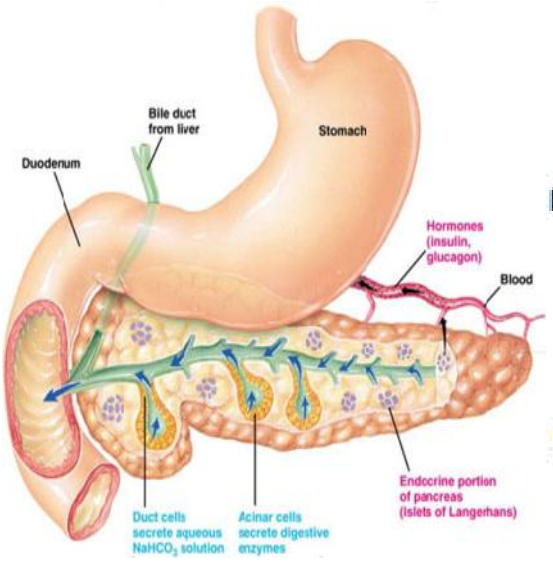
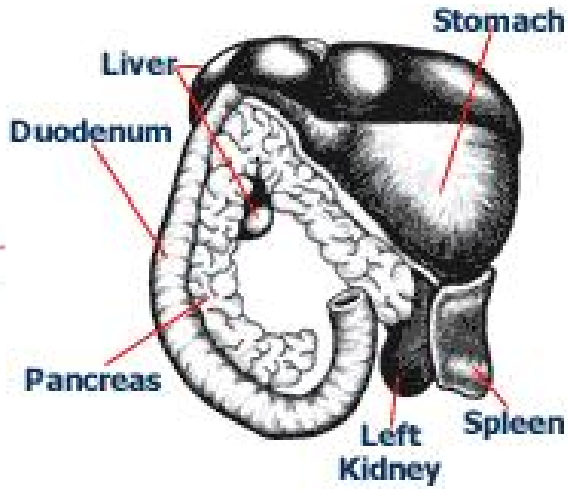
The Pancreas and Pancreas Function Test

- **Anatomy:** The pancreas is a small structure located near the stomach and attached to the wall of the small intestine. **The pancreas has two major functions. It produces hormones to aid in the maintenance of a proper blood sugar (glucose) level. The hormones are secreted into the bloodstream. It also produces important enzymes to aid in the digestion of protein and fats (lipids).** These enzymes travel from the pancreas to the small intestine through a small tube called the pancreatic duct.

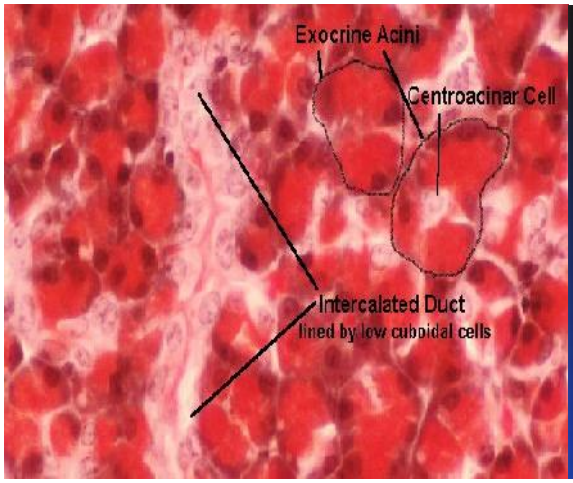


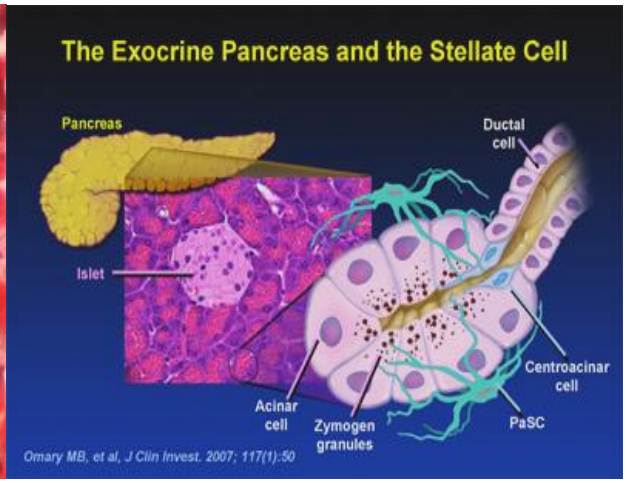


Pancreas of Human (one lobe) **Pancreas of Dog (tow lobes)**

- **The endocrine portion** of the pancreas takes the form of many small clusters of cells called **islets of Langerhans** or, more simply, islets. Humans have roughly one million islets. In a histological section of the pancreas, islets are seen as relatively pale-staining groups of cells embedded in a sea of darker-staining exocrine tissue. The image shows three islets in the pancreas of a horse.

- **The exocrine pancreas** is composed of closely packed **acini** draining into a series branched ducts. The acini are composed of several wedge-shaped **serous cells** surrounding a central lumen. These are typical polarized secretory cells with a spherical nucleus and basophilic cytoplasm. Eosinophilic secretory vesicles rich in digestive enzymes (**zymogen granules**) may be seen toward the apex of each acinar cell. The base of each pyramidal acinar cell lies on the basement membrane which surrounds each acinus. Beneath this basement membrane is a rich capillary network.





I. The exocrine pancreas:

◘ *Normal exocrine pancreas function:*

The exocrine pancreas secretes many different zymogens for digesting carbohydrates, fats, and proteins Protein digestion is catalyzed by the enzymes trypsin, chymotrypsin, and carboxypeptidase. These proteolytic digestive enzymes are initially released from the pancreas as the zymogens trypsinogen, chymotrypsinogen, and procarboxypeptidase, respectively, and are activated once they reach the small intestine. In the presence of chyme,

enterocytes release enteropeptidase, which activates some of the trypsinogen. Additionally, the newly formed trypsin assists in activating all three zymogens. This delayed activation prevents autodigestion of pancreatic proteins.

◘ ***Diseases of the pancreas:***

1) pancreatitis:

It is an inflammation of the pancreas, causing leakage of the digestive enzymes whereby the pancreas literally starts to "digest itself". Pancreatitis can be acute (sudden) or chronic (happening over a course of time). Both acute and chronic forms are serious and can be life-threatening, especially the acute form. For the majority of cases, the cause is unknown. ***Here are some potential risk factors:***

i. Hyperlipidemia (high fat content in blood).

ii. Obesity, Trauma, such as a severe abdominal injury.

iii. High fat meal (trigger for hyperlipidemia).

☙ Signs of pancreatitis:

The signs can vary from mild gastrointestinal upset to collapse and death.

A. Acute pancreatitis: Clinical signs :

1.Acute pain in abdomen and epigastric area. 2. Back arching. 3. Vomiting. **4.** Not eating. **5.** Fever or below-normal body temperature. **6.** Blood tinged or

fat-laden diarrhea.**7.** Depression. **8.** Dehydration, evaluated by noting sunken eyes, dry mouth, and increased skin turgor (skin tents when pinched).**9.**Shock.

➔ **Lab. findings of acute pancreatitis:**

1.Leukocytosis. 2. Hyperamylasemia, occur within few hours of onset of clinical signs, return to normal in 2-6 days. 3. Increase in urine amylase. 4. Increase in serum lipase within few hours of onset of clinical signs and remain elevated for 5-15 days. 5. Increase in blood glucose.

B. Chronic pancreatitis: Clinical signs

The same for acute but less severe with ravenous appetite without weight gain, voluminous, gray, fatty stool with feted odour.

➔ **Laboratory findings:** 1. Leukocytosis. 2. creatorrhea. 3. Steatorrhea. 4. Decrease in fecal trypsin. 5. Increase in blood glucose. 6. Increase in serum lipase.

N.B: A greater than 3-4 fold increase in serum activity of both amylase and lipase is considered supportive for acute pancreatitis. In chronic renal insufficiency, there will be 1-2 fold increase in serum lipase and amylase since they are mainly excreted through the kidney, in addition in renal insufficiency there will be increase in serum urea and creatinine.

2) Exocrine pancreatic insufficiency(EPI):

In patients with EPI, inadequate production of digestive enzymes by the pancreatic acinar cells leads to maldigestion and malabsorption of nutrients.

Fortunately, the pancreas has a high reserve capacity, so signs of maldigestion do not occur until 90% of the exocrine pancreatic function is lost.

- **Clinical signs:** Dogs with EPI present with signs of:-

a. maldigestion.

b. Primarily weight loss despite an increased appetite and diarrhea or loosely formed feces.

c. Feces are usually yellow or gray, increased in volume, and may appear undigested or pulpy. In most cases, fecal consistency is loosely formed, but dogs may experience severe watery diarrhea initially.

d. The diarrhea is usually accompanied by steatorrhea and creatorrhea.

e. Some dogs with EPI also experience vomiting.

f. Along with the weight loss, these dogs may have a poor coat.

g. They may also seem nervous, aggressive, or irritable as a result of abdominal discomfort.

- **Causes of EPI:-**

Causes of EPI include pancreatic acinar atrophy, chronic pancreatitis, pancreatic hypoplasia, and neoplasia.

3) Pancreatic acinar atrophy:

The most common cause of EPI in dogs is pancreatic acinar atrophy. The severity of this condition ranges from subclinical disease to a complete

absence of secretory capacity, Pancreatic acinar atrophy is thought to be an immune-mediated condition.

◘ *Clinical pathology:*

EPI rarely affects serum chemistry profile and complete blood count results. Amylase and lipase activities are not useful in diagnosing EPI. Occasionally, a serum chemistry profile may reveal hypocholesterolemia due to fat maldigestion.

A diagnosis of EPI and other pancreatic diseases is most reliably based on:

I. Clinical signs.

II. Pancreatic function test.

Pancreatic Function Test

I. Changes in serum pancreatic enzyme level:

1. Trypsin-like immunoreactivity (TLI) by radioimmunoassay:

This test measures the trypsinogen that has entered the blood stream directly from the pancreas. Enzymes that originated or were activated within the intestinal lumen are not measured, which eliminates any interference by intestinal inflammation. The test is also not affected by exogenous sources of pancreatic enzymes because it is species-specific. The reference range for canine TLI is 5.7 to 45.2 µg/L, with values below 2.5 µg/L being highly diagnostic for EPI when concurrent clinical.

N. B: Dogs with EPI may have normal TLI concentrations in the presence of kidney disease because trypsinogen is renally excreted.

2. Serum pancreatic lipase immuno-reactivity (PLI):

Serum pancreatic lipase immunoreactivity (PLI), used to detect pancreatitis, is also a sensitive test for diagnosing EPI.

Both PLI and TLI use radioimmunoassay to detect minute amount of serum lipase and trypsin using antibodies to the particular enzyme, they provide highly sensitive and species specific lab. Tests for diagnosing pancreatic diseases.

Normal TLI in a dog: 5-35 µg/L …….. µg/L in EPI: It is < 2.5.

- *In chronic pancreatitis:* may have normal or between 2.5-5 µg/L. TLI.
- *In acute pancreatitis:* TLI may increase.

II. Fecal examination:

More than one fecal sample on three successive days should be used before a conclusion is made.

i. Macroscopic examination:

1.Undigested food. 2. Bulky feces, semisolid or soft. 3. Pale in coloure- yellow-grey. 4. Rancid or putrid odour. 5. steatorrhea (increase in fat in feces) and creatorrhea (undigested muscle).

ii. Microscopic examination:

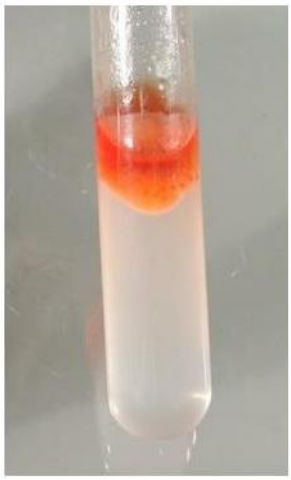
1. **Decrease in lipase enzyme** will lead to the presence of neutral fat globules detected by adding Sudan III to fecal emulsion on a slide; fat globules appear red or orange at low power.

Test for Fats (lipids)
Sudan III-IV

- Like lipids, the chemical Sudan is not soluble in water; it is, however, soluble in lipids.
- In this test dark red Sudan is added to a solution along with ethanol to dissolve any possible lipids.

Sudan III is used to identify the presence of lipids

- **What to do.**
- To a test tube, add equal parts of test liquid and water to fill about half full.
- If testing more than one liquid, label each test tube with a marker.
- Add 3 drops of Sudan III stain to each test tube. Shake gently to mix.
- A red-stained oil layer will separate out and float on the water surface if fat is present.



2. Striated muscle fibe Give the animal a meal of uncooked meat, stain fecal suspension on a slide with lugol's iodine or Sudan III. Light yellow cross striated undigested muscle due to lack of trypsin.

3. Starch: add lugol's iodine to a fecal sample a blackish-blue coloure is formed due to maldigestion of starch as a result of decrease or absence of amylase (amylorrhea).

III. Fecal trypsin:

It is a proteolytic pancreatic enzyme aid in the digestion by catalyzing the reaction that breaks down protein ingested with food.

1. Gelatin tube test: 9 ml (5% NaHCO3), add feces to 10 ml. 2ml 7.5 warm gelatin suspension + 1 ml diluted feces, mix and incubate at 37°C with blank. Put the tubes in the refrigerator.

Result: a. if gelatin solidifies at 4°C, trypsin is not present in the feces (in chronic pancreatitis it is not produced, in acute pancreatitis it leaks to the blood or in the area causing digestion of the pancreas).

2. Film test: Digestion of gelatin present on exposed x-ray film.

It is evident by clearing of the film. diluted feces, put it in a test tube then put a strip of x-ray film in the fecal mixture(use blank diluting fluid without feces)incubate for one hour at 37 °C, rinse both films gently with water

Result:

a. Clearance of x-ray film means trypsin is present in the feces (normal pancreatic function).

b. Absence of clearance means that trypsin is not present in the feces (pancreatic insufficiency.

3. Fecal proteolytic activity: Radial enzyme diffusion (RED), proteolytic activity is determined on a substrate such as azocasien or casien containing agar gel, activity of proteases is determined by measuring the zone of clearance surrounding the area in which fecal suspension is put in several dilutions.

4. Fecal elastase: Test kit for the quantitative detection of human fecal pancreatic elastase in stool in order to diagnose and quantify pancreatic insufficiency exclusively produced in the pancreas and therefore excellently suitable to diagnose pancreatic malfunctions like pancreatic insufficiency, it is extraordinary stable when passing through the bowels using ELISA.

II. The Endocrine pancreas:

It consists of about 1 million microscopic units, the islets of - langerhans, they are of 4 types:

1. α- cell Produce glucagon(20%).
2. β- Cells produce insulin (70%).
3. D- Cells produce somatostatin (5-10%).
4. F- Cells (1-2%) produce pancreatic polypeptide.

Diseases of endocrine pancreas:

1) B- cell hypofunction(diabetes mellitus):

In the absence of insulin, glucose will be inefficiently utilized. That will lead to continued activity of glucagon that will lead to hyperglycaemia, glucosuria + weight loss + polyphagia, in spite of large quantities of glucose in the blood, body tissues are starved, and fat catabolism to obtain energy will lead to ketoacidosis.

▣ **Causes of DM:**

a. Lack of secretion.
b. Abnormal insulin.
c. Insensitivity of B cells to stimuli.
d. Increase in insulin inhibitors and antagonists.
e. Cells are resistant to action of insulin.
f. Increase in ACTH, GH, corticosteroids.

Diabetes mellitus is caused by the inability of glucose to enter the cells, thereby causing an energy deficiency. Animals may have:-

i. Type I diabetes mellitus, where inadequate insulin is produced.
ii. Type II diabetes mellitus, which is non-insulin-dependent, and where the cells are resistant to the effects of insulin.

➤ Both types result in impaired utilization of carbohydrates, lipids and proteins.

➤ **Other chemicals and activities can also affect blood glucose:-**

a. There is a spike in blood glucose two to three hours postprandial.
b. The liver is responsible for glycogen production and breakdown, so liver disease may affect glucose levels.
c. Epinephrine increases gluconeogenesis and glycogenolysis.
d. Corticosteroids antagonize insulin, resulting in increased blood glucose and less glucose entering cells.

▣ **Laboratory diagnosis of DM:**

1. **Fasting blood glucose:** Fasting hyperglycemia plus glucosuria (but may be a physiologic response in cats). Blood glucose in excess of 200 mg/dl in dogs and 250mg/dl in cats.
2. **2. Glucose tolerance test:** Confirmatory test for the diagnosis of mild in apparent or subclinical DM. Oral glucose tolerance test for all animals EXCEPT ruminant, i.v. glucose tolerance test is used for all animals including ruminants. The patient is subjected to a challenging dose of glucose solution, and then repeated blood samples are collected at successive intervals.

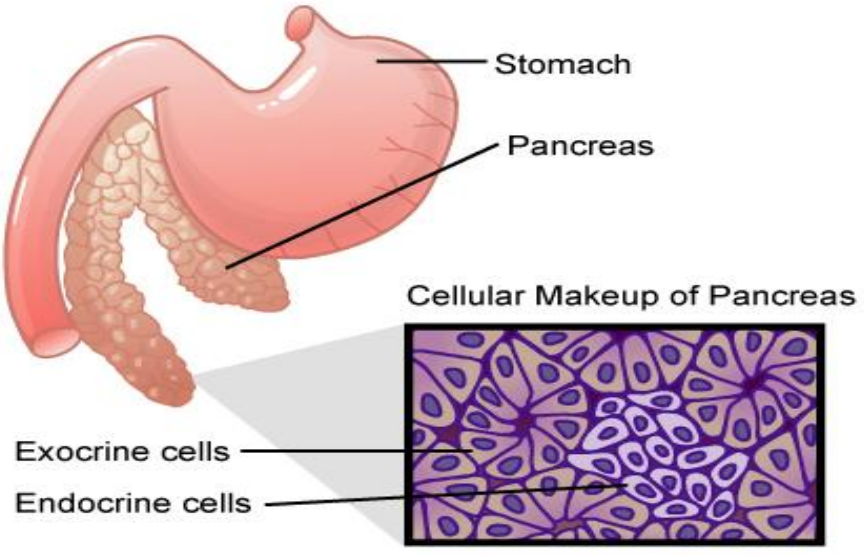
➔ **Good glucose tolerance** is indicated by limited rise in blood glucose, return to normal in 60-90m.

➔ **Low glucose tolerance:** The patient will show excessive rise in blood glucose that remains elevated, it takes more than 90 m to return to fasting level.

3. **Hypercholesterolemia and lipemia.**
4. **Ketonemia and ketonuria** (esp. with diabetic ketoacidosis). **Diabetic ketoacidosis** is an emergency and may be fatal. Animals show signs of diabetes mellitus, plus cachexia and hypothermia. Laboratory findings including increased blood glucose and ketones, metabolic acidosis and decreased electrolytes.
5. **Metabolic acidosis.**

➤ **<u>Long- term indices of DM control:</u>**

High concentration of glucose in the extracellular fluid will lead to non-enzymatic attachment of glucose to lysine residues of a variety of proteins until protein molecule is degraded, the concentration of glycated protein is a reflection of mean glucose level in the ECF during the life of that protein. An example is albumin and haemoglobin, it provides an index for glucose control over the 3 weeks prior to its measurement.



A diagram of the stomach and pancreas of Dog

The pancreas of dog consists of two lobes

▣ **Dysfunction of the endocrine pancreas should be suspected if an animal presents with the following signs:**

1) Polyuria and polydipsia.
2) Polyphagia.
3) Weakness.
4) Seizures.
5) Coma.

➔ **The small animal reference ranges for glucose should be memorized:**

- **Dog** 60 - 120 mg/dl.
- **Cat: 7**5 - 150 mg/dl.

2) β-cell hyper-function:

➔ *There is extra-production of insulin caused either by:-*

a) Beta cell tumors (insulinomas).
b) Extra-pancreatic tumours that secrets insulin, both are important causes of hypo-glycaemia.

➔ **There are other common causes of hypoglycemia:-**

Starvation, liver disease, insulin therapy overdose and pregnancy can also cause low blood glucose. Hunting and other hard-working dogs may develop a functional hypoglycemia related to the high glucose demands of their activities.