

## Lab #12: Digestive Physiology

### Background

In order for the nutrients in food to be absorbed, they must first be broken down into particles that are small enough to be transported through carrier proteins into the epithelial cells that form the mucosal lining of the digestive tract. This process of breaking down food is called *digestion*, and occurs primarily within three particular segments of the digestive tract: the mouth, the stomach, and the small intestine. Digestion occurs through two different processes: *physical digestion*, where large chunks of food are ground into tiny particles, and *chemical digestion*, whereby through the use of enzymes released into the digestive tract large polymeric biomolecules are broken into individual monomers or oligomers (e.g. dimers or trimers). Chemical digestion is essential for breaking food into particles that can be absorbed by the epithelium of the small and large intestine, and will be the focus of this lab exercise.

#### *The Importance of Enzymes in Chemical Digestion*

There are many different substances that are secreted into the different segments of the digestive tract. Mucus, bile salts, bilirubin, hydrochloric acid (HCl), and sodium bicarbonate (NaHCO<sub>3</sub>) are just some of the substances mixed with the food as it passes through the digestive tract, and many of these substances facilitate the breakdown of food. However, the most important substances secreted for the purpose of digestion are the digestive enzymes. Digestive enzymes greatly enhance the rate at which the covalent bonds that link subunits together to form polymeric biomolecules are broken. Indeed, without the presence of these enzymes, chemical digestion would essentially not occur. Although substances such as HCl and NaHCO<sub>3</sub> can alter noncovalent bonding patterns within and among biomolecules, they typically cannot break down covalent bonds.

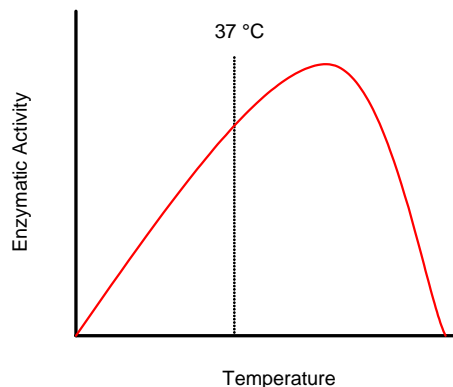


Fig 11.1. Influence of temperature on the rates of enzyme-catalyzed reactions. Note that both low and very high temperatures lead to the reduction of reaction rates.

We have previously discussed many aspects of enzyme structure, catalytic activity, and factors that influence this catalytic activity (e.g., enzyme, substrate, and cofactor concentrations, see Lab #4). Two factors that influence enzyme activity deserve further consideration here in the context of digestion: temperature and pH. Recall that temperature can have considerable influence on the rate at which enzyme-catalyzed reactions proceed. Low temperatures result in slow reaction rates because overall kinetic energy is reduced—since particles are moving more slowly, substrate binds to the enzyme less frequently, the reaction converting substrate to product lasts longer, and the random collisions that dislodge the product from the substrate occur less often and with less force. Very high temperatures can also slow the rate of an enzyme catalyzed reaction because high temperatures destabilize the noncovalent interactions that give enzymes the specific tertiary and quaternary structures that enable them to function as catalysts. This highlights the importance of the stable core body temperatures of humans in their digestive processes. The relatively high (but not extremely high) temperature of the human body enables digestive enzymes to break down food at near maximal rates. Organisms with more variable and lower core body temperatures (e.g. reptiles)

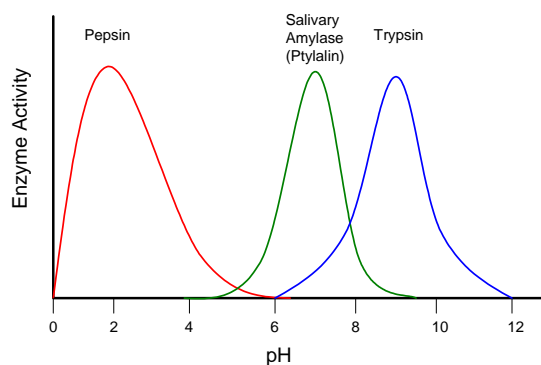


Fig 11.2. Effect of pH on catalytic activity in three digestive enzymes from different segments of the digestive tract.

will often bask after feeding to elevate body temperature and facilitate enzymatic digestion.

Enzyme activity is also influenced by the pH of the surrounding fluid. Recall that  $[H^+]$  can influence whether acidic and basic amino acid side chains are bound to a hydrogen ion or not, thus influencing the charge on those side chains and, in turn, alter ionic and hydrogen bonding patterns in those proteins, thus inducing changes in the tertiary and quaternary structure of proteins. Thus enzymes have a particular pH where they have the proper conformation to have maximal catalytic activity, and significant deviation from that pH will typically result in a decrease in catalytic activity. Interestingly, pH varies widely among different segments of the digestive tract, and different enzymes have maximum catalytic activity at these various pH's, effectively restricting the function of that enzyme to a particular region of the digestive tract (Fig 11.2). For example, gastric enzymes such as pepsin have maximum catalytic activity at the very low pH of the stomach, and no longer function once moved into the alkaline conditions of the small intestine. In contrast, intestinal and pancreatic enzymes, such as trypsin, function optimally at moderately alkaline pH. Altering pH among different regions of the digestive tract effectively enables a stepwise process of chemical digestion, where enzymes are activated for digestion at one point and then deactivated at the next.

### Chemical Digestion of Carbohydrates.

Carbohydrates are the first type of biomolecule to be chemically digested in the digestive tract, as chemical digestion begins in the oral cavity through a salivary enzyme called *salivary amylase* (or *ptyalin*). Salivary amylase begins the breakdown of the polysaccharide amylose (starch, the principle storage carbohydrate in plants) into the disaccharide maltose. However, when food is swallowed and transferred to the small intestine, chemical digestion of carbohydrates effectively stops. Only when the chyme passes into the small intestine will carbohydrate digestion resume. In the small intestine the chyme is exposed to *pancreatic amylase* (which continues the process of breaking down starch and glycogen into disaccharides and trisaccharides) and certain brush-border enzymes (e.g., lactase, sucrase, and maltase) that break down specific oligosaccharides into the monosaccharides that are absorbed by the intestinal epithelium.

### Chemical Digestion of Protein.

Chemical digestion of protein begins in the stomach. The lining of the stomach produces a mixture of fluids called gastric juice in response to neural stimulation (induced by smell, site and taste of food), by distension of the stomach as food enters, and by pH changes induced as the more neutral pH food enters the acidic stomach. Gastric juice contains a number of substances, but the two most important for initiating protein digestion are *hydrochloric acid* (HCl) and the protease *pepsin*. HCl is secreted by parietal cells in the gastric mucosa. The low pH (~2) of the gastric juice aids protein digestion in a couple of ways. First, the low pH denatures the tertiary structures of ingested protein, making them easier to digest enzymatically. Secondly, the low pH is required for the activation of pepsin. Pepsin is produced by chief cells of the gastric mucosa in an inactive (*zymogenic*) form called *pepsinogen*. Pepsinogen is inactive when released in the gastric pits, but once it diffuses into the lumen of the stomach the acidic conditions enable it to have a weak proteolytic activity (Fig 11.3). Pepsinogen can digest some

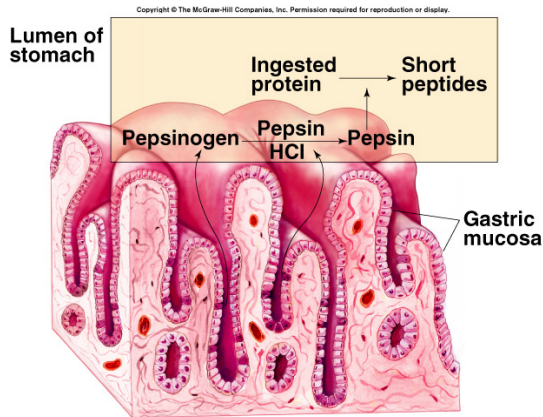


Fig 11.3. Activation of pepsin in the stomach. Image from S.I. Fox *Human Physiology*, 8<sup>th</sup> ed. McGraw Hill.

ingested protein, but more importantly pepsinogen molecules will partially digest one another, removing inhibitory segments of the polypeptide chain and thus converting each other into the fully active enzyme *pepsin*. Pepsin breaks peptide bonds between amino acids with hydrophobic side chains in the middle of polypeptides, thus it cleaves long polypeptides into shorter polypeptides.

Although the chemical digestion of protein begins in the stomach through the actions of pepsin, most of the digestion of protein takes place in the small intestine. Indeed, individuals with complete gastrectomies can still completely digest protein, although the homogenization of chyme through chemical and physical digestion in the stomach aids this process. The proteases that function in the small intestine come from

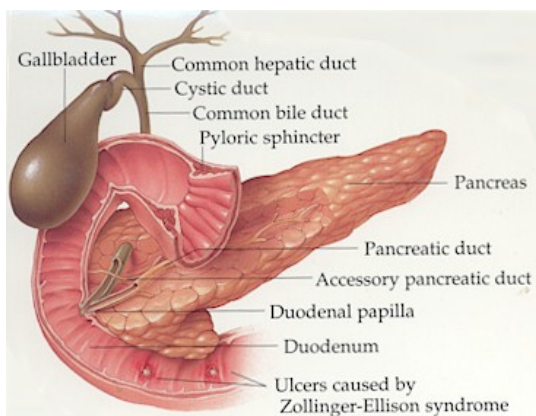


Fig 11.4. The pancreas, gall bladder, and duodenum. Image from <http://www.ucpancreas.org/pancreas.htm>

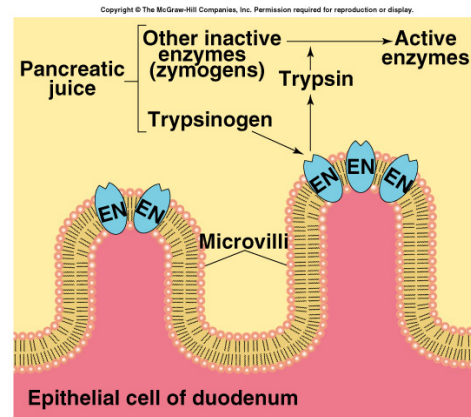


Fig 11.5. Activation of pancreatic zymogens in the small intestine by brush-border enzymes. The brush-border enzyme enterokinase (EN) phosphorylates the protease zymogen trypsinogen, converting it to its active form (trypsin). Trypsin, in turn digests other pancreatic zymogens into their active forms. Image from S.I. Fox *Human Physiology*, 8<sup>th</sup> ed. McGraw Hill

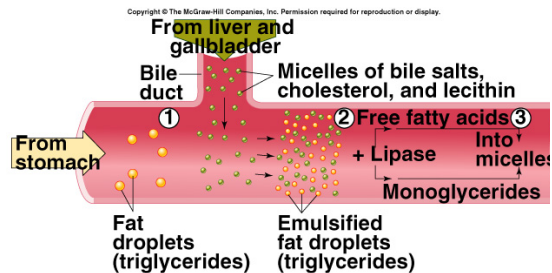
two major sources: membrane bound enzymes on the brush-border of the intestinal mucosa, and enzymes secreted into the small intestine from the pancreas. The pancreatic enzymes, along with bicarbonate salts, are components of pancreatic juice which is secreted primarily when food enters the small intestine through the pyloric sphincter. Chemicals in the chyme induce cells in the small intestine to secrete the hormones *secretin*, which stimulates water and bicarbonate secretion in the pancreas, and *cholecystokinin (CCK)*, which stimulates enzyme secretion in the pancreas. These hormones, in turn, cause the pancreas to release pancreatic juice through the duodenal papilla (Fig 11.4). A number of different proteases are found within pancreatic juice, but most are released as *zymogens* (inactive enzymes). Enzymes in the brush-border activate these zymogens (Fig 11.5), which ultimately digest the polypeptides into a combination of free amino acids, dipeptides, and tripeptides that are absorbed by the intestinal epithelium.

### *Chemical Digestion of Fat*

Lipids (principally triglycerides, or fat) are a particularly challenging group of biomolecules to digest chemically. This is because fats, being

hydrophobic, tend to aggregate in large droplets within the fluid of the digestive tract, minimizing the surface area of contact between the fat and the surrounding water. Since the digestive enzymes are water soluble, they can only come into contact with and digest those triglyceride molecules at the surface of the droplets. Thus, for fat digestion to be efficient, these large droplets must be broken into much smaller droplets and held in these smaller droplets in order to increase surface area and enable fat-digesting enzymes (*lipases*) adequate contact with their substrate.

Although there is a gastric lipase secreted in the stomach that causes a small amount of fat digestion, and infants produce a salivary lipase, almost all of the digestion of fat takes place in the small intestine. Like protein digestion, fat digestion is achieved through the activity of both pancreatic and intestinal brush border lipases. However, efficient fat digestion also involves the secretion of bile from the liver and gall bladder. Bile contains a mixture of bilirubin (formed from the heme units of dead and digested erythrocytes), cholesterol, phospholipids, inorganic ions, phospholipids, and negatively charged cholesterol derivatives called *bile salts*. Secretion of bile into the small intestine is triggered by CCK (the same hormone that induces pancreatic enzyme secretion). The bile salts are particularly important for fat digestion in that they serve as *emulsification agents*. Since bile salts are amphipathic molecules, they help to break the large fat droplets into tiny emulsification droplets and



- Step 1: Emulsification of fat droplets by bile salts
- Step 2: Hydrolysis of triglycerides in emulsified fat droplets into fatty acid and monoglycerides
- Step 3: Dissolving of fatty acids and monoglycerides into micelles to produce "mixed micelles"

Fig 11.6. Emulsification and digestion of fat in the small intestine. Image from S.I. Fox *Human Physiology*, 8<sup>th</sup> ed. McGraw Hill

prevent them from reforming into large droplets (Fig 11.6). This greatly increases the surface area over which lipases can come into contact with the triglycerides and hydrolyze them into amphipathic free fatty acids and monoglycerides, both of which can be absorbed by the intestinal epithelium. The liberation of free fatty acids, which are acids, would lead to a decrease in the pH of the fluid passing through the intestine, although normally the presence of bicarbonate in the pancreatic juices buffers against a decrease in pH.

## Experiment: Chemical Digestion of Nutrients.

**NOTE: Your group will be performing only one of these three experiments. However, you need to obtain data for all three experiments to fill out your datasheet, and will need to understand why all three experiments had the results they did.**

### Experiment I: Chemical Digestion of Carbohydrate

This experiment will examine the effects of the presence of enzymes, variable pH, and high temperatures on the digestion of starch (a polysaccharide) into maltose (a disaccharide) using salivary amylase.

- Obtain four test tubes and label them 1-4
- Add the following solutions to the four tubes (see Fig 11.7):
  - TUBE 1: 3.0 ml water
  - TUBE 2: 3.0 ml amylase
  - TUBE 3: 3.0 ml amylase solution and 10 drops of HCl solution
  - TUBE 4: 3.0 ml amylase solution, then place tube in boiling water for 5 min
- Add 5.0 ml of starch solution to each tube
- Place all four tubes in a 37 °C water bath for 1 hr to incubate
- After incubation, obtain another set of four test tubes labeled 1-4. Split the four incubated solutions evenly among the two sets of tubes. One set of solutions will be tested for the presence of undigested starch (Lugol's test), whereas the other will be tested for the presence of maltose (Benedict's test)
- Lugol's Test** (Fig 11.8) – add a few drops of Lugol's iodine solution to each of the four tubes in one set of solutions.
  - If the solution has an amber coloration (like the Lugol's reagent) then there is no starch present (-)
  - If the solution turns blue or black, starch is present in the solution (+)
- Benedict's Test** (Fig 11.9) – add 5 ml of Benedict's reagent to each of the four solutions, and place the four tubes in boiling water for 10 min.
  - If the solution is blue (like the Benedict's reagent), there is no maltose present in the solution (-)
  - If the solution has a greenish coloration, then a small amount of maltose is present (+)
  - If the solution has a yellowish coloration, then a moderate amount of maltose is present (++)
  - If the solution has an orange or reddish coloration, then a large amount of maltose is present (+++)

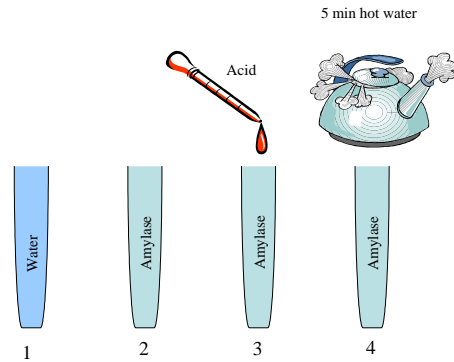


Fig 11.7. Preparation of samples for the carbohydrate digestion experiment

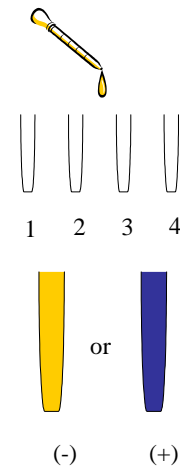


Fig 11.8. Lugol's test for the presence of undigested starch.

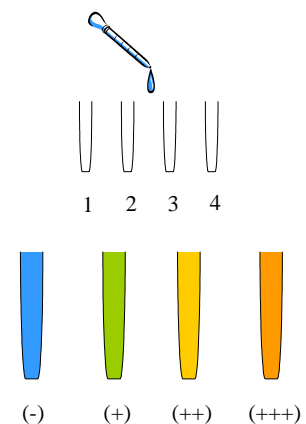


Fig 11.9. Benedict's test indicates the presence of and (qualitatively) the amount of maltose in solution.

### Experiment II: Chemical Digestion of Protein

This experiment will examine the effects of the presence of enzymes, variable pH, and low temperatures on the digestion of protein using a gastric protease (pepsin)

1. Obtain a set of five labeled test tubes
2. Add a small sliver of hard-boiled egg white to each of the five tubes
3. Add the following solutions to the tubes (see Fig 11.10):
  - TUBE 1: Add 5 ml pepsin solution and 10 drops of water
  - TUBE 2: Add 5 ml pepsin solution and 10 drops of HCl
  - TUBE 3: Add 5 ml pepsin solution and 10 drops HCl, then place on ice.
  - TUBE 4: Add 5 ml water and 10 drops HCl
  - TUBE 5: Add 5 ml pepsin solution and 10 drops of NaOH
4. Place Tubes 1, 2, 4 and 5 in a 37 °C water bath to incubate for 90 min.
5. Examine the egg white following incubation. Note any digestion that occurs to the egg white (Fig 11.11), and any color changes that may have happened to the solution.

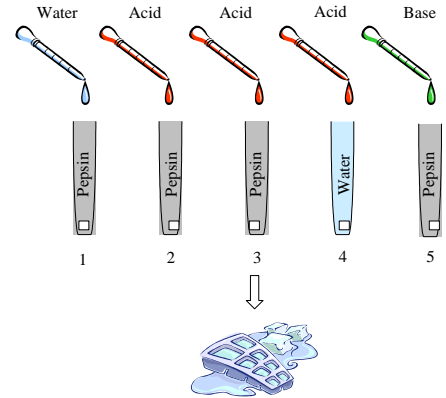


Fig 11.10. Preparation of samples for the protein digestion experiment.

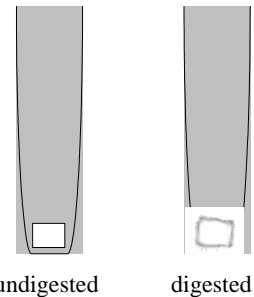


Fig 11.11. Comparison of undigested and digested egg white from the protein experiment

### Experiment II: Chemical Digestion of Fat

This experiment will examine the effects of the presence of enzymes and emulsifiers on the digestion of triglyceride using a pancreatic lipase (pancreatin)

1. Obtain a set of three test tubes
2. Add 3.0 ml heavy cream to each tube
3. Add the following to each tube (Fig 11.12):
  - TUBE 1: 5 ml water and a few grains of bile salts
  - TUBE 2: 5 ml pancreatin solution
  - TUBE 3: 5 ml pancreatin and a few grains of bile salt
4. Shake tubes rigorously to mix the contents

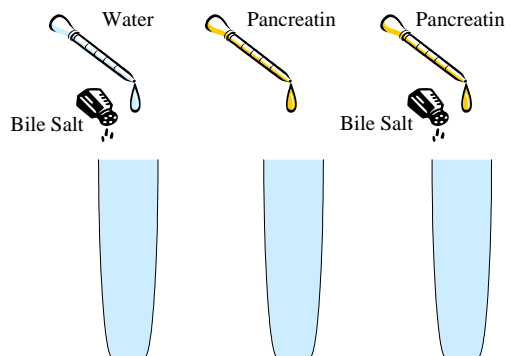


Fig 11.12. Preparation of samples for the fat digestion experiment

5. Measure the initial pH of each solution with the probe of a pH meter, cleaning off the probe in detergent and rinsing with distilled water between each measurement.
6. Place the tubes into the 37 °C water bath for incubation
7. Repeat the pH measurements of each solution at 20 min, 40 min, and 60 min after the beginning of incubation.