DIGESTION LAB

SFCC
A/P 242 (BIOL&242)
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Introduction:

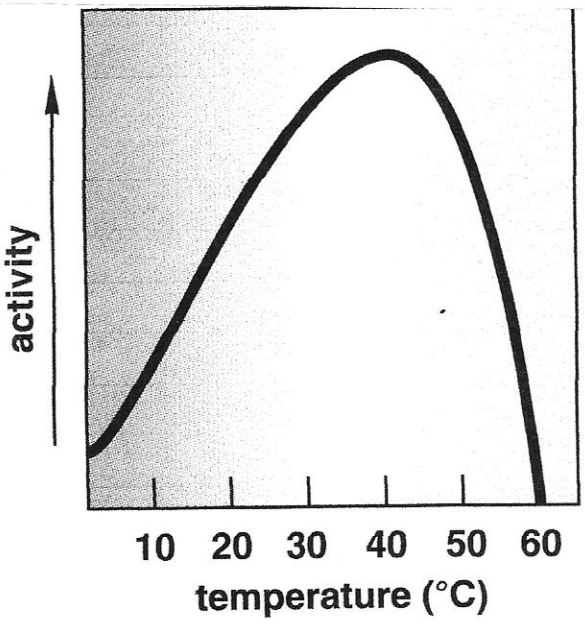
The function of the digestive system is to break down large food molecules (macromolecules) of carbohydrates, proteins and lipids into micromolecules small enough to be absorbed into the blood stream where the nutrients can be utilized by body cells.

Food is broken down by mechanical action, such as chewing, and by the *chemical action of digestive enzymes. Enzymes are proteins that catalyze* reactions. They increase the rate of reactions without being consumed by the reaction.

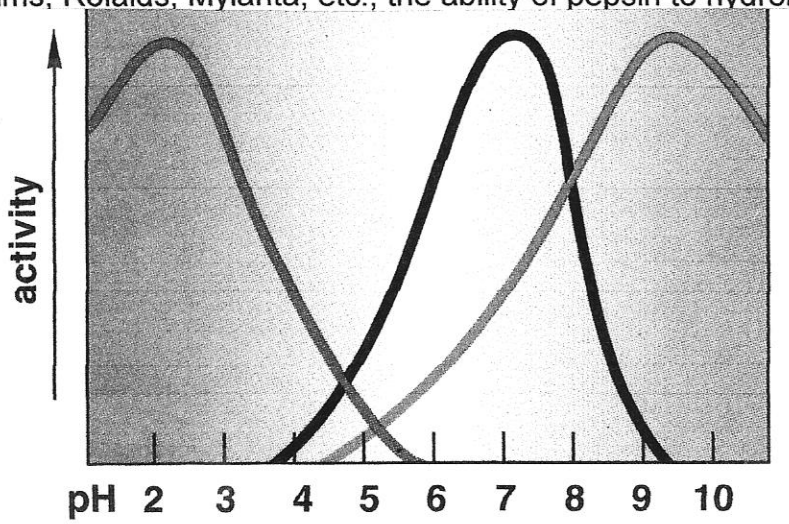
Enzymes in the human digestive tract catalyze the conversion of carbohydrates into disaccharide and monosaccharide sugars; convert proteins into short chains of amino acids called peptides, as well as individual amino acids; and convert lipids into glycerol and fatty acids.

Each enzyme found in the digestive system works on a SPECIFIC substrate (carbohydrate, protein or lipid). For example, amylase, an enzyme produced by the salivary glands and pancreas breaks down carbohydrates but has no effect on proteins or lipids. Another example is lipase, an enzyme that catalyzes the breakdown of fats but has no effect on carbohydrates or proteins.

Enzyme activity is also affected by temperature and pH. Human enzymes work best at human body temperature (37°C or 98.6°F). Since enzymes are proteins their molecular structure is denatured by heating to 60°C or higher and they no longer function. Conversely, as enzymes are cooled, their activity slows as the temperature decreases and activity ceases at 0°C.



Enzyme activity is also affected by pH. Various enzymes have an optimal pH at which they catalyze reactions the fastest rate. If the pH is higher or lower than optimal, the rate of reaction decreases. For example, the inactive enzyme pepsinogen, which is produced by "Chief cells" in the stomach, requires gastric acid containing HCl to convert it to the active enzyme pepsin, which works fastest at pH 2.0 to break down proteins into short chain peptides. If the stomach gastric *juice pH increases (becomes more alkaline) because a person ingests an antacid* such as Tums, Rolaids, Mylanta, etc., the ability of pepsin to hydrolyze proteins ceases.



In the following experiments we will use amylase to hydrolyze starch into sugar; trypsin (a pancreatic proteolytic enzyme) to break down protein into short chains of amino acids; and lipase to break down fat into glycerol and fatty acids.

Activity #1: Starch Hydrolysis by Amylase

Starch (Amylose) + water ----------→ Maltose + dextrins

Procedure:

1. Mark 5 clean test tubes "1A" through "5A" with a Sharpie marker.

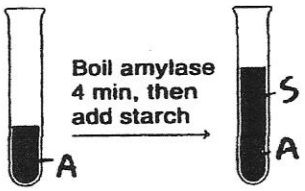
2. Follow the diagram to prepare each tube. (Note: Use a separate pipette for each additive to avoid cross contamination.

Tube 1A = 10 drops water plus 10 drops starch
Tube 2A = 10 drops water plus 10 drops amylase
Tube 3A = 10 drops water plus 10 drops maltose
Tube 4A = 10 drops amylase then BOIL for 4 minutes,
then add 10 drops starch
Tube 5A = 10 drops amylase plus 10 drops starch

3. Place all 5 tubes in the 37°C water bath for 45 to 60 minutes to allow time for the enzyme to catalyze the reaction.

Diagram: Starch Hydrolysis by Amylase

Salivary Amylase Digestion of Starch

Tube no.	1A	2A	3A	4A	5A
Additives (10 gtt ea)					
Incubation condition	37°C	37°C	37°C	37°C	37°C
IKI test (color change)					
Positive (+) or negative (−) result					
Benedict's test (color change)					
Positive (+) or negative (−) result					

Additive key:

■ = Amylase A ■ = Starch S □ = Maltose m □ = Water w

4. After the incubation period all tubes will be tested for the presence of starch using IKI (iodine potassium iodide) solution, and for the presence of sugar using Benedict's reagent.

Iodine Test for Starch:

AFTER INCUBATION, place a few drops from each tube into an appropriately marked "well" in the porcelain spot tray. Mark the wells "1A" through "5A". Add 2 or 3 drops of IKI solution to each well. If starch is present there will be a color change (dark purple). The test is negative for starch if there is no color change.

Benedict's Test for Sugar:

After completing the iodine test for starch add 5-10 drops of Benedict's reagent to each tube, "1A" through "5A" then place all tubes in a BOILING water bath for 5 minutes.
Benedict's reagent detects simple sugars. A yellow, green, orange or red color indicates maltose is present and is a positive test for sugar. A yellow color indicates a small amount of sugar while an orange or red color indicates a large amount of sugar is present. If the solution remains light blue in color (the color of Benedict's reagent) the test is negative for sugar.

Activity #1 Notes:

Tubes 1A, 2A and 3A are CONTROLS.
Tubes 4A and 5A are experimental.

Why boil tube 4A? (Heat denatures protein and amylase is a protein).
IKI yields a positive test for starch.

Benedict's Reagent yields a positive test for maltose (simple sugar).

IKI + starch = solution turns dark purple
IKI + dextrins = solution turns red
IKI + maltose = no color change (IKI solution remains yellow.the color of iodine)

Benedict's + maltose (after boiling for 5 minutes) = solution turns yellow, green, orange or red depending on the amount of sugar present

Activity #2: Protein Hydrolysis by Trypsin

Trypsin, a pancreatic enzyme, breaks proteins down into short chain peptides. BAPNA is a synthetic substrate with a dye bound to an amino acid. Trypsin cleaves the dye molecule from the amino acid causing the solution to change from colorless to bright YELLOW. The yellow color is direct evidence that trypsin hydrolyzes the peptide bonds in proteins to produce short chain peptides.

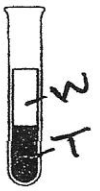
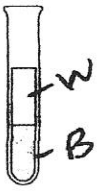
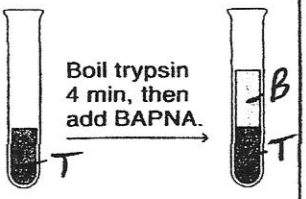
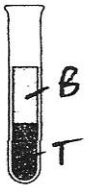
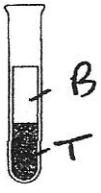
Procedure:
1. Mark 5 clean test tubes "1T" through "5T" with a Sharpie marker.

2. Follow the diagram to prepare each tube. (Note: Use a separate pipette for each additive to avoid cross contamination:
Tube 1T = 10 drops water plus 10 drops trypsin
Tube 2T = 10 drops water plus 10 drops BAPNA
Tube 3T = 10 drops trypsin then BOIL for 4 minutes,
then add 10 drops BAPNA
Tube 4T = 10 drops trypsin + 10 drops BAPNA (37°C)
Tube 5T = 10 drops trypsin + 10 drops BAPNA (0°C)

3. Place tubes 1T through 4T in the 37°C water bath for 45 to 60 minutes to allow time for the enzyme to catalyze the reaction. Place tube 5T in the bucket of ice at 0°C.

4. AFTER the incubation period, examine each tube for a color change of clear to yellow. The yellow color indicates a positive hydrolysis test. A clear color is a negative test result. Record your results on the diagram.

Trypsin Digestion of Protein

Tube no.	1T	2T	3T	4T	5T
Additives (10 gtt ea)					
Incubation condition	37°C	37°C	37°C	37°C	0°C
Color change					
Positive (+) or negative (−) result					

Additive key:

■ = Trypsin T □ = BAPNA B □ = Water W

Activity #2 Notes:

Trypsin + protein ----------→ peptides (10 – 100 amino acid chain)

Tubes 1T and 2T = Controls
Tubes 3T, 4T, and 5T = Experimental

Why incubate at 37°C? (body temp) Why 0°C?

BAPNA = protein (substrate) with a dye molecule bonded to it.
Trypsin + BAPNA ---------→ peptides + indicator
Indicator turns YELLOW when the peptide bonds are broken.

Activity #3: Lipid Hydrolysis by Lipase

Litmus cream contains fat and a litmus powder indicator. The indicator is blue in the presence of fat but turns pink if lipase hydrolyzes the fat into fatty acids. The free fatty acids lower the pH and the solution turns from blue to pink when the pH is acid.

Procedure:

1. Mark 7 clean test tubes "1L", "2L", "3L", "4L", "4B" and "5B" with a Sharpie marker.

2. Follow the diagram to prepare each tube. (Note: Use a separate pipette for each additive to avoid cross contamination.

Tube 1L = 10 drops water plus 10 drops lipase
Tube 2L = 10 drops water plus 10 drops litmus cream
Tube 3L = 10 drops lipase then BOIL for 4 minutes,
then add 10 drops litmus cream
Tube 4L = 10 drops lipase + 10 drops litmus cream (37°C)
Tube 5L = 10 drops lipase + 10 drops litmus cream (0°C)
Tube 4B = 10 drops lipase + 10 drops litmus cream + Bile (37°C)
Tube 5B = 10 drops lipase + 10 drops litmus cream + Bile (0°C)

3. Place tubes 1L through 4L and 4B in the 37°C water bath for 45 to 60 minutes to allow time for the enzyme to catalyze the reaction. Place tubes 5L and 5B in the bucket of ice at 0°C.

4. AFTER the incubation period, examine each tube for a color change of blue to PINK. The pink color indicates a positive hydrolysis test. A blue color is a negative test result. Record your results on the diagram.

Diagram: Lipid Hydrolysis by Lipase

Record your results on the diagram:

Pancreatic Lipase Digestion of Fats

Tube no.	1L	2L	3L	4L	5L	4B	5B
Additives (10 gtt ea)	W, L	W, C	Boil lipase 4 min, then add litmus cream. → C, L	C, L	C, L	B, C, L	B, C, L
Incubation condition	37°C	37°C	37°C	37°C	0°C	37°C	0°C
Color change							
Positive (+) or negative (−) result							

Additive key:

☐ = Lipase L ☐ = Litmus cream C ☐ = Water W △ = Pinch bile salts B

Activity #3 Notes:

Lipase + lipids ----------→ free fatty acids + glycerol

Tubes 1L and 2L = Controls; Tubes 3L to 5B = Experimental

Why add Bile?

Litmus is a pH indicator.
pH above 7.0 = solution is blue (alkaline)
pH below 7.0 = solution is pink (acidic)

Free fatty acids release hydrogen ions (H+) and H+ makes the solution acidic.

Activity #4: Effect of Rennin on Milk

The gastric juice of young mammals contains rennin, an enzyme that causes milk to curdle. This enzyme, while not present in the gastric juice of human adults, is present in the stomach of young infants. Rennin is produced by "Chief" cells in the stomach in the *inactive form, prorennin, and is converted by HCl in the stomach to the active enzyme* rennin.

Rennin converts casein (milk protein) to paracasein with the optimum pH of the reaction being 5.4. Calcium ions then react with the paracasein to form calcium paracaseinate which is an insoluble white curd.

Procedure:

1. Pipette 3 mls of rennin into a clean test tube.
2. Add 10 mls skim milk to the rennin; cover with parafilm and mix.
3. Place the tube in a 40°C waterbath. In a few minutes the tube will be "curdled" and can be inverted without spilling the contents.
4. Place your thumb over the end of the tube covered with parafilm and shake vigorously to dislodge the curd from the side of the test tube.
5. Place the tube back in the 40°C waterbath for approximately 30 minutes. You should see a yellow liquid called whey separate from the white curd.
6. Use a pipette to draw up 2-3mls of whey and place in another clean test tube.
7. Add 10 drops of Benedict's reagent to the tube containing 2-3mls of whey and BOIL for 5 minutes. An orange color indicates the presence of sugar in the whey.

Activity #4 Notes: (Effect of Rennin on Milk)

Rennin + milk -------→ paracasein + (Ca++) ------→ calcium paracaseinate (curds)

Casein is the protein in milk. Lactose is the sugar in milk.

Paracaseinate is the white solid. Whey is the yellow fluid.

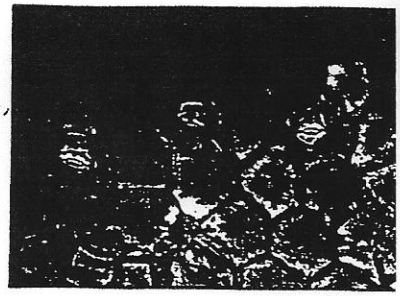
Where does the lactose go?.....curds or whey?

Benedict's reagent gives a positive test for lactose (glucose + galactose).
The color change will be the same as with maltose (glucose + glucose).
Both lactose and maltose are disaccharides (simple sugars).

Use Benedict's reagent to test the whey (yellow fluid) for the presence of sugar.

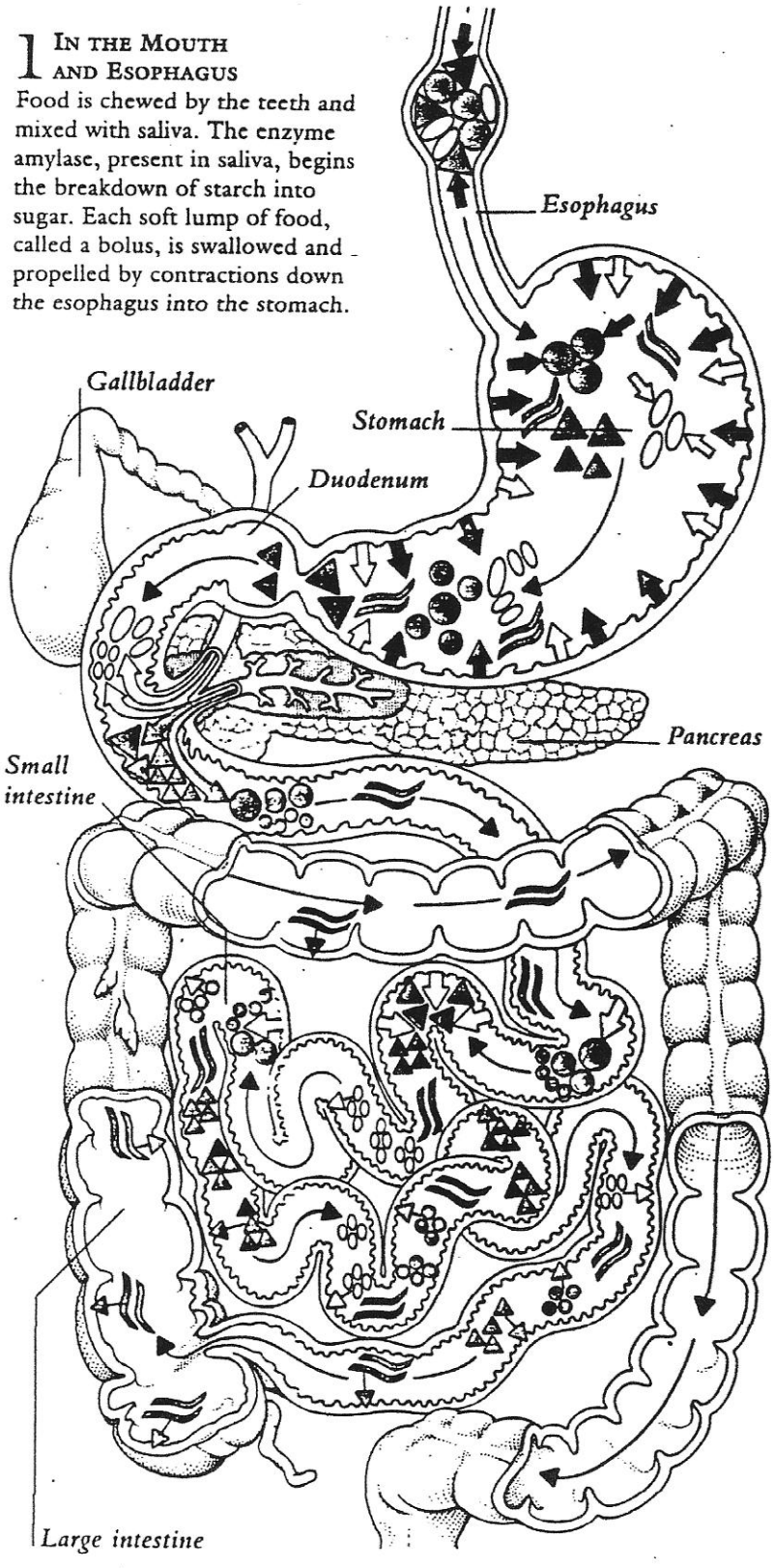
From your observations and results, does whey contain lactose sugar?

THE DIGESTIVE TRACT IS A MUSCULAR TUBE extending from the mouth through the stomach and intestines to the anus. Food moves along the digestive tract while it is changed into substances that can be absorbed into the bloodstream for distribution. The pancreas, the salivary glands, and the biliary system all connect to the digestive tract, producing various substances that are essential to healthy digestion.



Dietary fiber (cellulose)

1 IN THE MOUTH AND ESOPHAGUS
Food is chewed by the teeth and mixed with saliva. The enzyme amylase, present in saliva, begins the breakdown of starch into sugar. Each soft lump of food, called a bolus, is swallowed and propelled by contractions down the esophagus into the stomach.



THE BREAKDOWN OF FOOD

Certain nutrients, such as salts and minerals, can absorbed directly into the circulation. Proteins, f and carbohydrates, however, must be broken do into smaller molecules before they can be absorb Food is broken down both by mechanical action by the chemical action of digestive enzymes. Fat split into glycerol and fatty acids; carbohydrates monosaccharide sugars; and proteins into shorte chains and subsequently into individual amino ac

2 IN THE STOMACH
Pepsin is an enzyme produced when gastric acid catalyzes inactive pepsinogen. It breaks down proteins into smaller units called polypeptides and peptides.
Lipase breaks down a small proportion of fats into glycerol and fatty acids.
Hydrochloric acid is produced by the lining. Its acidity, needed for the action of pepsin, can also kill certain bacteria.

3 IN THE DUODENUM
Lipase, a pancreatic enzyme, breaks down fats into glycerol and fatty acids.
Amylase, another enzyme produced by the pancreas, breaks down starch into maltose, which is a disaccharide sugar.
Trypsin and **chymotrypsin** are powerful pancreatic enzymes that split proteins into polypeptides and peptides.

4 IN THE SMALL INTESTINE
Maltase, sucrase, and **lactase** are enzymes secreted by certain glands in the intestinal wall. They convert disaccharide sugars into monosaccharide sugars.
Peptidase, another enzyme secreted by glands in the intestinal wall, splits large peptides into smaller peptides and then into individual amino acids.

5 IN THE LARGE INTESTINE
Undigested food enters the large intestine, where water and salt are absorbed by the intestinal lining. The residue, together with waste pigments, dead cells, and bacteria, is pressed into feces and then stored for excretion.

KEY

- *Salivary an*
- *Pancreatic a*
- *Maltase, su and lactase*
- *Pepsin*
- *Trypsin anc chymotrypsi*
- *Peptidase*
- *Lipase*
- *Bile salts*
- *Hydrochlori*
- *Starch*
- *Disaccharid (maltose, su and lactose)*
- *Monosaccha (glucose, fru and galactos*
- *Proteins*
- *Peptides*
- *Amino acid:*
- *Fats*
- *Fatty acids*
- *Glycerol*
- *Water*