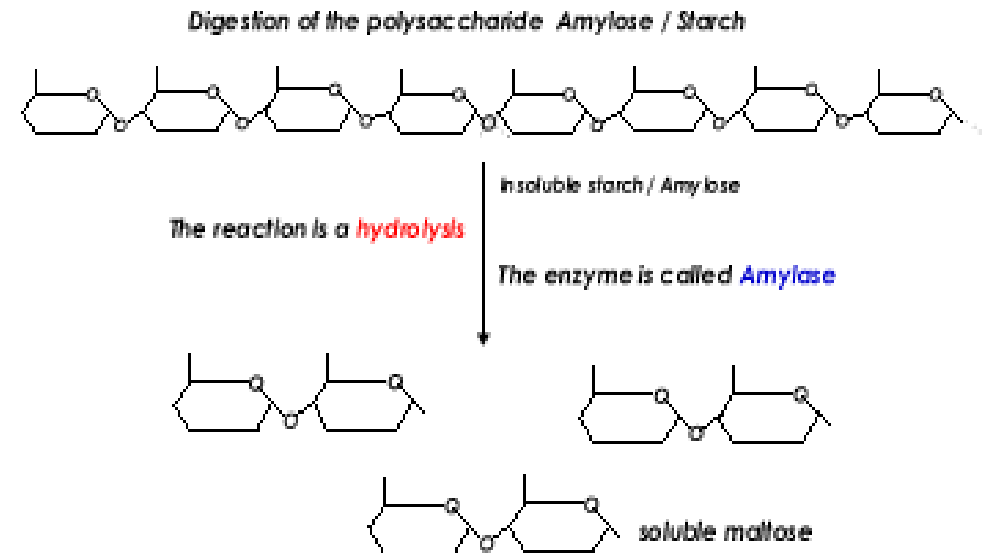


Determination of Plasma Amylase

-Amylase :

- Amylase is an enzyme that catalyze the breakdown of starch and glycogen by hydrolysis of internal **α -1,4-glycoside bonds** into smaller carbohydrate groups (maltose, oligosaccharides, glucose).
- It is produced in the **salivary glands, pancreas, liver, and fallopian tubes** and is **normally** excreted in **small** amounts in the urine.

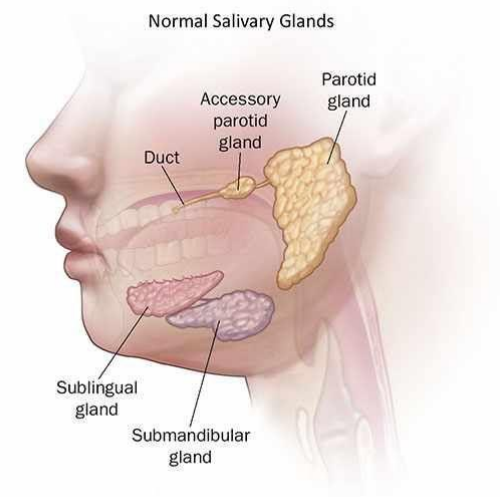
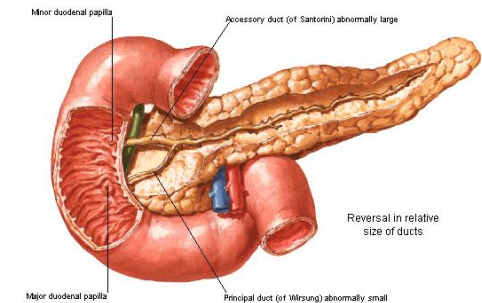


-Amylase main sources :

- Among healthy individuals, the **pancreas and the salivary glands** account for almost all serum amylase, 40-45% from the pancreas and 55-60% from the salivary glands.

- Electrophoresis shows that serum amylase is of **2 main types:**

1. **P**-type amylase from the **p**ancreas.
2. **S**-type amylase from the **s**alivary glands.

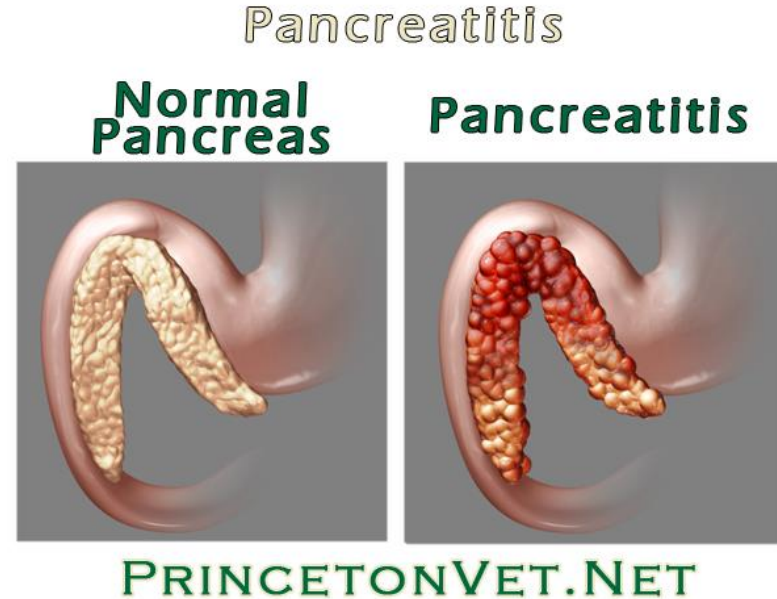


-Amylase in Serum and Urine :

- This test of **blood and urine** is most often **used to distinguish acute pancreatitis and other causes of abdominal pain** that require immediate surgery.
- If the pancreas or salivary glands are inflamed, much more of the enzyme enters the blood and, consequently, more amylase is excreted in the urine.
- Serum and urine amylase measurement in addition to other laboratory tests, **amylase clearance**, **amylase isozyme** , and measurement of **serum lipase levels**, increase the specificity of amylase measurement in the diagnosis of acute pancreatitis.

Pancreas Function Test:

- Blood levels of the pancreatic enzymes **amylase** and **lipase** are measured.
- This test used to diagnose and monitor treatment of **acute pancreatitis**.



-Range of expected values of amylase:

- **Serum** : 16-108 U/L
- **Urine**: 0 - 14 U/hour

-Increased plasma amylase (hyperamylasemia):

- Salivary gland inflammation.
- Pancreatitis.
- Pancreatic cancer.
- Obstruction of pancreatic duct.

-Decreased plasma amylase:

- Pancreatic insufficiency.
- Severe liver disease

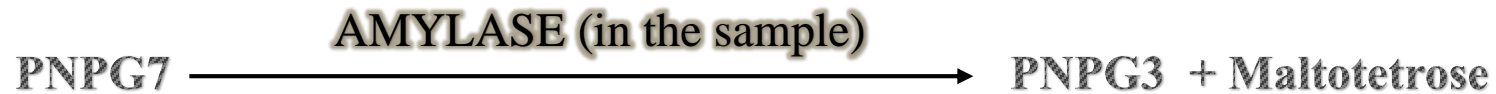
Practical Part

-Objective:

- To estimate the concentration of amylase in serum.

-Principle (of the used kit) :

1- **Amylase** hydrolyzed p-nitrophenyl D-maltoheptoside (**PNPG7**) to P-nitrophenylmaltotriose (**PNPG3**) and **maltotetrose**:



2- **Glucoamylase** hydrolyzes **PNPG3** to P-nitrophenylglycosie (**PNPG1**) and **glucose**:



3-Then **PNPG1** is hydrolyzed by **glycosidase** to **glucose** and **P-nitrophenol** which produce a **yellow color** which absorb at 405nm, the rate of **increase** in Ab is measured at 405 nm and is proportional to the amylase activity in the sample:



-Method:

CHEMICALS	SAMPLE
Amylase substrate	1.0 ml
Pre-warm at 37C for 5 minutes and add:	
Serum	0.025 ml

1. Mix and incubate at 37°C for **90 seconds** and read the absorbance at **405 nm** against distilled water.
2. Continue readings every **30 seconds for 2 minutes** and determine **$\Delta A/\text{min}$** .

-Results:

Time (Seconds)	Absorbance at 405 nm
0	
30	
60	
90	
120	

-Calculations:

-Amylase Activity in TEST (U/L) = $\Delta A/\text{min}$ x 4824

$$\Delta A/\text{min} = (\Delta A_1 + \Delta A_2) \div 2$$

$$\rightarrow \Delta A_1 = (A_{60s} - A_{30s}) + (A_{30s} - A_{0s})$$

$$\rightarrow \Delta A_2 = (A_{120s} - A_{90s}) + (A_{90s} - A_{60s})$$

-Homework:

- Mention other biochemical markers of acute pancreatitis?

-References:

- Fischbach FT, Dunning MB. A Manual of Laboratory and Diagnostic Tests. Lippincott Williams & Wilkins, 2009 .p. 419-420.
- BCH472 practical note.