جامعة الملك سعود
King Saud University

DETERMINATION OF PLASMA AMYLASE

BCH 472 [Practical]

Amylase

- Amylase is an enzyme that catalyzes 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.

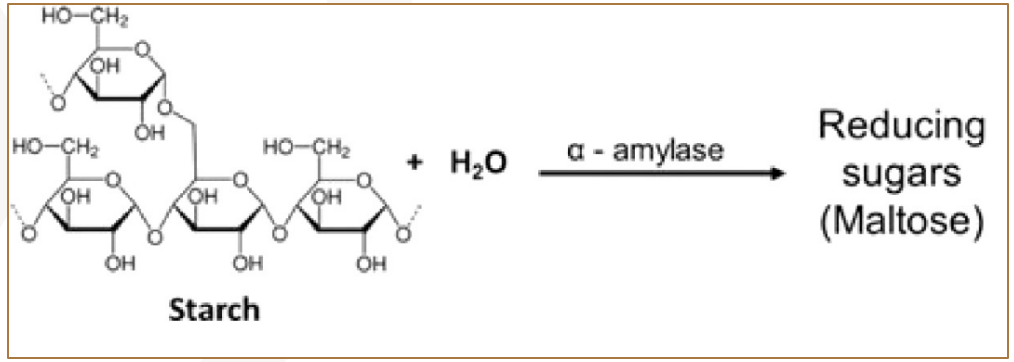


Figure 1. Chemical reaction of amylase

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

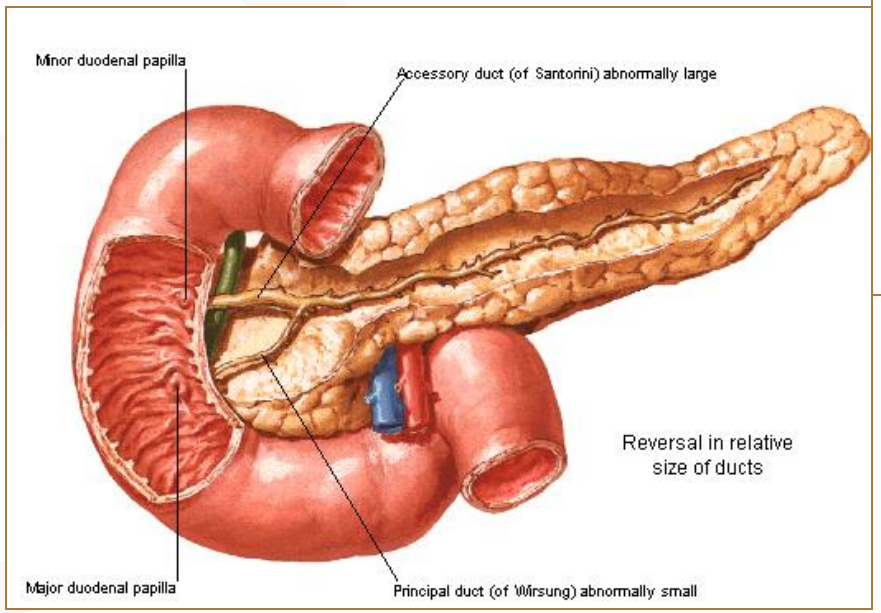


Figure 2. The anatomy of the pancreas

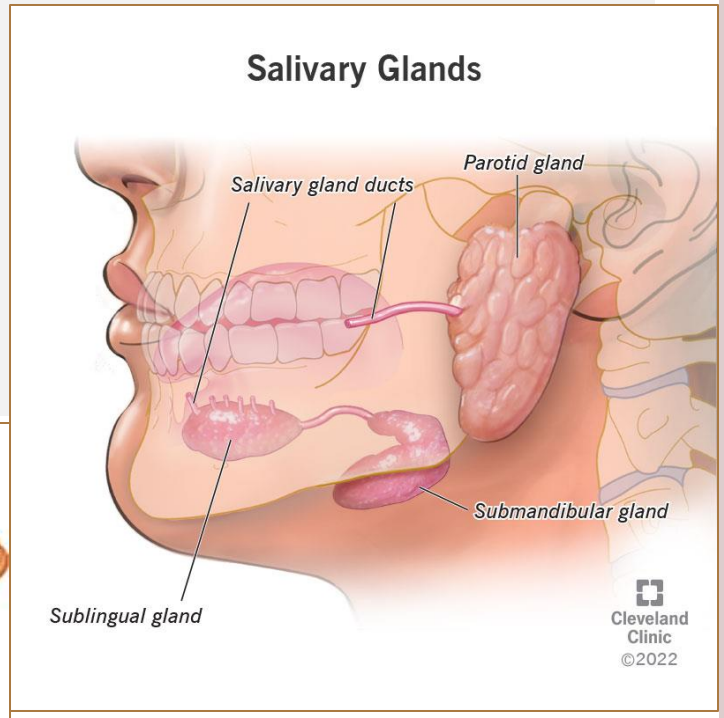


Figure 3. The locations of salivary gland.
Source: my.clevelandclinic.org

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.

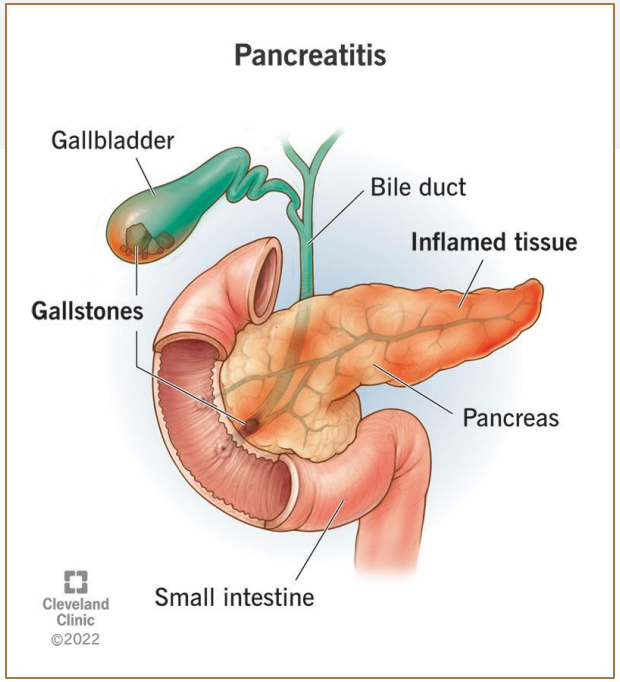


Figure 4. Pathology of acute pancreatitis. *Source: my.clevelandclinic.org*

Range of expected values of amylase:

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

Increased plasma amylase **(hyperamylasaemia):**

1. Salivary gland inflammation
2. Acute pancreatitis
3. Pancreatic cancer
4. Obstruction of pancreatic duct

Decreased plasma amylase:

1. Pancreatic insufficiency
2. Liver disease
3. Kidney disease
4. Cystic fibrosis
5. Pregnancy

Practical Part

Objective:

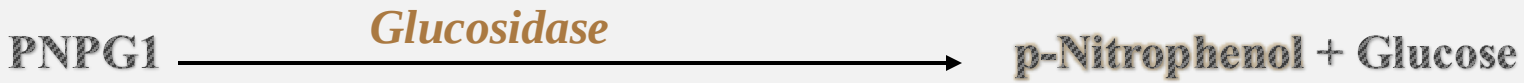
To estimate the concentration of amylase in serum.

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).

2) Applications → 2) Simple Kinetics → wave-length (405 nm) → 1) Seconds → Duration (120 sec = 2 min) → Intervals (30 sec) → Print Data Table (off) → Press start (2 times)

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

ΔA/Min = (ΔA1+ ΔA2) ÷ 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.
- Amylase Test: MedlinePlus Medical Test. (2025, Mar 3). Retrieved from https://medlineplus.gov/lab-tests/amylase-test/
- BCH472 practical note.