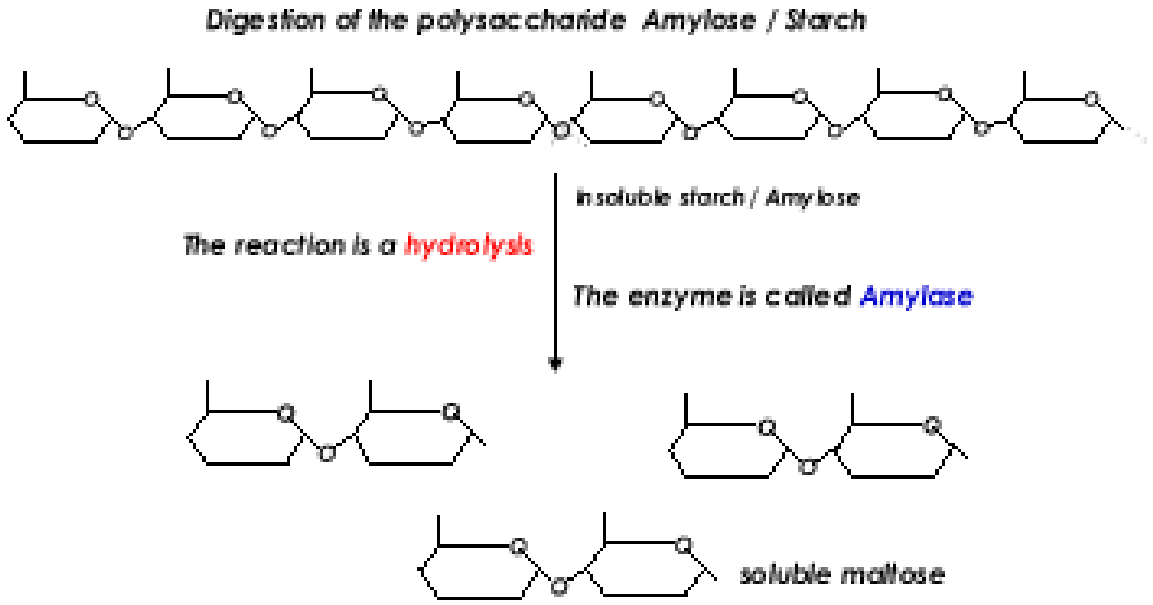
Determination of Plasma Amylase

-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.

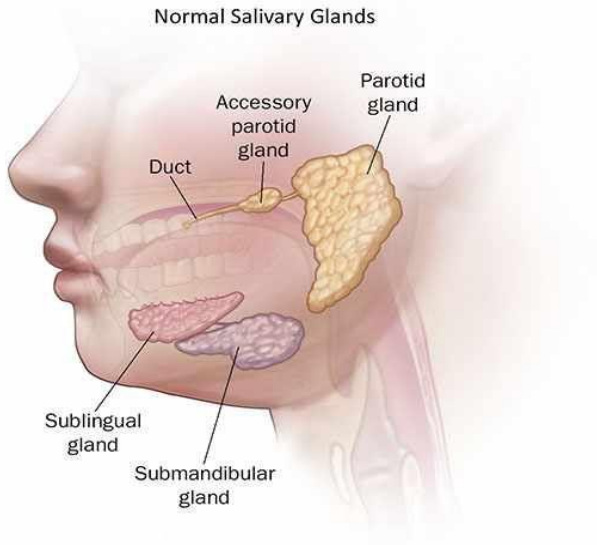


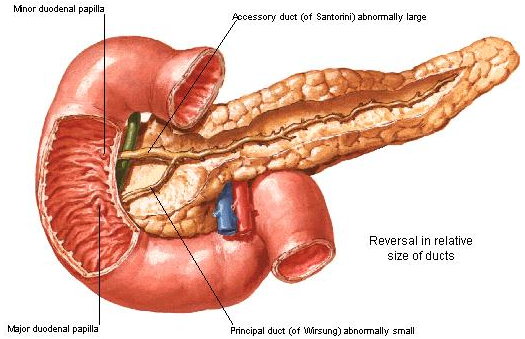
-Amylase main sources :

- 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 pancreas.
2. S-type amylase from the salivary glands.





-Amylase in Serum and Urine :

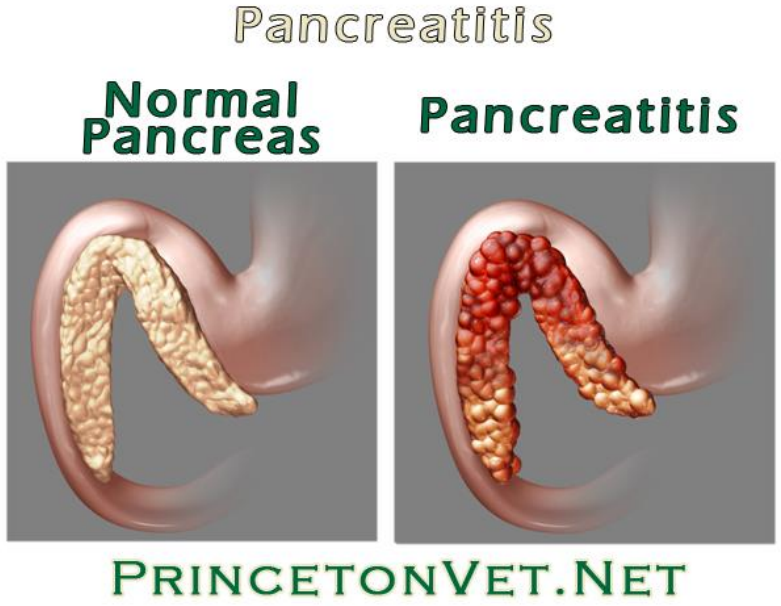
- This test of blood and urine is most often **used to distinguish acute pancreatitis and other causes of abdominal pain** that require immediate surgery.

- 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 specify 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.**
- **lipase** test has become a much more **sensitive and specific biomarker** in diagnosing acute pancreatitis.



-Range of expected values of amylase:

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

-Increased plasma amylase (hyperamylasaemia):

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

-Decreased plasma amylase:

- Pancreatic insufficiency.
- Severe liver disease.

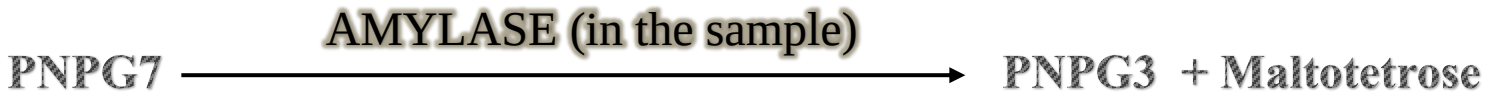
Practical Part

-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 **PNG3** 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:

-Materials:

Amylase (color/kinetic) kit (UDI).

-Method:

CHEMICALS	SAMPLE
AMYLASE SUBSTRATE	1.0 ml
Pre-warm at 37^{o}C for 5 minutes and add:	
Sample1	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 **ΔA/min.**

-Results:

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

-Calculations:

-Amylase Activity in TEST (U/L)= ΔA/min x 4824

$$\Delta\ A/Min = (\Delta A_1 + \Delta A_2) \div 2$$

➔ **Δ A1=** (A60s – A30s) **+** (A30s – A0s)

➔ **Δ A2=** (A120s – A90s) **+** (A90s – A60s)

-References:

- Fischbach FT, Dunning MB. A Manual of Laboratory and Diagnostic Tests. Lippincott Williams & Wilkins, 2009 .p. 419-420.
- Ismail OZ, Bhayana V. Lipase or amylase for the diagnosis of acute pancreatitis? Clin Biochem. 2017 Dec;50(18):1275-1280. doi: 10.1016/j.clinbiochem.2017.07.003. Epub 2017 Jul 16. PMID: 28720341.
- BCH472 practical note.