2. DIGESTIVE CHEMISTRY

Introduction

References: ***Lab Manual, Exercise 34, p. 188-191.***
Directions for the lab experiments are written into the lab.

1. Instructions for this experiment are NOT in the Lab Manual. Please read ALL of the material on the lab table before you begin. This information will be needed for the quiz and practical.
2. If you do not understand a procedure given on the lab table, ask for help.
3. Be sure that you know the following information:
    A. The name of each of the following tests
    B. The name of the test solution
    C. The name of the substrate that the test solution reacts with
    D. The color or appearance of negative and positive results for each test

Station 1: Enzymatic Action

Q1A. Water combines with large molecules and chemically splits the large molecules into smaller molecules in the process known as -?-.
Q1B. Is the process in Q1A a synthesis or decomposition reaction? Is the reaction anabolic or catabolic?
Q2A. What is the function of an enzyme in this type of reaction?
Q2B. What two things are most likely to interfere with an enzyme or destroy its function?

Station 2: Starch Hydrolysis, The Effect of Salivary Amylase

Salivary amylase hydrolyzes starch. Amylase is an enzyme that speeds up the chemical breakdown of starch into a disaccharide, maltose. Iodine is the test solution for the substrate, starch. Iodine causes a solution containing starch to turn blue-black. However, if starch is not present because it has been hydrolyzed, the solution will remain amber, the original color of the iodine solution.

This experiment is testing to see if starch hydrolysis did occur. Therefore, a POSITIVE test for starch hydrolysis is indicated by an AMBER color (the color of Lugol's Iodine) and a NEGATIVE test for starch hydrolysis is indicated by a BLUE-BLACK color, showing starch is still present.

COLLECTION OF SALIVARY AMYLASE

1. Rinse your mouth well with water at the sink. (Food or chewing gum will influence the result.)
2. Obtain a piece of parafilm, remove the paper backing, and place the parafilm in your mouth.
3. Stimulate the flow of saliva by chewing the parafilm.
4. Collect about 10 ml of saliva in a plastic collection cup.
5. Discard the chewed parafilm in the biohazard waste container.
6. Save the saliva not used in the experiment in case you need to repeat the experiment. If the results are not clear, you may need to do it again.

INSTRUCTIONS FOR THE STARCH HYDROLYSIS TEST
Work in groups of 2 or 3 students.

1. Label three test tubes, #1, #2 and #3.
2. Into each test tube, put 25 drops f 0.05% starch solution.
3. Into test tube #1, put about 25 drops of the pH 2 buffer solution.
4. Into test tube #2, put about 25 drops of the pH 7 buffer solution.
5. Into test tube #3, put about 25 drops of the pH 12 buffer solution.
6. Using **three different disposable pipettes, one for each tube** add about 10-15 drops of saliva to each test tube. Leave the pipettes in the test tubes when finished.
7. Label a depression plate with #1, #2, and #3.
8. Using pipette #1, put a few drops of the solution in test tube #1 into depression #1.
9. Using pipette #2, put a few drops of the solution in test tube #2 into depression #2.
10. Using pipette #3, put a few drops of the solution in test tube #3 into depression #3.
11. Add a few drops of Lugol's Iodine to each depression.
12. Observe the results and record below.

	Test Tube #1 pH 2	Test Tube #2 pH 7	Test Tube #3 pH 12
Color			
Starch Test (+ or -)			
Is Starch Present?			
Hydrolysis Test (+ or -)			

Q3A. What color is a positive starch hydrolysis test? Which test tubes are positive for starch hydrolysis?
Q3B. Which pH showed the most starch hydrolysis? Therefore, what pH would you expect to find in the mouth?
Q4A. What color is a negative starch hydrolysis test? Why?
Q4B. Which test tube showed the least starch hydrolysis? Why might this be?
Q5A. Which test tube showed hydrolysis as it most likely happens in the mouth?
Q5B. Which test tube showed what happens to hydrolysis when it enters your stomach?
Q6A. How is the pH of the stomach different from the pH of the mouth?
Q6B. What happened to the enzyme when starch hydrolysis did not occur?
Q7A. The higher the pH, the lesser/greater the hydrogen ion concentration.
Q7B. Name two conditions besides pH that could affect the effectiveness of enzymes.
Q8A. What is the function of an enzyme in the body?
Q8B. True or False: A denatured enzyme retains its ability to act as a catalyst in chemical reactions.

Station 3: Benedict's Test for Glucose and Reducing Sugars

Benedict's Reagent contains the cupric (copper) ion which reacts with reducing sugars to form a precipitate. The precipitate varies in color and intensity according to the amount and type of saccharide (sugar) present.

Blue	=	-	=	negative (no reducing sugar present)
Green-yellow	=	+	=	small amount of reducing sugar
Orange	=	++	=	more reducing sugar
Red	=	+++	=	greater amounts of reducing sugar
Red-brown	=	++++	=	greatest amounts of reducing sugars

INSTRUCTIONS FOR THE BENEDICT'S TEST

1. Label a large test tube D and a second large test tube E.
2. Put 10 drops of the test solution D in test tube D.
3. Put 10 drops of the test solution E in test tube E.
4. Add one dropper-full of Benedict's solution to both tubes and mix contents.
5. Place the two test tubes in the hot water bath for about 5 minutes.
6. Examine the tubes and answer the following questions.

Q9A. What color is the solution in test tube D? What color is the solution in test tube E?
Q9B. Which test tube shows a positive result for reducing sugar? What color is it?

Station 4: Iodine Test for Starch

In this experiment, Lugol's Iodine is used as the test solution, but you are looking **for** the presence of starch. Lugol's Iodine turns blue-black in the presence of starch. A blue-black color indicates a positive test for the presence of starch. This is opposite to the results of the test for starch hydrolysis.

INSTRUCTIONS FOR THE STARCH TEST

1. Label one test tube B and the second test tube C.
2. Put one dropper-full of test solution B in test tube B.
3. Put one dropper-full of test solution C in test tube C.
4. Add 3 drops of Lugol's Iodine to each tube.
5. Examine the tubes and answer the following questions.

Q10A. What color is the solution in test tube B? What color is the solution in test tube C.
Q10B. Which tube tests positive for the presence of starch?

Station 5: Biuret Test for Proteins

The copper sulfate ($CuSO_4$) in the Biuret reagent reacts with protein in an alkaline environment to form a light purple color. Therefore, a positive result is light purple or lavender. A negative result stays the same blue color of the Biuret reagent. To make the alkaline environment, a mild base, Sodium Hydroxide (NaOH) is added.

INSTRUCTIONS FOR THE BIURET TEST

1. Label one test tube H and the second test tube I.
2. Put 20 drops of test solution H in test tube H.
3. Put 20 drops of test solution I in test tube I.
4. Add two full droppers of NaOH to each tube.
5. Add 10 drops of Biuret reagent ($CuSO_4$) to both tubes. Stir contents well.
6. Examine the tubes and answer the following questions.

Q11A. What color is the solution in test tube H? Is this positive or negative for protein?
Q11B. What color is the solution in test tube I? Is this positive or negative for protein?

Station 6: The Grease Spot Test for Lipids

The solution that contains lipids will leave a grease spot on paper that is translucent to light, even after the spot dries. A solution that does not contain lipids will not leave a translucent spot after the paper dries.

INSTRUCTIONS FOR THE GREASE SPOT TEST

1. Draw a line down the middle of a piece of filter paper.
2. Label one side of the paper J and the other side, K.
3. Put one drop of solution J in the area labeled J on the filter paper.
4. Put one drop of solution K in the area labeled K on the filter paper.
5. Examine both areas by holding the paper up to the light.
6. Wait 5 minutes.
7. Again examine both areas by holding the paper up to the light.
8. Answer the following questions.

Q12A. Which side of the filter paper, J or K, is translucent to light?
Q12B. Which solution is positive for lipids?

Station 7: Bile and Emulsification of Lipids

References: Textbook, p. 693-694.

INSTRUCTIONS TO OBSERVE THE ACTION OF BILE SALTS

1. Shake the two test tubes vigorously and observe immediately.
2. Allow the test tubes to stand for 2 minutes and observe again.
3. Determine which test tube contains smaller fat droplets after 2 minutes.
4. Continue to observe to determine if the fat droplets stay suspended longer in one tube than in the other.

Q13A. Define emulsification.
Q13B. What organ releases concentrated bile?
Q14A. What organ produces bile?
Q14B. What hormone stimulates both of the organs in Q13B and Q14A?
Q15A. Which tube in the experiment contains bile salts?
Q15B. How can you tell?

Station 8: Absorption

References: Textbook, p. 698-700.
Workbook, p. 247, #9.

On the model of the intestinal wall:

Q16A. Identify this structure. What is its function? Label it in purple on Fig. 14-5, p. 247 in your workbook.
Q16B. Identify this vessel. What system does it belong to? Color it green on Fig. 14-5, p. 247.
Q17A. What is absorbed into the vessel in Q16B?
Q17B. The substance absorbed into the vessel in Q16B is made out of two kinds of molecules. Name them. Color the cells that assemble these molecules orange on Fig. 14-5, p. 247.
Q18A. Identify this cell. What does it secrete? Color these cells red on Fig. 14-5, p. 247.
Q18B. What is the function of the secretion named in Q18A?
Q19A. Identify these vessels. Color them pink on Fig. 14-5, p. 247.
Q19B. What system do the vessels in Q19A belong to?
Q20A. The vessels in Q19A will join together to form what large vessel that carries nutrient-rich blood?
Q20B. To what organ will the vessel in Q20A transport its contents?

Q21: **Clinical Application Thought Question:** (Answer at the bottom of your lab report.)

A patient comes to you complaining of intestinal bloating and increased flatulence. You question her about her recent activities and learn that she has drastically modified her diet, cutting out most of the meat and greatly increasing the number of fruits and vegetables, especially legumes (peas, beans, etc.) that she consumes each day. Is there any relationship between her symptoms and her new diet? If so, what is it?

*Turn in p. 247 with your lab report.