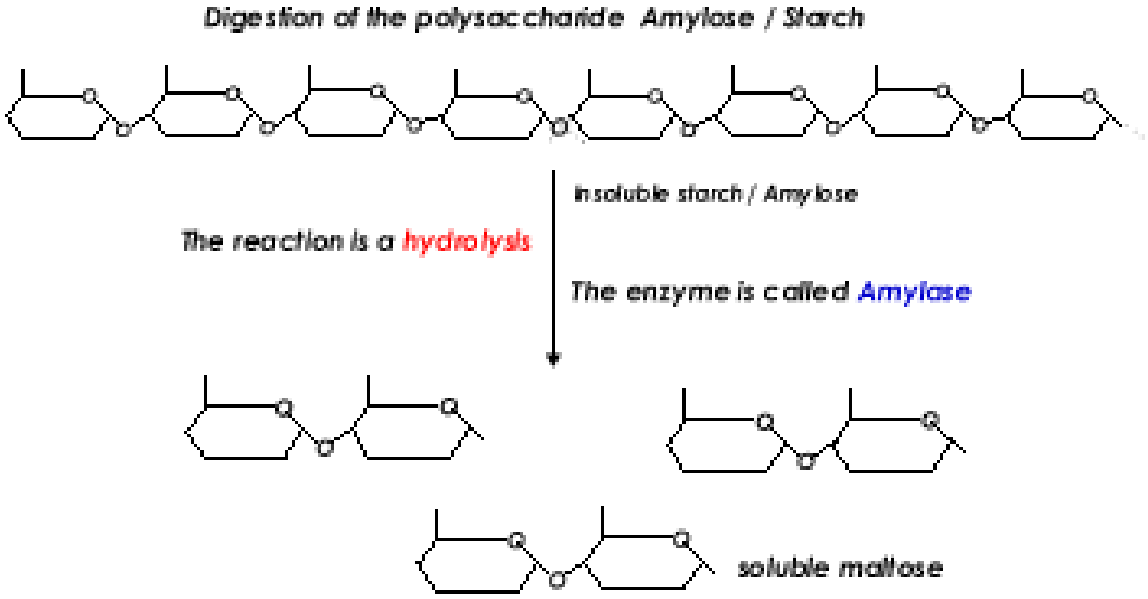
Determination of plasma amylase

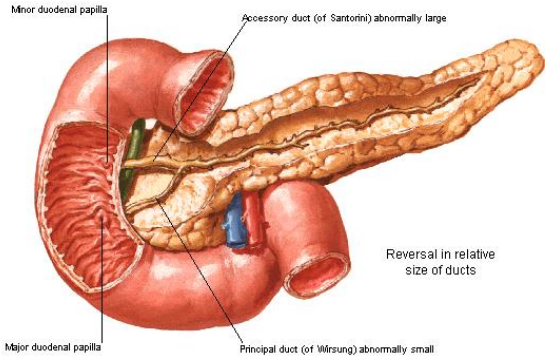
Amylase:

- Amylase is an enzyme that catalyze the breakdown of starch and glycogen 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.
- 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.



- 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.
- It may also detect some digestive tract problems.
- 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.



-RANGE OF EXPECTED VALUES of amylase:

- Serum : 16-108 U/L
- Urine: 0 - 14 U/Hour

-Increased plasma amylase:

- salivary gland inflammation.
- Pancreatitis.
- pancreatic cancer.

-Decreased plasma amylase:

- Pancreatic insufficiency.

Practical Part

-Objective:

- To estimate the concentration of amylase in serum.

- Amylase kit:

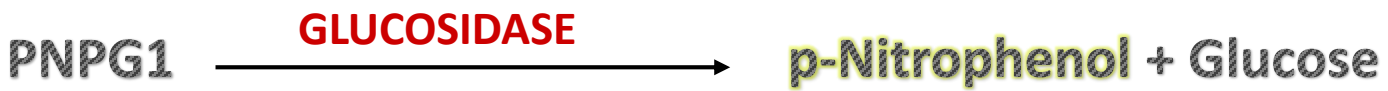
- P-Nitrophenyl D-Maltoheptoside
- Glucosidase
- Glucoamylase
- Sodium Chloride 50 mM,
- Calcium Chloride
- Buffer , pH 6.9 + 0.01

-Principle:

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 405nm and is proportional to the amylase activity in the sample:



-Method:

CHEMICALS	SAMPLE 1
AMYLASE SUBSTRATE	1.0 ml
Pre-warm at 37oC 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

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

$$\Delta \text{ A1} = (\text{ A60s} - \text{A30s}) + (\text{ A30s} - \text{A0s})$$

$$\Delta \text{ A2} = (\text{ A120s} - \text{A90s}) + (\text{ A90s} - \text{A60s})$$