomes and make themselves familiar with all three iterations of this activity, we posted the *Chemical Digestion Lab Expected Results* (provided above) online for students to review outside of lab. Students were expected to use this document to prepare for questions on practical exams with no additional class time or input from their instructors on this topic. While there have been some grammatical and clarifying revisions made to this document over time, the material presented has not changed.

Lab 8 Worksheet Name: _____ Section: _____

Activity 3

Although you only have to complete one of the three parts of Activity 3, you will be responsible for knowing how to complete and analyze each of them, so be sure to talk to other groups about their results!

8. **Email the photos you took** of your tubes to your instructor, and **copy Dr. Cantwell** at ecantwel@su.edu. Be sure to include the names of everyone in your group! (2pts)

What nutrient were you assigned to digest? _____

9. Write a hypothesis regarding your assigned experiment. Your hypothesis should address how your manipulations will affect enzyme activity… it should not mention color. It could be a hypothesis about one particular tube, or it could address all tubes that you prepare. (1pt)

10. Describe the outcome of the experiment you conducted in your own words, addressing each test tube. It is appropriate to report color here, but also indicate what that means. (2pts)

11. Draw a conclusion regarding the hypothesis you wrote in #8. Did you support it? Disprove it? (1pt)

Over the course of several semesters, it became clear that students were uncomfortable writing their own hypotheses, and were not successful in answering the more open questions that were asked on the worksheet. The first change we made to address this was to shift the timing of scientific method instruction, discussing it before students began the lab rather than discussing it in the midst of activity in the hope that performance would improve. While some students benefited from this change in timing, many were still confused. Therefore, we made the decision to change the nature of the worksheet questions. As is shown in the current version of the worksheets, hypotheses are given and students are asked to analyze their data to address those hypotheses. This change was welcomed by both instructors, who saw an improvement in student performance, as well as students, who indicated they better understood the results they had obtained.

From an instructional standpoint, we were pleased that students were developing a better understanding of their group's results; however, part of the point of conducting an experiment such as this is to reinforce the scientific method, and the changes made detracted from that. Further, our goal remained to provide students with an overall understanding of how chemical digestion takes place and can be affected by environmental conditions, which would require an understanding of all three experiments, and the changes we made did not specifically address that objective. We continued to solicit student and instructor feedback on this exercise, and there were several crucial pieces of information that shaped the third revision of this lab to its current form. Students indicated that: 1) there was no time during the lab period during which they could talk to other groups to find out what they were doing, 2) they were not spending time outside of lab reviewing the posted write-up of expected results, and 3) they had no idea how we might test them over this material on practical examinations. Instructors indicated that there was sufficient time in the next week's lab to consider adding a review component at that time.

Based on this feedback, we revised the lab to: 1) re-integrate the scientific method in a more meaningful way, 2) make clear to students what the takeaways from this activity are and 3) ensure that all students are exposed to all three experiments. To accomplish these goals, we made several major changes to the lab manual. We first decided to expand the introduction of digestive enzymes in general and included a discussion of conditions that affect digestive enzyme activity. Next, we added the section titled, *Review: The Scientific Method* to the introduction, since we eventually ask students to develop their own hypotheses as a part of this activity. While students still do not have significant time for interaction and sharing of knowledge between groups during the first week of lab, and while we still post expected results immediately following the lab, we have now added time for discussion in the first hour of the next week's lab. During this discussion, students complete the follow-up worksheet, in which they must consider elements of each of the three different nutrient digestion experiments, as well as a theoretical fourth digestion of nucleic acids. The novel consideration of nucleic acids gives students the opportunity to develop their own hypotheses and, using theoretical data reported by their instructor, form conclusions. Both hypotheses and conclusions are discussed by the class as a whole.

Feedback from both instructors and students has been very positive. Instructors have found that students better understand the material and are engaged in the follow-up discussions. Students have reported that they like knowing what other groups did, what they learned, and the importance of those findings. They also report having a better understanding of chemical digestion as a whole. Finally, students report that they feel more comfortable about the knowledge they are meant to be carrying forward to practical examinations.

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