DIAGNOSTIC BIOCHEMISTRY

Blood sugar and ketone bodies

Blood glucose:
The blood glucose is supplied from two sources:

- Dietary glucose (intestinal absorption).
- Hepatic glucose production (such as gluconeogenesis).

Intestinal absorption of glucose is affected by

- Systemic hormonal activity (E.g. Thyroid hormone).
- Gastro-intestinal hormone activity (E.g. Secretin).

In post-absorptive state:

- Epinephrine and glucagon causes release of glucose from glycogen stored in liver.
- Glucocorticoids promote gluconeogenesis.

Role of liver in maintenance of blood glucose:

I. Liver supply glucose to blood (by the action of glucocorticoids, epinephrine and glucagon).
II. Liver removes glucose from blood (by the action of hormone insulin).

Methods of glucose estimation in blood

1. Folin-wu method.
2. Ortho-toludine method.
   Ortho-toludine reacts with Glucose giving blue green colour complex.
3. Glucose oxidase method

Glucose
↓ Glucose oxidase
Gluconic acid + H_2O_2

H_2O_2 reacts with orthophenyl diamine producing coloured product.

4. Hexokinase Method (HK method)

Glucose + ATP $\xrightarrow{\text{Hexokinase}}$ Glucose-6-Phosphate + ADP

Glucose-6-Phosphate + NADP $\xrightarrow{\text{Glucose-6-phosphate dehydrogenase}}$ 6-Phosphogluconate + NADPH

The NADPH produced is measured spectrophotometrically.

5. Glucose Dehydrogenase Method (GD method)

This method relies on production of NADH upon the action of Glucose Dehydrogenase on glucose that is measured spectrophotometrically.

Glucose + NAD^+
↓ Glucose Dehydrogenase
Gluconolactone + NADH

Precautions to be taken during blood collection for blood glucose estimation

After blood collection, the blood glucose breakdown occurs at the rate to 10% per hour by RBCs by glycolysis. So, for prevention of loss of blood glucose following precautions can be taken-

1. Plasma or serum should be separated as quickly as possible.
2. Refrigeration of blood sample.
3. Addition of sodium fluoride (NaF) at the rate of 10 mg sodium fluoride per ml of blood.

Causes of hyperglycemia:

1. Diabetes mellitus.
2. Emotional stress.
3. General anaesthesia.
4. Hyperthyroidism.
5. Acute pancreatic necrosis.
6. Hyperpitutarism.
7. Chronic liver disease.
8. Shock (due to depressed action of insulin).

Hypoglycemia

1. Hyperinsulinism in dogs: this conditions leads to hypoglycaemia. The blood glucose falls below 55 mg per dl of blood and insulin level in blood falls below 20 µU/ml of blood.
2. Hypoglycemia in piglets: this occurs in piglets when the newborn piglets fail to suckle shortly after birth. In calves, foals, lambs and other newborns of other animals have enough glycogen in liver at birth. This occurs due to :
   - Very less glycogen in liver at birth in piglets.
   - Gluconeogenic enzymes (enzymes required for gluconeogenesis) are underdeveloped in newborn pigs.

   At the time of death of piglets due to hypoglycaemia, the glycogen content in liver is almost zero and glucose level is less than 40 mg per 100 ml of blood.

3. Hypoglycaemia in ruminants: this is associated with bovine ketosis. This is observed in high milk yielding cows during the time of lactation and pregnancy (especially seen in ewes). During this period, the large amount of glucose is being drained out of body (in milk and placenta).

Ketosis

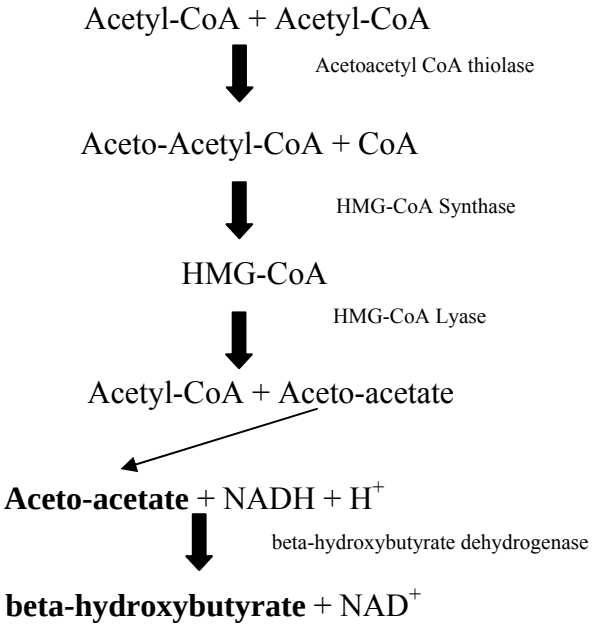
The ketosis is caused by presence of three types of compounds in blood called ketone bodies that include Aceto-acetic acid, β-hydroxy-butyric acid and acetone. Aceto-acetic acid and β-hydroxy-butyric acid exist in ionized form that is they are present in blood as Aceto-acetate and β-hydroxy-butyrate. The acetone is volatile ketone body.

Source of ketone bodies

The most important source of ketone bodies is fatty acids. Apart from that glucose, lactic acid, glycerol, and amino acids also can be converted to ketone bodies.

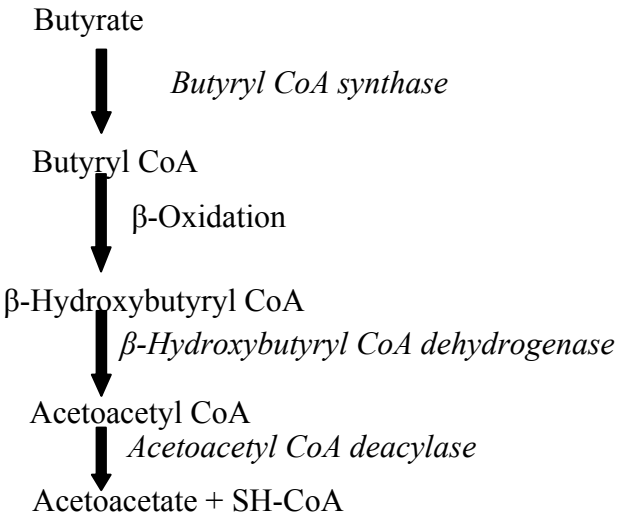
During the time of less glucose in blood (such as diabetes), the level of insulin decreases and level of glucagon increases. Glucagon activates an enzyme in adipose tissues called hormone-sensitive lipase. Upon activation, the hormone-sensitive lipase causes break down of tri-acyl-glycerol in adipose tissues. This releases fatty acids into blood. These fatty acids now move to liver. In liver, the fatty acids undergo

β-oxidation ultimately producing acetyl-CoA. Acetyl-CoA is the precursor of ketone bodies. The ketone bodies are formed in liver from acetyl-CoA.



Ketogenesis of GI tract in ruminants

In rumen, the butyrate is produced during rumen fermentation that is converted into ketone bodies.



Pathophysiology of ketosis:

Increased ketone bodies in blood plasma causes metabolic acidosis called *ketoacidosis*. As acidosis progresses, it leads to increased catabolism of muscle protein. Much of the nitrogen produced from protein degradation is diverted into ammonia rather than urea because the ammonia (NH_4^+) is used for balancing the negative charge of ketone bodies excreted in urine. Thus the ketone bodies production leads to increased catabolism of muscle protein.

The alimentary ketosis occurs when the cattle are fed with spoiled silage that contains excessive amounts of butyric acid.

Causes

1. Diabetes mellitus.
2. High fever.
3. In starvation.
4. In cows, it is met with in acetonemia, milk fever and in anorexia of high yielding animals.
5. In ewes in pregnancy disease.
6. Cachectic conditions.

Qualitative tests for ketosis

1. Rothera's test:
   Saturate 5 ml of urine with ammonium sulphate. Add 2 or 3 drops of 5% sodium nitroprusside solution and then add strong ammonium hydroxide. A permanganate color is indicative of ketones.

Quantitative tests for ketosis

1. Microdiffusion test:
   Acetone reacts with salicylaldehyde producing coloured product.
2. Williamson's method.
   When Hydroxybutyrate gets converted to acetoacetate, it produces NADH that is read spectrophotometrically.

Blood urea nitrogen

In healthy animals, the rate of synthesis and degradation of protein is in equilibrium but in disease condition, this gets altered. Normally, the proteins are digested in gut and digested protein yields amino acids. These amino acids are absorbed into portal blood circulation and moved to liver and other tissues. In theses tissues and liver, these amino acids are utilized for protein synthesis. These synthesized proteins upon degradation yields amino acids. These amino acids have two fates. First, these amino acids are used for fatty acid synthesis and gluconeogenesis. Second, some of these amino acids are put into urea cycle producing urea that is excreted in urine.

The proteins are the major source of synthesis of urea. Urea is the main form in which the nitrogen is eliminated in mammals. The nitrogen is freely filtered by kidney and reabsorbed by collecting tubules. The reabsorption of urea is increased when urine flow to tubules is reduced.

Causes of increased BUN:

1. Hypovolemia (lower glomerular filtration rate).
2. High protein intake.
3. Inadequate fluid intake.

Causes of increased BUN:

1. Liver disease or damage.
2. Malnutrition.

Uric acid in blood

Uric acid is catabolic product of purines (Adenine and Guanine).

High level of uric acid in serum may be present due to:

1. Genetic factors.
2. Kidney disease: due to reduced renal excretion of uric acid.
3. Fasting or rapid weight loss can temporarily elevate uric acid level.

Pathophysiology of uric acid

- Increased uric acid can form free radicals that can damage cell membranes by lipid oxidation. The cell membrane damage is more pronounced in vascular smooth muscle

cells causing hypertension.

- The uric acid can induce rennin-angiotensin system.
- Uric acid can increase chemokine and cytokine expression and C-reactive protein expression that cause vascular damage.

Clinical Enzymology

The diagnostic accuracy various enzymes depends on

a) Organ specificity.
b) Sub-cellular location of enzyme.
c) The mechanism of enzyme release from cells.
d) The clearance from blood.
e) Rate of induction of enzyme synthesis.

Factors affecting serum enzyme activity

1. Organ mass and enzyme tissue concentration: Enzymes with high concentration in organs have high potential to cause greater increase in serum enzyme activity with disease. E.g. ALT (Alanine Amino Transferase) has the ratio in liver to extra-cellular fluid (ECF) is 1000:1. Thus the injury to hepatocytes, the ALT is released to ECF and then from ECF to serum.
2. Cell-location: the cell location (having a marker enzyme) relative to blood, urine or other fluids is an especially significant determinant. This determines the presence of released enzyme in blood or urine or other body fluids.
   E.g. Enzyme Gamma Gutamoyl Transferase (GGT) in tubular epithelial cells of kidney is released into urine not to blood upon damage to tubular cells.
3. Mechanism of release of cytoplasmic enzymes: the enzyme is released from injured cell not by leakage but by formation membrane blebs. The membrane blebs are formed in many disease conditions. The membrane blebs are released into blood vessels and release enzyme.
4. Mechanism of release of membrane bound enzymes: enzymes like ALP are present on the surface of cell membranes being anchored to cell membranes towards the exterior. These are released during any kind of disease conditions.
5. Blood clearance rate of enzymes: this depends on degradation by proteases in blood. Small enzyme molecules such as amylase and lipase are filtered through glomerulus. The enzymes that are glycoproteins are removed from blood by liver cells.
6. Enzyme induction: drugs or other pathophysiological conditions induce enzyme production causing increase in enzyme level in blood (this may not be a tissue injury).

Enzymes of clinical importance:

1. Alanine Aminotransferase (ALT) also called glutamate pyruvate transaminase (SGPT): this enzyme is associated with hepatic and/ or muscular injury
   Indications of ALT:
   - Hepatic injury in dogs and cats.
   - Hepatic or muscular injury in cattle and horse
   - Hypoxia due to anaemia.
   - Inflammatory and infectious diseases.
   - Neoplastic disease.

   Drugs that induce ALT are CCl_4, acetaminophen, mushroom alkaloids.
2. Aspartate aminotransferase (AST) also called SGOT (Serum Glutamate Oxaloacetate Transaminase): AST activity is high in liver, skeletal muscle, cardiac muscle.
   Routinely used in equine and food animals as a screening test for all three organs.

3. Sorbital Dehydrogenase (SDH):
   This enzyme catalyzes the reaction
   Sorbitol + NAD^+ ↔ Fructose + NADH.
   This enzyme is found in highest concentration in liver followed by kidney.
   This enzyme is indicated only in hepatic diseases. It is used in conjunction of ALT to rule out hepatic disease as ALT is increased in kidney disease also.

4. Glutamate Dehydrogenase (GDH): this enzyme is a mitochondrial enzyme and its highest activity is found in liver. The sensitivity of GDH is more than SDH in hepatic disease. It is used most commonly in food animals.
5. Gamma glutamyl transferase (GGT): the highest concentration of this enzyme is found in kidney, pancreas, intestines and mammary glands of cattle, sheep, goat and dogs. *The horse mammary gland has very low concentration of GGT*. This is a membrane bound enzyme and increased serum concentration of GGT is not associated with affection of kidney and pancreas. As the enzyme is released from these organs not to blood but to urine and gut.

Indications of increased GGT: cholestasis in cattle

6. Alkaline phosphatase (ALP): this enzyme functions in bone mineralization. The organs with increased ALP activity are liver, bone, kidney, intestinal mucosa and placenta. Intestinal ALP is not found in serum of most animals that may be due to short half life (less than 5 minutes) in blood. The half life of renal ALP and placental ALP in dogs is less than 6 months.
   Indications of ALP:
   - Increased Liver ALP is seen in cholestasis, hyperthyroidism, and hepatic lipidosis.
   - Increased Bone ALP is associated with increased osteoblastic activity (this is the marker enzyme of bone formation). The Bone ALP is very high in young and growing animals.
   - Disease conditions associated with increased ALP are hyperparathyroidism, renal diseases, fracture healing.
   - Serum ALP level can be used to monitor the healing of fracture.
   - Osteocarcinoma caused the elevation of Bone ALP.
   - Administration of adrenocorticosteroids or the condition of hyper-adrenocorticism causes the elevation of ALP.

7. Lipase: this enzyme is present in pancreas and can be used for diagnosis of pancreatic disease.
   The non-pancreatic causes the can elevate the serum lipase are
   - Renal failure.
   - Enteritis and gastroenteritis.
   - Some hepatic disorders.
   - Bile duct carcinomas.
   - Lymphosarcoma of GIT.
   - Treatment with glucocorticoids.

8. Amylase: this enzyme is used in screening test for acute pancreatitis. Renal disease may also increase the level of serum amylase due to decreased glomerular filtration rate.
9. Trypsinogen and trypsin: trypsinogen in serum is thought to be derived primarily from the pancreas.
   Indications: canine exocrine pancreatic insufficiency.

10. Creatine kinase (CK): high concentration of this enzyme is found in heart muscle followed by heart muscle, other smooth muscle and brain.
    Creatine kinases occur in three isoenzymes:
    i. CKMM: this isoenzyme is present in skeletal muscle and heart muscle.
    ii. CKBB: this isoenzyme is present in brain.
    iii. CKMB: this isoenzyme is present in small amounts in muscle, heart and brain.
    Indications of CK:
    - Skeletal muscle injury.
    - Nutritional myopathies.
    - Congenital myopathies.
    - Neurological disorders.
    - Intra-muscular injection of drugs.
11. Lactate Dehydrogenase (LDH): the LDH has five isoenzymes found in different tissues.
    LDH-4: this isoenzyme is found in skeletal muscle. Its level increases during muscular and hepatic disorders.
    LDH-1: this isoenzyme is found in heart muscle. Its level increases during myocardial infarction.