PEPSIN DIGESTION OF PROTEINS AND EFFECTS OF ANTACIDS

I. OBJECTIVES:

1. To study how pepsin digests proteins in our diet.
2. To study the effects of antacids on pepsin's activity.

II. BACKGROUND:

In the human digestive system, there are many enzymes to help digest our food. In Biology 114, we demonstrated the effects of salivary amylase on starch. In the stomach, the enzyme **pepsin** functions to break proteins into smaller polypeptides. The pancreas secretes a number of enzymes including maltase, sucrase, and lactase (which break down the corresponding disaccharides), trypsin and chymotrypsin (which further digest protein), and pancreatic lipase (which breaks fats into glycerol and fatty acids) into the small intestine where most digestion and absorption takes place.

Enzymes like pepsin and trypsin are secreted in inactive forms (**pepsinogen** and trypsinogen) and require certain conditions for conversion to their active forms. Pepsinogen, for example, needs HCl for conversion to pepsin and a pH range of 1 to 3. This pH range is also necessary for proper functioning of the pepsin. Too great of a change in pH can **denature** proteins such as these enzymes, thus causing them to cease functioning.

The pH of the stomach environment is generally around 1.5 to 3.5, which as mentioned, is necessary for the activation and optimal activity of pepsin. Most over-the-counter antacid medications combat the normal stomach acid (HCl) to produce a more nearly neutral pH (closer to a pH of 7), and as the pH rises above 4, pepsin activity decreases or stops. Additionally, it has been observed that because our bodies are designed to maintain a normal, constant internal environmental balance – **homeostasis** – typically, consumption of antacids results in the stomach actually secreting *more* hydrochloric acid to try to compensate for the imbalance caused by the antacids. Thus, while consumption of antacids may be helpful when prescribed by a doctor to soothe gastric ulcer, casual use is probably not a good idea. TV advertisers are only interested in your money, not your heath.

III. MATERIALS NEEDED:

16 × 150 test tubes with caps
wax pencil
1% pepsin solution (1 g pepsin in 100 mL or 10 g/L)
boiled 1% pepsin solution
0.5% $NaHCO_3$ (sodium bicarbonate – baking soda) (= 5 g/L)
0.1N HCl
hard-boiled egg white and knife
antacid(s) of your choice (record which you use)
250 mL beaker

IV. PROCEDURE:

1. Obtain (at least) eight 16 × 150 test tubes and number them. Obtain an additional test tube for each antacid you will be testing.

2. In these tubes place the following solutions:

#1	10 mL dH_2O
#2	5 mL dH_2O + 5 mL 1% pepsin solution
#3	5 mL dH_2O + 5 mL 0.5% $NaHCO_3$
#4	5 mL dH_2O + 5 mL 0.1 N HCl
#5	5 mL 0.5% $NaHCO_3$ + 5 mL 1% pepsin
#6	5 mL 0.1 N HCl + 5 mL 1% pepsin
#7	5 mL 0.1 N HCl + 5 mL BOILED 1% pepsin solution
#8	5 mL 0.1 N HCl + 5 mL 1% pepsin solution

3. Mix each solution with a vortex.
4. Use pH paper to determine the pH of each test tube. Record pH values in your lab notebook.
5. In a 250 mL beaker, obtain 100 mL of 0.1 N HCl. Use pH paper to determine the pH of this solution and record in your lab notebook. This would be in the range of how much HCl might be present in your stomach.
6. To this solution, add one dose of the antacid you are testing (if more than one antacid is being tested, each needs a separate beaker of solution). This would be analogous to the effect on your stomach acid caused by taking a dose of that antacid. Stir to completely dissolve and mix (note: tablets may need to be ground, first, with a mortar and pestle). Determine the pH and record in your lab notebook. How much did that dose of antacid change the pH of your "stomach?"
7. Obtain a test tube for each antacid you are testing, label (#9, #10?, etc.), and into it (them), place 5 mL of the corresponding solution(s) you just mixed + 5 mL of 1% pepsin solution. Check the pH again. Remember to record tube number(s), contents, and pH in your lab notebook.
8. Obtain 5 mm-sized cubes of egg white and add one piece to each test tube.
9. Cap the tubes and incubate all <u>EXCEPT #8</u> at 37°C for 48 hours. Store #8 at room temperature in the designated location.
10. The next class period, obtain your tubes and record what happened in each. Is there egg white still present or not? Is it still white or has it turned translucent? Is it still in one piece or broken up? Is there any smell or any other change?
11. *Optionally, repeat this whole process with the enzyme trypsin, an enzyme which is active in the small intestine.*

V. DATA:

Create a table in your notebook similar to the following:

tube #	contents	pH	reaction?

The following rating system can be used to indicate the results of this experiment:

(3)	+++	no egg white observed
(2)	++	some egg white present but decreased mass and very transparent
(1)	+	egg white slightly transparent and only slightly changed in size
(0)	–	no change in the condition of the egg white

VI. CONCLUSIONS:

1. What effect(s) does pH have on pepsin's ability to digest egg white?
2. What effect(s) does the addition of antacid have on stomach pH and on pepsin's ability to digest egg white? Of what significance would this be in "everyday life?"
3. *If trypsin was also tested, how do the results compare with the pepsin? Is the effect of pH the same or different?*