

Digestive Physiology – Laboratory 8

The digestive system consists of the digestive tract or alimentary canal and the accessory organs such as the tongue, pancreas and liver. The digestive tract is a continuous tube that is open to the external environment at both ends, the mouth and the anus (Figure 1). Material passing through the digestive tract is outside the body in the sense that it is only in contact with cells that line the tract similar to substances in contact with skin cells outside the body. For materials in the digestive tract to reach the inside of the body they must pass through epithelial cells of the tract in a process called **absorption** into the blood. Before food can be absorbed however, it must be broken down by physical processes such as chewing, and by enzymatic processes that transform large molecules into smaller monomers that can pass into digestive tract cells and into the blood for distribution to cells throughout the body. This breakdown of food into smaller, absorbable units is the process of **digestion**.

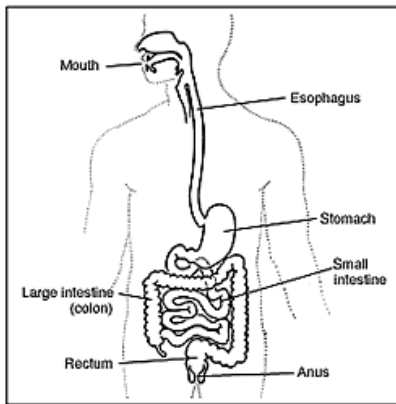


Figure 1.

Movement of food through the digestive tract from the esophagus to the anus is controlled by complexes of nerves and hormones that influence propulsion of food through contraction of smooth muscles lining the digestive tract and that influence the secretion of enzymes that chemically breakdown the food. Food moves through the digestive tract by means of alternating waves of contraction and relaxation of smooth muscles in the process known as **peristalsis** (Figure 2). Similar smooth muscle contractions occur in the large and small intestines, mixing food with digestive enzymes and moving material through these parts of the digestive tract.

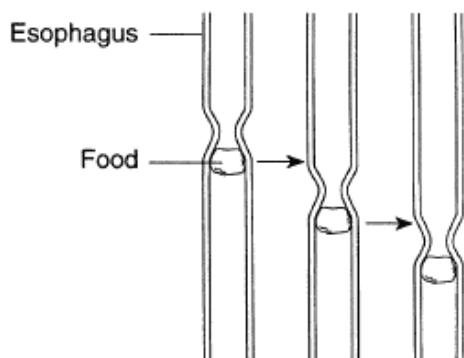


Figure 2.

A number of accessory organs compliment the digestive tract to result in the digestion and absorption of food. Cells in the mouth secrete the enzyme **amylase**, which begins to breakdown carbohydrates. The teeth and tongue also physically breakdown food into smaller particles with more surface area exposed to action of enzymes. Cells that line the stomach secrete protein-digesting enzymes along with hydrochloric acid (HCl) that produces an acidic environment for the initial phases of protein breakdown. The stomach also physically breaks down food particles by the contraction of smooth muscle lining the stomach. As the now liquefied food passes from the stomach into the small intestine, proteins, carbohydrates and lipids are broken down by action of enzymes that come from several sources. Cells that line the small intestine secrete digestive enzymes and the small intestine consists of many folds and finger-like projections (**villi**) that greatly increase the surface area of contact between cells and foodstuffs. A second source of enzymes is the **pancreas**, which delivers enzymes and bicarbonate (to buffer the acidity of the material from the stomach) to the small intestine through the common bile duct. Bile salts produced in the **liver** and stored in the gall bladder also pass into the small intestine via the common bile duct and bile acts to emulsify fats, which increases the surface area of fats that are in contact with lipid digestive enzymes. Large macromolecules must be broken down to an absorbable size by enzymes; similar to breaking down mortar that holds bricks together in a wall. Each enzyme binds with a specific **substrate**. For example, enzymes such as amylase break down carbohydrates to monosaccharides or disaccharides, proteins are broken down by peptidases to amino acids and lipids are broken down by lipase to glycerol and fatty acids (Figure 3).

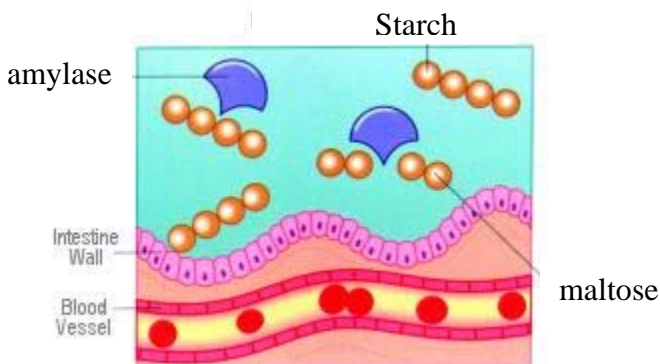


Figure 3. Breakdown of starch by the enzyme amylase to form the sugar maltose.

Materials for Digestive Physiology Exercises

- Water bath (37°C).
- Boiling water bath.
- Test tubes, test tube holders, graduated cylinders, droppers.
- Starch solution: 1 g cooked starch per 100 ml water.
- Iodine solution (Lugol's reagent): 1 g iodine, 2 g potassium iodide per 300 ml water
- Benedict's reagent: 50 g sodium carbonate, 85 g sodium citrate and 8.5 g copper sulfate in 5 L water
- White of hard-boiled eggs.
- Pepsin solution (5 g per 100 ml), 2N HCl, and 10 N NaOH.
- pH meter

EXERCISE 1 – DIGESTION OF CARBOHYDRATE BY SALIVARY AMYLASE

This exercise investigates the chemical breakdown and disappearance of a substrate (starch) and the appearance of a product (maltose) by action of the enzyme salivary amylase (a starch digesting enzyme produced by salivary glands in your mouth) and the effects of temperature and pH on the reaction. Therefore it is important to be able to determine the presence of these substances to examine the level of activity of the enzyme. The appearance of maltose will be determined by the Benedict's test – formation of a yellow-red colored precipitate in the presence of maltose.

Procedures:

1. Label four clean test tubes 1-4.
2. Obtain a paraffin stick from the laboratory instructor and chew it. Chewing paraffin (which is chemically inert) causes secretion of salivary glands with altering the composition of saliva. Obtain 10 ml of saliva in a test tube.
3. Examine the physical characteristics of saliva (color, viscosity, pH) and record your observations.
4. Add 3 ml of distilled water to test tube 1.
5. Add 3 ml of saliva to test tubes 2 & 3.
6. Add 3 drops HCl to tube 3.
7. Boil the remaining saliva and add 3 ml of the saliva to tube 4 when it has cooled.
8. Add 5 ml cooked starch to each of the four test tubes.
9. Incubate all tubes for 1 hour in a 37°C water bath.
10. Divide the contents of each sample by pouring ½ into four new test tubes.
11. Test one set of the four solutions for starch by adding a few drops of Lugol's reagent – a purple-black precipitate indicates a positive test.
12. Test the other set of four solutions for the presence of maltose by the following method:
 - a. Add 5 ml of Benedict's reagent to each of the four test tubes and immerse them in a rapidly boiling water bath for 2 minutes.
 - b. Remove the tubes from the boiling water with a test tube clamp and rate the amount of maltose present according to the following scale:
 - Blue (no maltose) -
 - Green +
 - Yellow ++
 - Orange +++
 - Red (most maltose) ++++

Salivary Visual Analysis

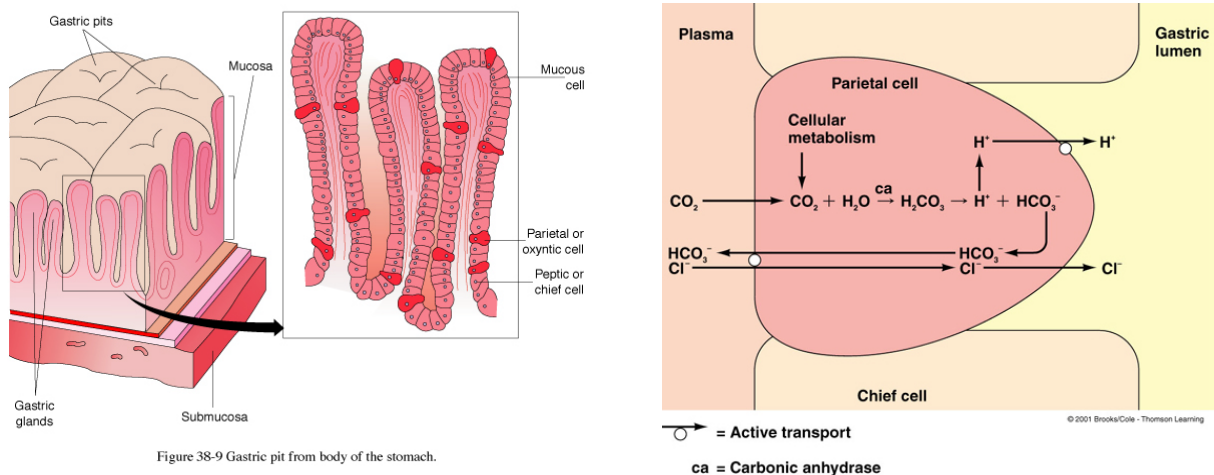
Color	Viscosity	pH

Tube No.	1	2	3	4
Additives	Water	Saliva	HCl	Boiled saliva
Lugol's test				
Benedict's				

Describe your conclusions concerning the effects of temperature and pH on the digestion of starch by the enzyme salivary amylase.

EXERCISE 2 – DIGESTION OF PROTEIN BY PEPSIN

The protein digesting enzyme pepsin has a pH optimum that is adapted to the normal very low pH of the stomach (a pH of less than 2). The pH of the stomach is due to the secretion of hydrochloric acid by parietal cells in gastric glands that line the stomach. Another type of cell (chief cells) produces pepsin, which is transformed to pepsinogen in the presence of HCl and is responsible for about 15% of protein digestion in the human digestive tract (Figure 4). The remainder of protein digestion occurs in the small intestine as the result of the action of enzymes such as trypsin (secreted by the pancreas) and peptidases (located in the cells that line the small intestine).



Procedures:

1. Label 5 clean test tubes 1-5.
2. Cut five slices of egg white about the size of a fingernail and as thin as possible.
3. Place an egg slice into each of the five test tubes.
4. Add 1 drop distilled water into tube 1.
5. Add 1 drop of concentrated HCl into tubes 2-4.
6. Add 1 drop of NaOH to tube 5.
7. Add 5 ml pepsin solution to tubes 1,2,3 and 5.
8. Add 5 ml distilled water to tube 4.
9. Place tubes 1,2,4 and 5 in a 37°C water bath.
10. Place tube 3 in an ice bath.
11. Incubate all tubes 1 hour.
12. Remove the incubated tubes and record the appearance of egg white in each tube.

Tube No.	1	2	3	4	5
Additives	Water Pepsin	HCl Pepsin	HCl Pepsin	HCl Water	NaOH Pepsin
Incubation	37°C	37°C	Ice bath	37°C	37°C
Egg white digestion					

Review Questions

1. In exercise 1, which tube(s) contained the most starch following incubation? Which tube(s) contained the most maltose?

2. What conclusions do you draw from these results?

