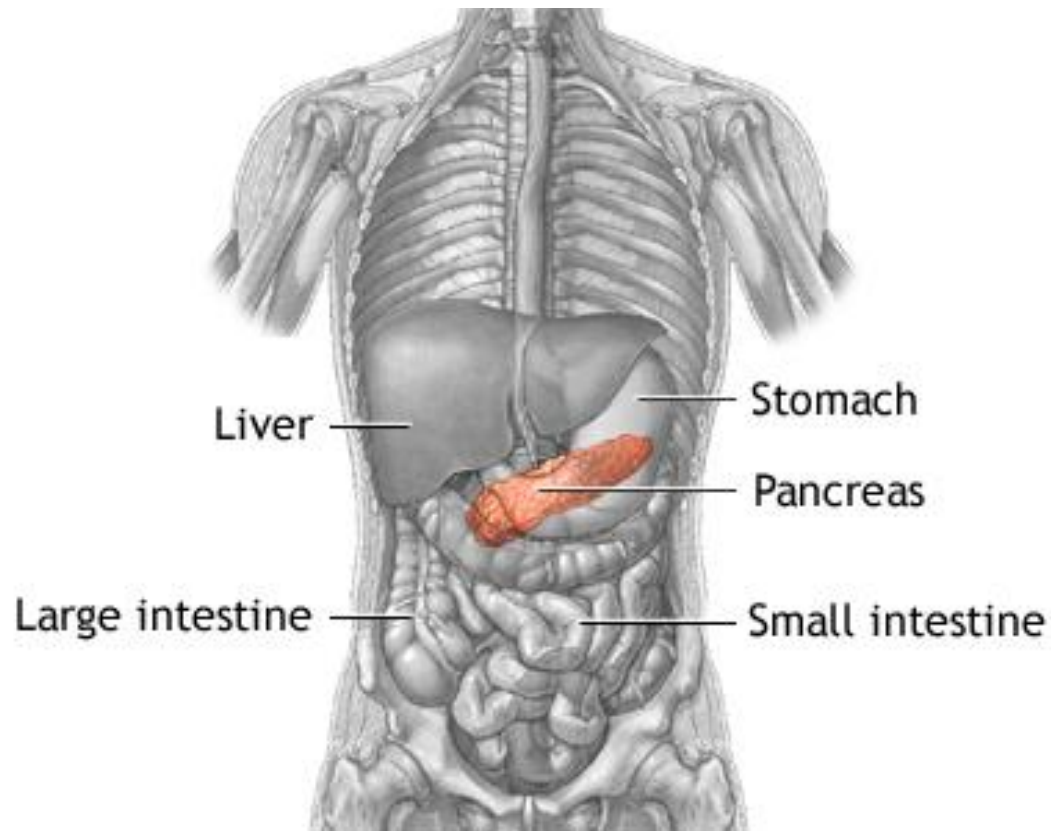
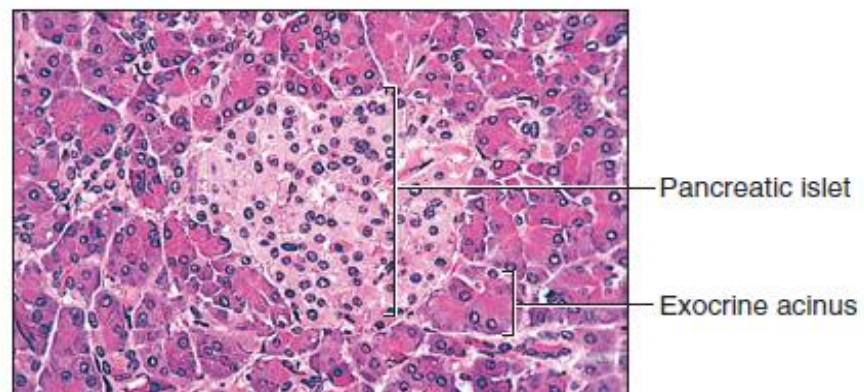
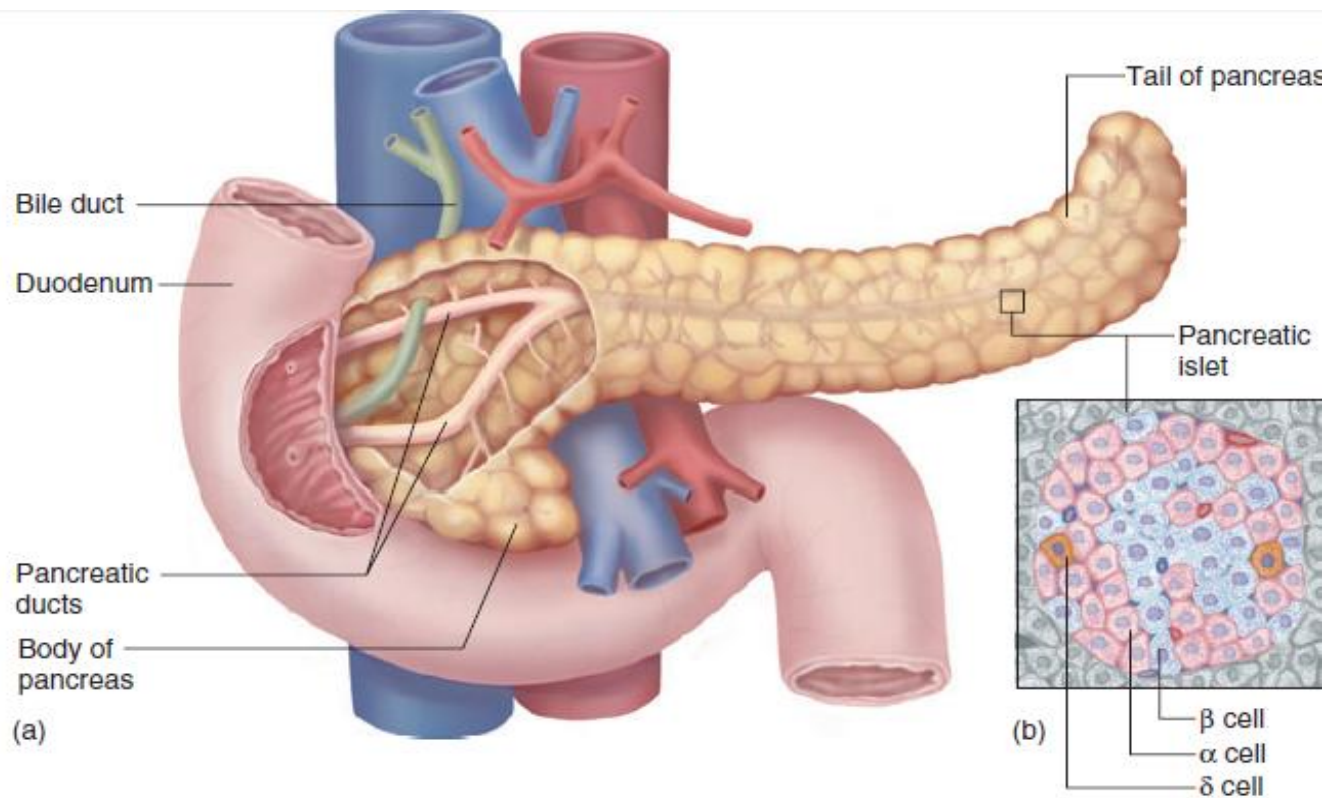


Determination of amylase in serum



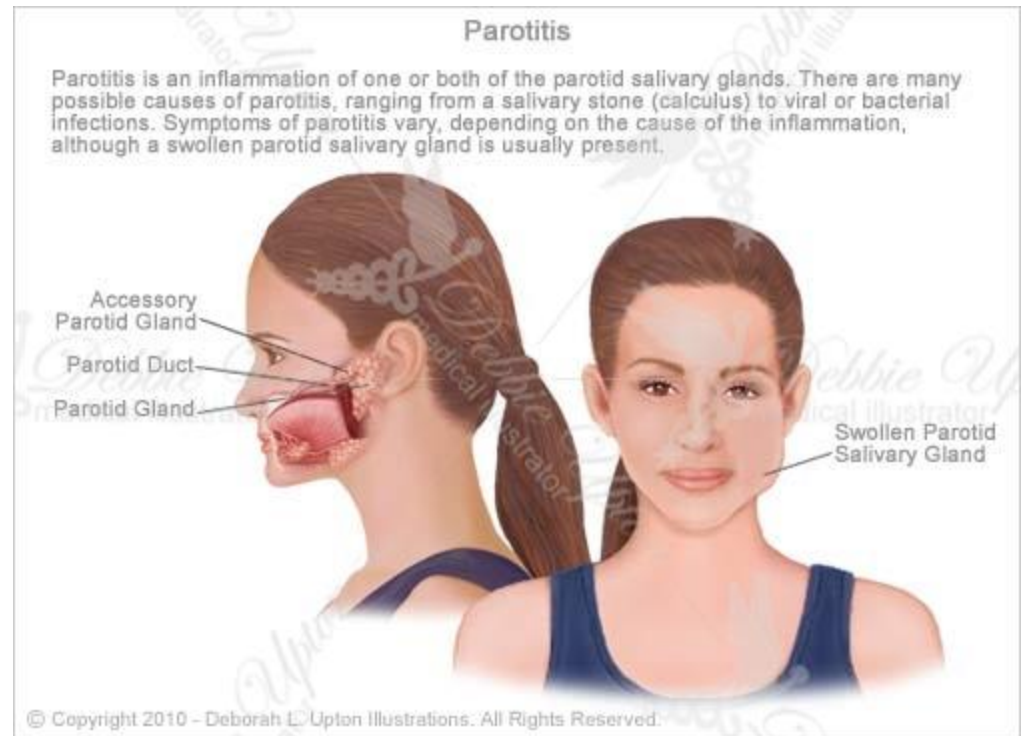


Parts of the Pancreas gland

- Amylase is an enzyme that helps digest carbohydrates. It is produced in the pancreas and the glands that make saliva. 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.
- Amylase may also be measured with a urine test.
- Amylase in serum arise mainly from the pancreas (P-amylase) and the salivary gland (S-amylase). Serum P- amylase activity is a more sensitive and more specific test than total amylase for the detection of acute pancreatitis.

- Serum amylase activity greater than 10 times the normal value are virtually diagnostic of acute pancreatitis. Maximum value of more than five times the upper reference limit are found in about 50% of cases, but are not pathognomonic of acute pancreatitis, since similar high values sometimes occur in the afferent loop syndrome, mesenteric infection and acute biliary tract disease, as well as in acute parotitis.

parotitis



How to Prepare for the Test

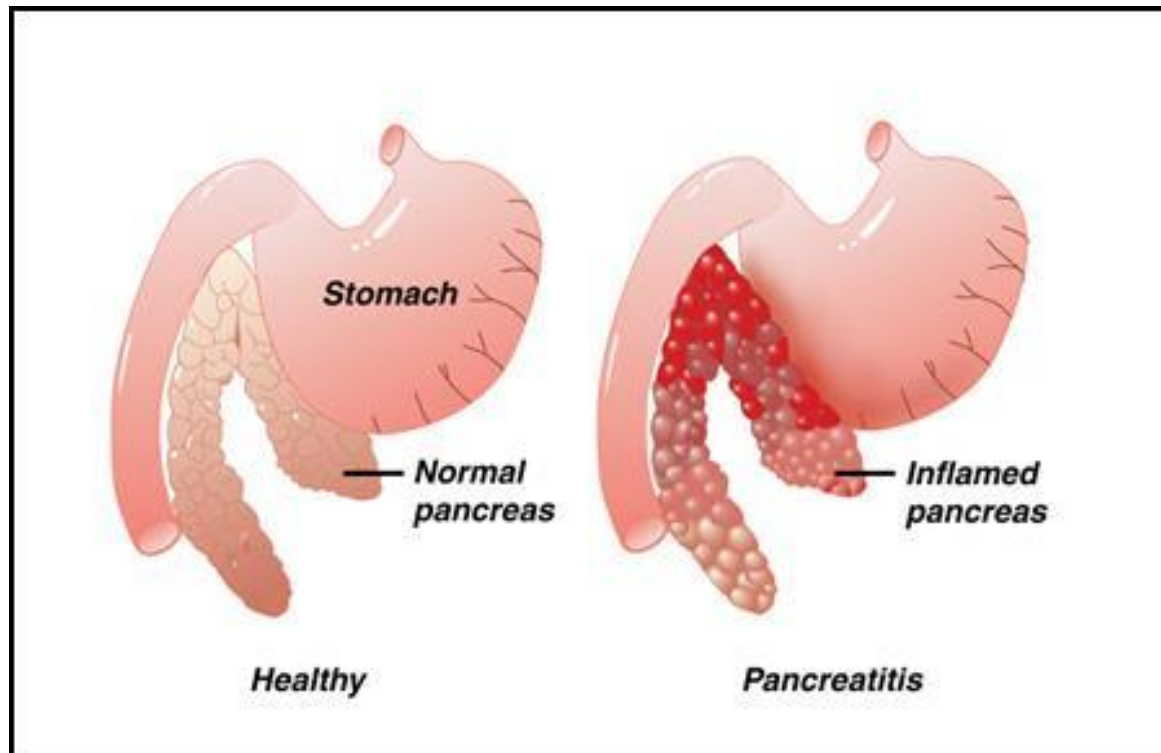
- No special preparation is needed. However, alcohol should be avoided before the test..
- Drugs that can increase amylase measurements include:
 - Asparaginase
 - Aspirin
 - Birth control pills
 - Cholinergic medications
 - Ethacrynic acid
 - Thiazide diuretics

Why the Test is Performed

- This test is most often used to diagnose or monitor acute pancreatitis. It may also detect some digestive tract problems.
- The test may also be done for
- Chronic pancreatitis
- Pancreatic pseudocyst

Chronic pancreatitis

Chronic pancreatitis is inflammation of the pancreas that does not heal or improve, gets worse over time, and leads to permanent damage.



Symptoms of Chronic pancreatitis

Abdominal pain

- Greatest in the upper abdomen
- May last from hours to days
- Eventually may be continuous
- May get worse from eating or drinking
- May get worse from drinking alcohol
- May also be felt in the back

Digestive problems

- Chronic weight loss, even when eating habits and amounts are normal
- Diarrhea, nausea, and vomiting
- Fatty or oily stools

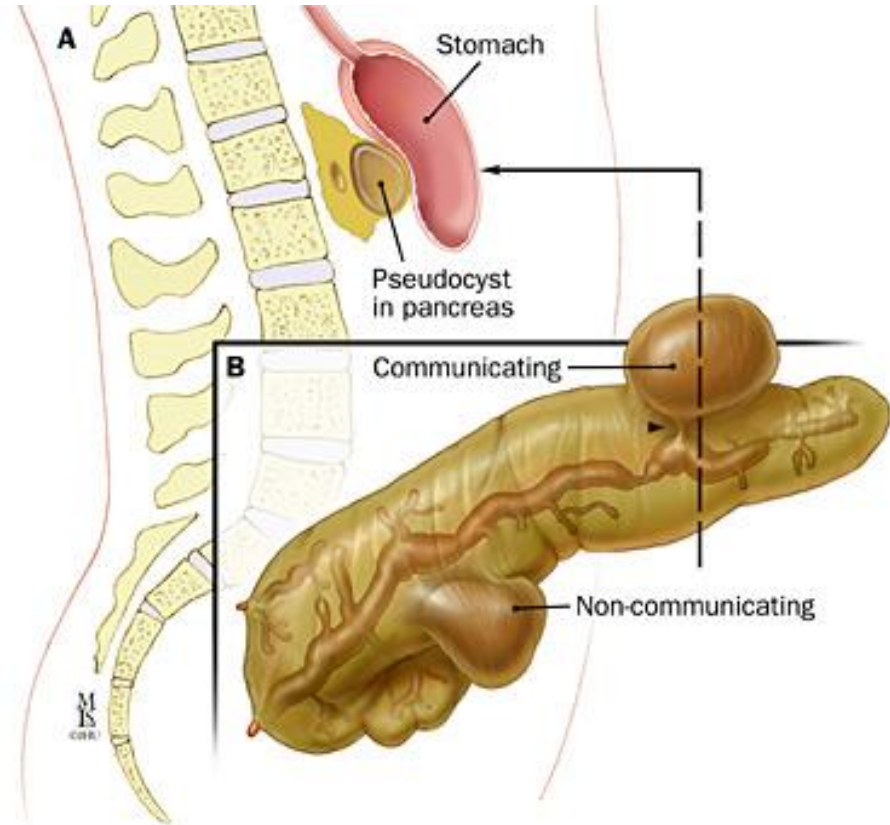
The symptoms may become more frequent as the condition gets worse. The symptoms may mimic pancreatic cancer. Sitting up and leaning forward may sometimes relieve the abdominal pain of pancreatitis.

Treatment of Chronic pancreatitis

- Pain medicines
- Fluids given through a vein (IV) to replace lost fluids and maintain your blood pressure to replace lost fluids and maintain your blood pressure.
- Stopping food or fluid by mouth to limit the activity of the pancreas, and then slowly starting an oral diet
- Eating a low-fat diet
- Eating small, frequent meals (this helps reduce digestive symptoms)
- Getting enough vitamins and calcium in the diet, or as extra supplements
- Limiting caffeine
- The doctor may prescribe pancreatic enzymes, which you must take with every meal. The enzymes will help you digest food better and gain weight

Pancreatic pseudocyst

- A pancreatic pseudocyst is a fluid-filled sac in the abdomen, which may also contain tissue from the pancreas, pancreatic enzymes, and blood



Symptoms and Treatment of Pancreatic pseudocyst

- Symptoms can occur within days to months after an attack of pancreatitis, and include:
- Bloating of the abdomen
- Constant pain or deep ache in the abdomen, which may also be felt in the back
- Difficulty eating and digesting food

Treatment depends on the size of the pseudocyst and whether it is causing symptoms. Many pseudocysts go away on their own. Those that remain for more than 6 weeks and are larger than 5 cm in diameter often need treatment.

- Possible treatments include:
- Drainage through the skin using a needle, usually guided by a CT scan
- Surgical

RANGE OF EXPECTED VALUES

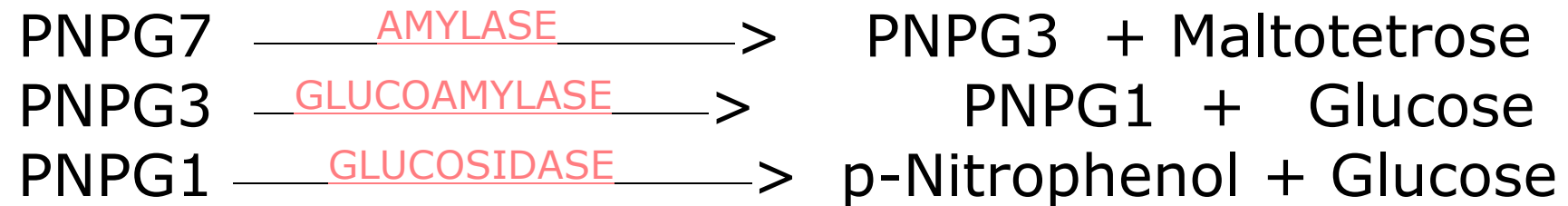
Serum : 16-108 U/L

Urine: 0 - 14 U/Hour

low values in Serum is may due liver diseases and pancreatic insufficiency

Principle:

Amylase hydrolyzed p-nitrophenyl D-maltoheptoside (PNPG7) to P-nitrophenylmaltotriose (PNPG3) and maltotetrose . Glucoamylase hydrolyzes PNG3 to P-nitrophenylglycosie (PNPG1) and glucose . Then PNPG1 is hydrolyzed by glycosidase to glucose and P-nitrophenol which produce a yellow color . The rate of increase in Ab is measured at 405nm and is proportional to the amylase activity in the sample.



MATERIALS

CHEMICALS:

- **AMYLASE SUBSTRATE (PNPG7):**

p-Nitrophenyl D-Maltoheptoside , Glucosidase ,
Glucoamylase , Sodium Chloride 50 mM, Calcium
Chloride and Buffer , pH 6.9 ± 0.01 .

- **2 SERUM SAMPLES**

PNPG7	— <u>AMYLASE</u> —	>	PNPG3 + Maltotetrose
PNPG3	— <u>GLUCOAMYLASE</u> —	>	PNPG1 + Glucose
PNPG1	— <u>GLUCOSIDASE</u> —	>	p-Nitrophenol + Glucose

MATERIALS

GLASSWARE:

- **1.** Accurate pipetting devices.
- **2.** Test tubes / rack
- **3.** Timing device.
- **4.** Heating block /bath (37 °C).
- **5.** Spectrophotometer capable of reading at 405 nm (400-420 nm). The cuvette compartment should be temperature controlled to maintain temperature (37 °C) during the assay.

METHOD

CHEMICALS	SAMPLE 1	SAMPLE 2
AMYLASE SUBSTRATE (PNPG7):	1.0 ml	1.0 ml
Pre-warm at 37oC for 5 minutes and add:		
Sample1	0.025 ml	
Sample2		0.025 ml

Mix and incubate at 37oC for 90 seconds and read the absorbance at 405 nm against distilled water.

Continue readings every 30 seconds for 2 minutes and determine $\Delta A/\text{Min}$.

RESULTS

ABSORBANCE AT 405	SAMPLE 1	SAMPLE 2
A 0 S		
A 30 S		
A 60 S		
A 90 S		
A 120 S		



CALCULATIONS

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

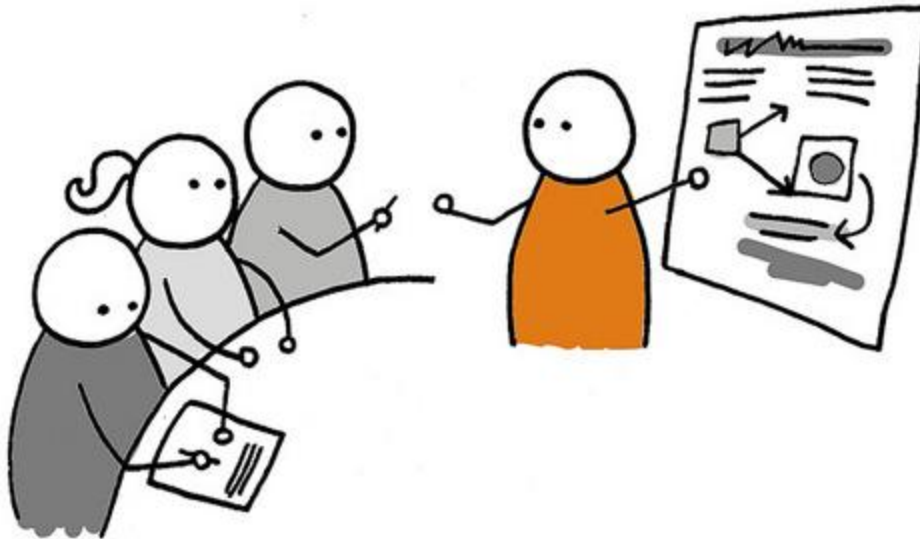
$$\Delta A/\text{Min} = (\Delta A1 + \Delta A2) \div 2$$

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

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

DISCUSSION

Comment on the concentration of amylase in sample 1 and sample 2 .



QUESTIONS

- *What Is the Difference between Salivary Amylase and Pancreatic Amylase?*
- *What are the enzymes secreted by pancreas?*
- *Why doctor prescribe enzymes for Chronic pancreatitis patients?*



REFERENCES

- **UDI AMYLASE (COLOR/KINETIC) KIT**
- <http://www.nlm.nih.gov/medlineplus/ency/article/003464.htm>
- **Lecture Notes: Clinical Biochemistry** Geoffrey Beckett, Simon W. Walker, Peter Rae
- <http://heartsfortheclasse.blogspot.com/2012/07/bio-202-endocrine.html>

