# **Lab 2: cell metabolism**

## **Pre-lab Reading**

In this lab, you will demonstrate the enzyme-catalyzed hydrolysis of proteins, lipids, and carbohydrates. You will test for the presence of the original substrate (protein, lipid, or carbohydrate) and for the presence of its hydrolysis products amino acids, fatty acids, or (monosaccharides).

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| **Digestion:** process of breaking down food into nutrients to absorb | **Digestive enzymes:** specialized proteins synthesized in specialized cells, then released into digestive tract to accelerate extracellular breakdown of biological nutrients (proteins, carbs, lipids) |
| **Mechanical digestion:** physical manipulation of food (ex: chewing, mixing chyme, peristalsis) | **Chemical digestion:** breaking down food in a chemical reaction with the aid of digestive enzymes |

**General Digestion Vocabulary**

The absorption of nutrients from foods that are consumed first requires that those foods be broken down into small absorbable units by the process of **digestion**. Digestion in the human body includes both a **mechanical** phase (chewing, mixing of chyme, peristalsis) and a chemical phase. **Chemical digestion** occurs with the aid of special proteins known as digestive enzymes.

After being synthesized in specialized cells, digestive enzymes are released into the digestive tract to accelerate the extracellular breakdown of the biological nutrients (carbohydrates, proteins, and lipids) in the food and fluids consumed. The products of chemical digestion are absorbed into the bloodstream or lymphatic vessels, carried to the cells of the body, transported into these cells, and then used in **intracellular metabolism**. You are probably familiar with the various types of intracellular metabolism, such as aerobic respiration, glycolysis, lipolysis, protein synthesis, etc.

The extracellular digestion of nutrients occurs by a process known as **enzyme-catalyzed hydrolysis**. **Hydrolytic enzymes** or **hydrolases** are used to catalyze or speed up the chemical reaction where water is added to help break down larger food molecules into their monomers. Your textbook reviews the hydrolysis of the 3 major nutrient groups and demonstrates that:

Proteins + H20 + enzyme 🡪 amino acids +enzyme

Fats + H20 + enzyme 🡪 fatty acids + glycerol(or monoglyceride) +enzyme

Carbohydrates + H20 + enzyme 🡪 monosaccharides +enzyme

In the body, each of the above reactions can only occur if the proper hydrolytic enzyme is present and active. Notice that the enzymes are not used up or changed in the reaction; they are present on both the reactant (**substrate**) side and the **product** side of the equation. The specific enzymes for the digestion of carbohydrates, proteins, and lipids are discussed in the following sections. You may wish to review in your textbook how enzymes act as catalysts and which factors control their activity, such as pH, temperature, enzyme and substrate concentrations, and cofactors.

## **Protein Digestion Vocabulary**

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| **Pepsin**: enzyme produced by chief cells of stomach, optimal pH2, hydrolyzes proteins into peptides | **Aminopeptidase, dipeptidase**: enzymes produced in the brush border of small intestine, break down peptides into amino acids |
| **Peptides**: short strands of amino acids, formed from broken down proteins | **Biuret Test**: tests for the presence of proteins, positive test=blue to violet, partial digestion into polypeptides=pink |
| **Chyme**: partially digested food | **Albumin**: protein used in this lab |
| **Trypsin, chymotrypsin, and carboxypeptidase**: pancreatic enzymes released into small intestine, optimal pH9 | **Ninhydrin Test:** test for the presence of amino acids or peptides, positive test=colorless to blue-violet |

Protein digestion begins in the stomach with the enzyme **pepsin**. Pepsin is produced by the chief cells of the stomach and hydrolyzes proteins into smaller **peptides** (short strands of amino acids). Pepsin works best at a stomach pH of 2 and denatures when the **chyme** (partially digested food) moves into the small intestine where the pH is around 9. Hydrolysis of proteins into peptides is continued in the small intestine by the pancreatic enzymes **trypsin**, **chymotrypsin**, and **carboxypeptidase**. The peptides produced by these first four enzymes are further broken down into amino acids by **aminopeptidases** and **dipeptidases**, which are enzymes that are produced in the brush border of the small intestine. The amino acids are then absorbed into the capillaries of the small intestine and carried to the cells of the body for intracellular metabolism.

You will be observing the digestion of protein (**albumin**) by the enzyme **trypsin** according to the following hydrolysis reaction:

 Protein + trypsin + H2O 🡪 peptides + trypsin

 You can monitor the progress of the above reaction using two tests, the **biuret test** for the presence of protein and the **ninhydrin test** for the presence of amino acids. Before the reaction takes place, the biuret test should be positive and the ninhydrin test negative. After the reaction has gone to completion (all of the protein has been hydrolyzed to amino acids), the biuret test should be negative and the ninhydrin test should be positive. For the biuret test, the biuret reagent turns from blue to violet when positive for proteins. A pink color in the biuret test indicates partial digestion of the protein into polypeptides. For the ninhydrin test, a color change from colorless to blue-violet is positive for the presence of amino acids.

**Protein Digestion Process**

**Proteins** partially hydrolyze into **peptides** in the stomach (pH2) via **pepsin**

**Chyme** moves to small intestine (pH9) where **pepsin** is denatured

Pancreatic enzymes **trypsin, chymotrypsin, and carboxypeptidase** take over breakdown of **proteins** into **peptides** in small intestine

**Peptides** broken down into **amino acids** via **aminopeptidase** and **dipeptidase**

**Amino acids** absorbed by capillaries of small intestine

**Amino acids** carried to cells of body for intracellular metabolism

## **Carbohydrate Digestion Vocabulary**

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| **Starch**: the carbohydrate used in this lab | **Maltase**: enzyme which breaks down disaccharides to monosaccharides, produced in brush border of small intestine |
| **Disaccharide**: 2-sugar chain (eg: **maltose**) | **Iodine test**: tests for the presence of starch |
| **Monosaccharide**: 1-sugar chain (eg: **glucose**) | **Benedict’s test**: tests for the presence of maltose |
| **Amylase**: enzyme which breaks down starch into maltose (amylo- =starch; -ase =enzyme)**\*Salivary amylase**: amylase produced in the salivary glands and secreted into oral cavity**\*Pancreatic amylase**: amylase produced in the pancreas and secreted into the duodenum (sm. Int.) |  |

You will be observing the hydrolysis of carbohydrate (**starch**) by the enzyme **amylase**. Salivary amylase is produced in the salivary glands while pancreatic amylase is made in the pancreas. Both digest starch according to the following hydrolysis reaction:

Starch + amylase + H2O 🡪 maltose + amylase

Starch is the substrate for the enzyme amylase while maltose is the product of the hydrolysis reaction. Maltose is a **disaccharide** which is further broken down into two glucose units (**monosaccharides**) by the enzyme **maltase**. Maltase is found in the brush border of the small intestine. The resultant glucose is then absorbed into the capillaries of the small intestine and carried to the cells of the body for intracellular metabolism.

 You can monitor the progress of the above reaction using two tests, the **iodine test** for the presence of starch and the **Benedict's test** for the presence of maltose. Before the reaction takes place, the iodine test should be positive and the Benedict's test negative. After the reaction has gone to completion (all of the starch has been hydrolyzed), the iodine test should be negative and the Benedict's test positive.

**Expected results for the iodine test include:** Iodine + starch 🡪 blue-black color +++ positive

 Iodine + partially digested starch 🡪 purple-red color ++ partially positive

 Iodine + almost completely digested starch 🡪 brown-red-orange + slightly positive

 Iodine + completely digested starch 🡪 yellow color - negative

 Iodine + no starch 🡪 yellow color – negative

**Expected results for the Benedict's test include:** Benedict's solution + starch 🡪 blue color- negative

 Benedict's solution + partially digested starch 🡪 greenish-blue ++ slightly positive

 Benedict's solution + digested starch 🡪 yellow-orange ++ partially positive

 Benedict's solution + completely digested starch 🡪 red color (precipitate) +++ positive

**Carbohydrate Digestion Process**

**Carbs** partially hydrolyze into **disaccharides** in the mouth (pH7) via **salivary amylase**

**Chyme** moves to stomach (pH2) where **salivary amylase** is denatured (NO **CHEMICAL** DIGESTION OF CARBS IN STOMACH! ONLY ***MECHANICAL*** DIGESTION!)

**Chyme** continues to duodenum (pH9) where **pancreatic amylase** takes over breakdown of **carbs** into **Maltose**

**Maltose** broken down into **glucose** via **maltase**

**Glucose** absorbed by capillaries of small intestine (via Na+/Glucose linked transporters)

**Glucose** carried to cells of body for intracellular metabolism (glycolysis)

## **Lipid Digestion Vocabulary**

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| **Lipid:** a fat-like molecule, unable to dissolve in water | **Lipase**: enzyme produced in pancreas, digests lipids |
| **Micelles**: tiny oil droplets (which provide more surface area for easier digestion) | **Bile salts**: perform emulsification, produced in the liver, stored in gallbladder, and released into small intestine |
| **Emulsification**: breakdown of lipids into micelles | **Lacteals**: lymphatic capillaries |

Because lipids are not water soluble, they must first be emulsified before enzymes can efficiently break them down. In **emulsification**, **bile salts** break the large lipids into minute oil droplets. Bile salts are produced by the liver, stored in the gallbladder, and released into the small intestine when needed. The small oil droplets, called **micelles**, have more surface area available for the enzymes to act upon.

You will be observing the emulsification and digestion of lipids (olive oil) by bile salts (soap). Lipase is produced in the pancreas and digests lipids according to the following hydrolysis reaction:

Lipid (olive oil) + lipase + H2O 🡪 monoglycerides (or glycerol) + fatty acids + lipase

The olive oil is the substrate for the enzyme while the glycerol and fatty acids are the products of the hydrolysis reaction. The glycerol and fatty acids are absorbed into the epithelial cells of the small intestine and packaged into special lipoproteins known as **chylomicrons**. The chylomicrons are absorbed into the **lacteals** (lymphatic vessels) of the small intestine and carried to the liver for intracellular metabolism.

**Lipid Digestion Process**

**Lipids** undergo **emulsification** into **micelles** via **bile salts** in duodenum

**Micelles** are then broken down into **fatty acids and monoglycerides** (or glycerol) via **lipase**

**Fatty acids and glycerol** absorbed into epithelial cells of small intestine where they are synthesized into triglycerides

Triglycerides are coated in protein (for water solubility), forming chylomicrons

Chylomicrons transported into **lacteals**, then into bloodstream

Chylomicrons carried into cells of body

## **Chemical digestion reactions in today’s lab:**

**Bio Nutrient Hydrolase Monomers** (with enzyme still intact)

 Protein + Trypsin (+H₂O) amino acids +trypsin

 Carbs + Amylase (+H₂O) monosaccharides +amylase

 Lipids + Lipase (+H₂O) fatty acids + glycerol + lipase

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| **Protein reaction**: when required substrates are placed in test tube, Biuret test will be positive, Ninhydrin test will be negative. After hydrolysis is complete, Biuret test will be negative, Ninhydrin test will be positive | **Carb reaction**: when required substrates are placed in test tube, iodine test will be positive, Benedict’s test will be negative. After hydrolysis is complete, iodine test will be negative, Benedict’s test will be positive |