

The exploration of exocrine pancreas

The pancreas is a large compound gland with most of its internal structure similar to that of the salivary glands. The pancreatic digestive enzymes are secreted by pancreatic acini and large volumes of sodium bicarbonate solution are secreted by the small ductules and larger ducts leading from the acini. The combined product of enzymes and sodium bicarbonate then flows through a long pancreatic duct that normally joins the hepatic duct immediately before it empties into the duodenum through the papilla of Vater, surrounded by the sphincter of Oddi.

Exocrine secretion of pancreas is represented by the pancreatic juice with a flow of 1500-3000 ml/daily. It is iso-osmotic, alkaline (pH>8) and contains around 20 enzymes. Pancreatic juice assures the optimal pH and enzymes for the intestinal digestion. Pancreatic juice is secreted most abundantly in response to the presence of chyme in the upper portions of the small intestine.

Pancreatic secretion contains multiple enzymes for digesting all of the three major types of food: proteins, carbohydrates, and fats. It also contains large quantities of bicarbonate ions, which play an important role in neutralizing the acidity of the chyme emptied from the stomach into the duodenum. Most pancreatic diseases remain clinically silent until approximately 90% of the gland is destroyed. Earlier recognition of pancreatic dysfunction may improve the management of the patient's disease and his or her quality of life.

1. Functional investigation of exocrine pancreas

1. Collecting and exploration methods of pancreatic juice

Tube tests require an oroduodenal tube that aspirates pancreatic secretion from the duodenum near the papilla of Vater so that the response to stimulating factors can be measured. The stimulants used are secretin, cholecystokinin. Duodenal juice, uncontaminated with gastric juice or duodenal secretion, is collected using an Einhorn tube, equipped with balloons (filled with air) that prevent contamination with gastric juice and jejunal reflux. Contamination with salivary secretion is avoided, by placing a pipe in oral cavity that allows saliva to drain in a tray. During collection, gastric secretion is continuously taken off, in order to prevent the negative effect on the sample, which is bicarbonate neutralization and enzyme degradation. Biliary secretion should be evacuated before pancreatic juice is collected. For this, MgSO₄ 33% is administered through a tube that reach the duodenum mucosa. After all biliary secretion is gathered, a stimulatory substances for pancreatic juice is administered. The stimulation of the pancreas can be accomplished directly by intravenous injection of secretin alone or in combination with cholecystokinin. The combination allows the assessment not only of bicarbonate secretion (with secretin) but also of enzyme secretion, mainly trypsin.

Other stimulants for pancreatic secretion:

1. Pancreosimine - 100 u in a single dose; collect the pancreatic juice for 20 min;
2. Secretin(0,5 u/kgc) + pancreozimine(100 u), administrated in perfusion for 1 hour;
3. Secretin(0,5 u/kgc) + caerulina (0,1µg/kgc);
4. Secretin (0, 5 u/kgc) + Bombesin (15mg/kgc).

The collection period varies from 45 to 120 minutes.

A more physiological stimulation test of the pancreas by a meal is called the Lundh test. It assesses the response of the pancreas to endogenous secretin and pancreozymin (or CCK) released in response to a test meal of protein, fat and carbohydrates. The concentration of trypsin and the volume of secretion are measured in samples obtained in the duodenal aspirate. The Lundh meal is virtually always abnormal in pancreatic insufficiency. Unfortunately there is a borderline zone of abnormal values that are uninterpretable. In addition, many other factors influence the results of a Lundh meal, including small bowel

mucosal disease, rate of gastric emptying, and surgical interruption of gastro duodenal anatomy. Although this is a more physiological test, its sensitivity and specificity are lower (70-80%) than those of direct hormonal stimulation.

Quantitative and qualitative tests are made on the collected juice. In most situations, the secretions of the three types of enzymes are parallel, so is sufficient to measure only one enzyme, to evaluate the presence of the other enzymes in the pancreatic juice.

Parameter	Value
Volume of pancreatic juice (ml/kg)	2 – 7.5
Maximum concentration of bicarbonates (mEq/l)	90 -152
Maximum concentration of amylase (U/Kg)	6 -57
Maximum concentration of lipase (ml NaOH N/10)	35 -55
Concentration of trypsin (U Gross / Michaelis)	18 - 43

1. AMYLASE

Plasmatic (urinary) amylase determination by Wolgemuth method

Principle: pancreatic amylase hydrolyzes the starch (at the body temperature). This enzyme hydrolyzes the starch to produce maltose, dextrans (achrodextrans and erythro dextrans). When iodine is used as an indicator of amylase activity, starch produces a blue color, dextrans a red color and maltose a yellow color. We are going to determine the maximum dilution in which the contained amylase (serum, urine) hydrolyzes completely a certain amount of starch, in an examined product.

Materials: solution of starch 0,1g%, iodine solution (Lugol solution IKI), test tubes, stand, pipettes, thermostat, serum or urine, gloves, NaCl 0,9%, beakers, marker.

Procedure: The test tubes are marked from 1 to 10 with a marker. In 9 test tubes, in the beginning test tube 2 1 ml of NaCl is introduced, 1 ml in each of them (with the exception of a tube 1). In the first test tube 2 ml of serum are introduced. 1ml from the first test tube is passed in the second test tube and is stirred again. The procedure is repeated until the last test tube, from where 1 ml of solution is removed, so that the volume to be the same in all test tubes. The purpose is to increase the dilution of serum: 1/1, 1/2, 1/4, 1/8, 1/16, 1/32... and so on. After that, in all test tubes are added 2 ml of starch sol. 0,1g %. All test tubes are introduced into the thermostat at 37°C for 30 minutes. The test tubes are cooled under cold tap water after they are taken off from the thermostat. In all test tubes 1- 2 drops of Lugol sol are added and the content of test tube is shaken.

Results: Iodine gives a color reaction with starch and its hydrolysis products. Blue color means unhydrolezed amidone, red- partial hydrolysis until dextrans, yellow/no color- total hydrolysis until maltose. We notice the last glass tube with uncolored content, where exist enough amylase to hydrolyze the entire amount of starch; the result is expressed in Wolgemuth units (1 Wolgemuth unit is the amount of amylase necessary to hydrolyze 1 mg of amidone in 30 minutes at 37°C);

For instance, let's say that the last test tube where the hydrolysis is complete is the test tube no.5 (here the dilution is 1/16), the titer of amylase is calculated in the following way:

If 1/16 ml of serum hydrolyzes 2 ml of starch 0,1 g% , than 1 ml of serum will hydrolyze X ml of starch 0,1 g% \Rightarrow

$$X = \frac{1 \times 2}{\frac{1}{16}} = 32 \text{ Wohlgemuth Units}$$

Practically, 2 is multiplied by serum fraction denominator dilution.

Normal values:

Amylasemia 8-32 uW

Amyllasuria 16-64 uW

Amylase is not specific for the pancreas because it is secreted by other organs too (salivary glands, liver, small bowel, kidney) or by different tumors (breast carcinoma, lung and ovary tumors). (there are several isoenzymes: P secreted by pancreas, S from other sources, and they can be used to precisely identify the cause of hyper-amylasemia; serum amylases level should be correlated with the clinical symptoms and signs of the patient).

Conditions associated with hyperamylasemia:

- 1. Pancreatic amylase:** pancreatitis/carcinoma/trauma, including surgical and post-ERCP complications of pancreatitis, drugs, diabetic ketoacidosis
- 2. Salivary amylase:** malignant neoplasms, pulmonary diseases / pneumonia / tuberculosis /carcinoma, diabetic ketoacidosis, ruptured ectopic pregnancy, ovarian cyst
- 3. Mixed or unknown:** renal insufficiency, thermal burns.

- Values in serum are increased in pancreatic inflammation, are very easy to determine and have to be taken into consideration when they exceed 3 times the upper level of normal. In acute pancreatitis serum amylases increase in the first 24 hours and remain elevated for 1 to 3 days and then they start to decrease slowly for the next 3 to 5 days.

- Urinary level is increased for a longer period in acute pancreatitis compared with serum levels. Urinary amylase could be increased even if the serum levels are within normal ranges.

2. PANCREATIC LIPASE

It is organ specific, pancreas inflammation generates elevation of its serum levels (normal range 7-60 U/L). It is increased in acute pancreatitis in 70-85% of cases and remains so for 7 to 14 days.

3. SERUM TRIPSINOGEN

Organ specific, acute pancreatic injury will generate high serum levels of tripsinogen, while in chronic injuries its levels will be decreased along with steatorrhea (lipids present in stool). (normal range 15- 65 ng/ml)

4. CHOLECISTOKININ TEST (CCK)

Evaluates de pancreatic juice secretion after bolus or continuous administration of CCK (0.2g/kg). Secretin will increase pancreatic juice secretion over 2 ml/kg per hour, bicarbonate concentration (HCO_3^-) > 80 mmol/L, and HCO_3^- flow over 10 mmol/L per hour. CCK will increase enzymes secretion too. The response is proportional with functional tissue(decreased in chronic pancreatitis).

II. Functional investigation of exocrine pancreas with coprological tests

a. Macroscopic examination of the stool

Examination of the stool is a very important preliminary test for recognizing a pancreatic insufficiency. An abundant stool, with a light yellow color, an oily appearance and a musty smell means steatorrhea. Besides these, if undigested alimentary residue, intact muscular fibers (creatorrhea) and alkaline reaction of the stool are present we have the coprological aspect of pancreatic insufficiency.

b. Microscopic examination of the stool

In the stool are discovered many fat drops, which are colored with Sudan III (neutral lipids). Muscular fibers are evidenced with Lugol coloration and glycogen granules with Schiff reactive.

III. Imagistic methods for pancreas investigation

1. Abdominal radiography. Could provide pancreatic calcifications (chronic pancreatitis), pancreatic enlargement (tumor, cyst), dilated small bowel (acute pancreatitis).
2. Abdominal ultrasound. Usual method to explore pancreas, provides information of gland structure (homogeneous, Wirsung's dimensions), edema, cysts, tumor or calcifications.
3. Computer tomography. Allows pancreas and neighboring tissues visualisation. It is very useful for the diagnosis of calcifications, tumors or pancreatic ducts dilation. It can not always make the difference between inflammatory or tumoral pancreas injuries.
4. Endoscopic retrograde cholangio-pancreatography (ERCP). Cannulation of the pancreatic duct during endoscopic retrograde cholangiopancreatography (ERCP) has been combined with direct stimulation of the pancreas. This technique allows the measurement of pure pancreatic juice uncontaminated by biliary or intestinal secretions, but this method is possibly no more sensitive than other tests in the diagnosis of pancreatic disease. Allows the visualization of biliary and pancreatic ducts by endoscopy.
5. Endoscopic ultrasound. Provides images about pancreatic structure, Wirsung's dimensions (dilated in chronic pancreatitis), ductal or tissue lithiasis.
6. Cholangio MRI. It is used to provide a 3D image of biliary and pancreatic ducts.
7. Ultrasound guided pancreatic biopsy.