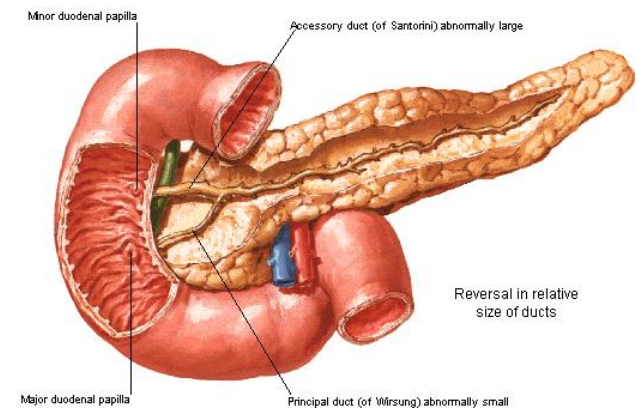


Determination of plasma amylase

BCH 472

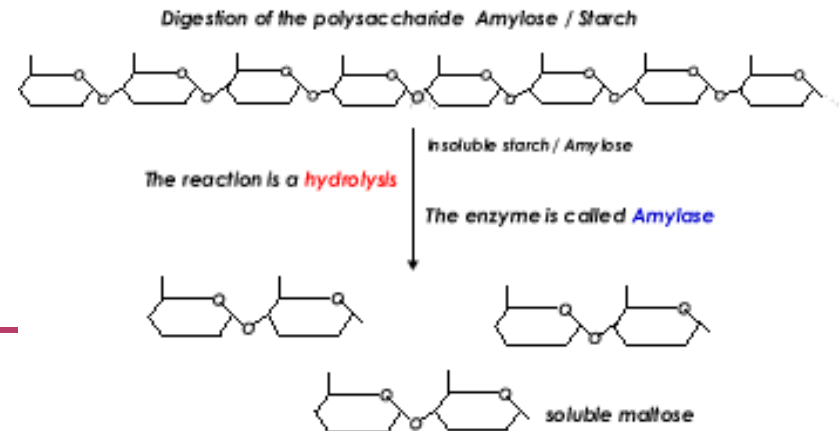


Amylase:

Amylase is an enzyme that catalyze the **breakdown of starch and glycogen** into smaller carbohydrate groups (maltose, oligosaccharides, glucose)

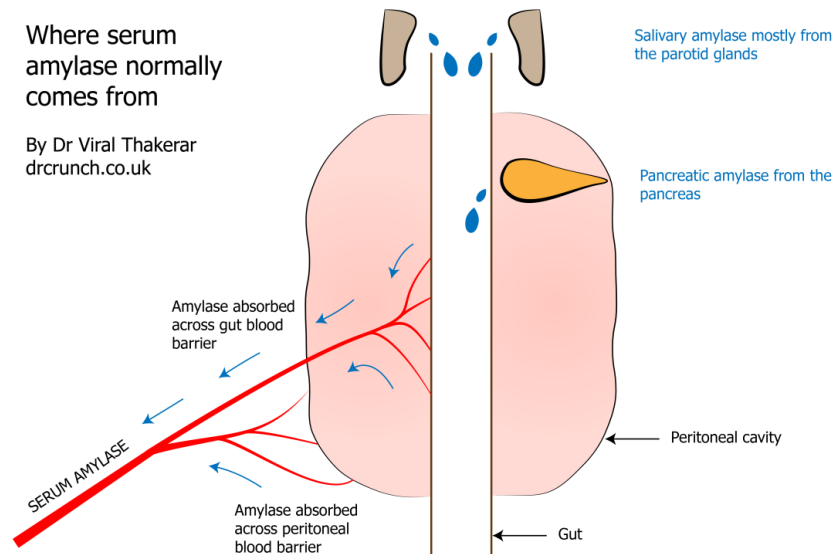
It is produced mainly in the **salivary glands** and **pancreas**, and is normally excreted in small amounts in the urine.

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.



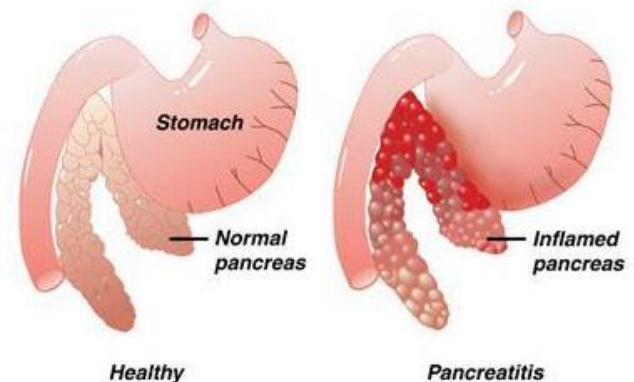
Amylase:

- When the pancreas is diseased or inflamed, amylase releases into the blood.
- A test can be done to measure the level of this enzyme in a blood.



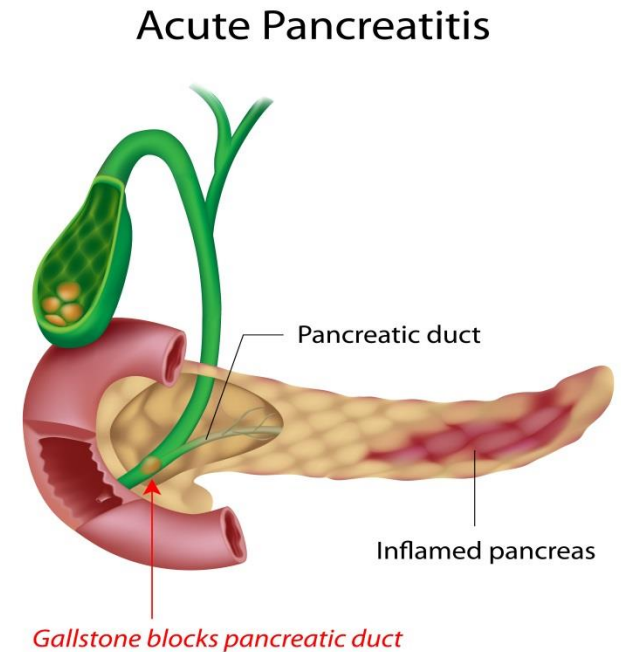
Determination of amylase level:

- **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 specificity of amylase measurement in the **diagnosis of acute pancreatitis**.



Amylase in Serum and urine :

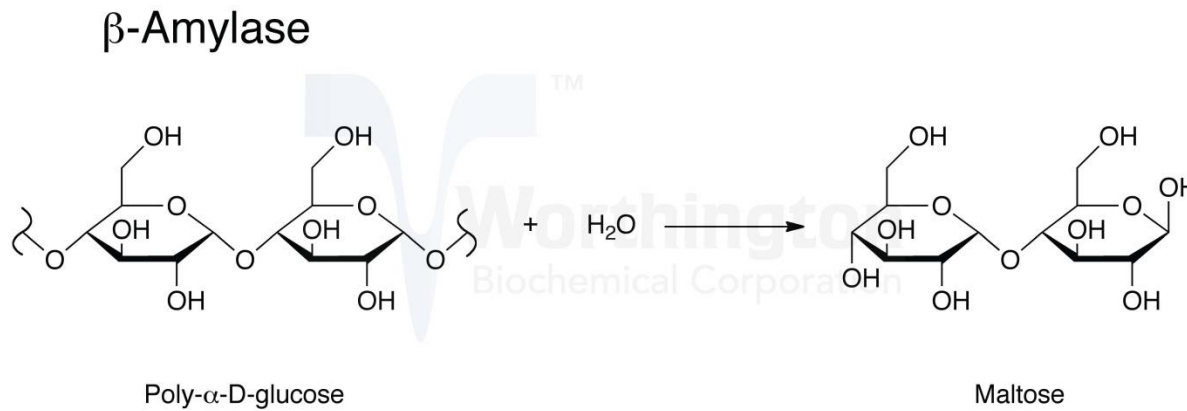
- **Increased blood and urine amylase levels may occur due to:**
 - **Acute** pancreatitis(a sudden inflammation of the pancreas).
 - Obstruction of the pancreatic duct.
 - Infection of the salivary glands or a blockage.
-
- **Decreased level :**
 - Damage to the pancreas.



Practical Part

Objective:

To estimate the concentration of amylase in serum

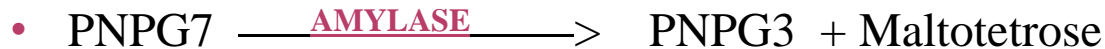


Amylase Kit

- **Amylase kit:**
 - P-Nitrophenyl D-Maltooligoside
 - Glucosidase
 - Glucoamylase
 - Sodium Chloride 50 mM,
 - Calcium Chloride
 - Buffer , pH 6.9 ± 0.01
-

Principle:

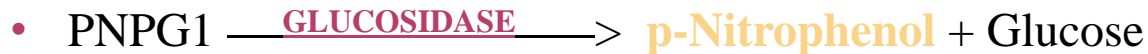
1- **Amylase** hydrolyzed p-nitrophenyl D-maltoheptoside (**PNPG7**) to P-nitrophenylmaltotriose (**PNPG3**) and maltotetrose .



2- **Glucoamylase** hydrolyzes **PNPG3** to P-nitrophenylglycose (**PNPG1**) and glucose .



3-Then **PNPG1** is hydrolyzed by **glycosidase** to glucose and **P-nitrophenol** which produce a **yellow** color.



Method:

CHEMICALS	SAMPLE 1
AMYLASE SUBSTRATE (kit)	1.0 ml
Pre-warm at 37°C for 5 minutes and add:	
Sample (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 **$\Delta A/\text{Min}$** .
- The rate of increase in Ab is measured at 405nm and is **proportional** to the amylase activity in the sample.
-

Results:

Seconds	Absorbance at 405 nm
0	
30	
60	
90	
120	

CALCULATIONS:

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

$$\Delta A/\text{Min} = (\Delta A1 + \Delta A2) \div 2$$

$$\Delta A1 = (A_{60\text{ s}} - A_{30\text{ s}}) + (A_{30\text{ s}} - A_{0\text{ s}})$$

$$\Delta A2 = (A_{120\text{ s}} - A_{90\text{ s}}) + (A_{90\text{ s}} - A_{60\text{ s}})$$

RANGE OF EXPECTED VALUES of amylase:

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

Discussion :

Comment on the amylase concentration in the sample.
