CLINICAL CHEMISTRY IV (CCH 5512)

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COURSE CONTENTS OUTLINE

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- Pathophysiologic importance of lipoproteins and lipids
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- Explain the Clinical Significance of tumour markers
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General principles of toxicology

Intoxication forms
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Patho-chemical aspect of drug toxicology

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- Phenytoin
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- Digoxin
- Theophylline bronchodilator
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- Amino glycoside antibiotics

Drug poisoning with specific agents like
- Paracetamol
- Salicylates
- Alcohol
- Carbon monoxide
CHAPTER I

METABOLIC DISORDERS

Introduction

A metabolic disorder can happen when abnormal chemical reactions in the body alter the normal metabolic process. It can also be defined as inherited single gene anomaly, most of which are autosomal recessive.

Metabolism is the process your body uses to get or make energy from the food you eat. Food is made up of proteins, carbohydrates, and fats. Chemicals in your digestive system break the food parts down into sugars and acids, your body's fuel. Your body can use this fuel right away, or it can store the energy in your body tissues, such as your liver, muscles, and body fat.

A metabolic disorder occurs when abnormal chemical reactions in your body disrupt this process. When this happens, you might have too much of some substances or too little of other ones that you need to stay healthy. There are different groups of disorders. Some affect the breakdown of amino acids, carbohydrates, or lipids. Another group, mitochondrial diseases, affects the parts of the cells that produce the energy. You can develop a metabolic disorder when some organs, such as your liver or pancreas, become diseased or do not function normally. Diabetes is an example.

Symptoms

Some of the possible symptoms that can occur with metabolic disorders are: lethargy, weight loss, jaundice, seizures, to name a few. The symptoms expressed would vary with the type of metabolic
There are four categories of symptoms: acute symptoms, late-onset acute symptoms, progressive general symptoms and permanent symptoms.

Causes

Inherited metabolic disorders are one cause of metabolic disorders, and occur when a defective gene causes an enzyme deficiency. These diseases, of which there are many subtypes, are known as inborn errors of metabolism. Metabolic diseases can also occur when the liver or pancreas do not function properly.

1. Glucose-6-phosphate Dehydrogenase Deficiency

Definition

A condition causing red blood cells to break down in response to certain medication, infections or stresses.

G6PD Deficiency: An inherited disease characterized by hemolytic anemia.

Most common disease-producing enzyme abnormality in humans. Over 200 million people. Has the highest prevalence in the Middle East, tropical Africa and Asia, and parts of the Mediterranean. Life span of individuals is shortened as a result of complications arising from chronic hemolysis.
Increased resistance to malaria shown by female carriers.

**Note:** Red blood cell, while biochemically complex, is a relatively simple cell (no nucleus, organelles, and protein-synthesizing machinery).

So, defects in any of the remaining components—enzymes, membrane, and hemoglobin—can lead to hemolysis.

**Pathogenesis of hemolysis in G6PD**

**Glutathione:** Glutathione is a substance found in every cell in the body, where it acts as an antioxidant to neutralize free radicals and prevent cellular damage. Chemically, glutathione is a simple molecule composed of three protein building blocks or amino acids—cysteine, glutamine and glycine.

Glutathione, is its reduced forms, can chemically detoxify free radicals.

**Free radicals:** are atoms or groups of atoms with an odd (unpaired) number of electrons and can be formed when oxygen interacts with certain molecules. Once formed these highly reactive radicals can start a chain reaction.
Take electrons from other compounds and are thus very destructive to cell membranes, proteins, and DNA.

Implicated in cancer, inflammatory disease, and aging.

If the G6PD is missing, it can no longer convert NADP⁺ to NADPH.

Since NADPH is essential for the maintenance of the reduced glutathione, glutathione is now oxidized, not reduced.

No protection against free radicals and peroxides formed within the cell.

Other tissues have alternative sources of NADPH production that can keep glutathione reduced.

The erythrocyte has no nucleus or ribosomes and can not renew its supply of the enzyme.

**Result:** hemolytic anemia

**Clinical symptoms**

Patient may complain of dyspnea or fatigue (caused by anemia)

Dark urine and, occasionally, back pain may be reported by patients (caused by hemolysis).

Skin appear jaundiced or pale.

A resting heartbeat with a flow murmur may be present if the anemia is pronounced.
There are many variations of G6PD Deficiency - most individuals never show any clinical manifestations.

**Some patients, however, develop hemolytic anemia if they are**

1) treated with an oxidant drug
2) ingest fava beans
3) contact a severe infection

1. **Oxidant drugs**: commonly used drugs that produce hemolytic anemia in patients with G6PD deficiency are best remembered from the mnemonic AAA:
   
   “Antibiotics” (e.g. sulfamethoxazole) “Antimalarials” (e.g. primaquine) “Antipyretics” (e.g. acetanilid)

2. **Favism**: some forms of G6PD Deficiency are susceptible to the hemolytic effect of the fava bean, which is a staple in the Mediterranean region.

   Note: favism is not observed in all individuals with G6PD Deficiency, but all patients with favism have G6PD Deficiency.

3. **Infection**: most common factor of hemolysis in G6PD deficiency:

   Reaction: infection → inflammatory response → free radicals → diffuse into RBC.

   Glucose-6-phosphate dehydrogenase (G6PD) deficiency is an inherited condition usually occurring in males. It's more common in those of African and Mediterranean descent. Triggers include infections, stress, fava beans, aspirin and other drugs.

   Where symptoms are triggered, they include fever, dark urine, abdominal and back pain, fatigue and pale skin
Treatment

Stop taking AAA drugs
Stop eating fava beans
Treat infection promptly

2. Cystic Fibrosis

Definition

• An inherited life-threatening disorder that damages the lungs and digestive system.
• It is caused by the presence of mutations in both copies of the gene for the cystic fibrosis transmembrane conductance regulator (CFTR) protein.
is a genetic disorder that affects mostly the lungs, but also the pancreas, liver, kidneys, and intestine. Long-term issues include difficulty breathing and coughing up mucus as a result of frequent lung infections.

It is an:

- Autosomal recessive
- Generally manifests as lung disease
- Most common lethal genetic disease affecting Caucasian populations

Results from a mutation in the Cystic Fibrosis transmembrane conductance regulator gene.

- Usually diagnosed in children
  - 3% diagnosed as adults
  - Only 9% of patients live past 30 years.
  - Only recently with advances in therapy do 25% of patients live to adulthood (age 18-20)

**Pathophysiology**

- **Lung**
  
  Due to the abnormal or absent CFTR protein, the movement of Na⁺ and Cl⁻ from the apical airway increased resulting in what is considered dehydrated mucous that cannot be cleared from the lungs.
This leads to the accumulation of more viscous, nutrient-rich mucus in the lungs that allows bacteria to hide from the body's immune system.

- **The lungs of individuals with cystic fibrosis** are colonized and infected by bacteria from an early age.
- These bacteria thrive in the altered mucus, which collects in the small airways of the lungs.
  - This mucus encourages the development of bacterial microenvironments (biofilms) that are difficult for immune cells (and antibiotics) to penetrate.
  - The lungs respond to repeated damage by thick secretions and chronic infections by gradually remodeling the lower airways (bronchiectasis), making infection even more difficult to eradicate.
  - Over time common bacterial infections such as Staphylococcus aureus and Hemophilus influenzae colonize and infect the lungs.
  - However, Pseudomonas aeruginosa dominates.
  - Once within the lungs, these bacteria adapt to the environment and develop resistance to commonly used antibiotics. Pseudomonas can develop special characteristics that allow the formation of large colonies, known as "mucoid" Pseudomonas, which are rarely seen in people that do not have CF.
• **GI tract**
  
  – Pancreatic
    
    • The failure to secrete Na⁺ and HCO₃⁻ are the consequence of the CFTR gene.
    
    • **This results in the** poor hydration and transport of the digestive enzymes from the lumen of the pancreatic ducts resulting in auto destruction of the pancreas.
      
      • **This can result in poor processing and absorption of food materials and malnourishment.**
      
      • **Can result in Type 1 diabetes**

• **Sweat glands**
  
  – CF patients have sweat normal volumes however the concentration of NaCl is increased due to the inability of the sweat duct to reabsorb NaCl prior to excretion.

**Diagnosis**

• Because of the large number of mutations associated with CF DNA analysis is incomplete.
  
  – >200 mutations are responsible for CF have been identified.
• The newborn screen initially measures for raised blood concentration of immunoreactive trypsinogen.
  – Infants with an abnormal newborn screen need a sweat test in order to confirm the CF diagnosis.
  – Trypsinogen levels can be increased in individuals who have a single mutated copy of the \textit{CFTR} gene (carriers).

• Sweating is induced by pilocarpine iontophoresis. At the test site, an electrode is placed over gauze containing pilocarpine and electrolyte solution that will not interfere with the sodium and chloride measurement.

• A second electrode (\textit{without pilocarpine}) will be placed at another site and a mild electrical current will draw the pilocarpine into the skin where it stimulates the sweat glands.

The test site is carefully cleaned and dried, then a piece of preweighed filter paper is placed over the test site and covered with parafilm to prevent evaporation. Specialized collection devices may also be used.

Sweat is collected for \textbf{30 minutes}. The filter paper is retrieved and weighed to determine the weight of sweat collected.
Several laboratory methods are then used to determine the sodium and chloride concentrations.

**Sweat Chloride test**

- **Reference ranges**
  - >60 mEq/L, the test is positive
  - 40-60 mEq/L is borderline
  - <40 mEq/L is negative.

- **Interpretation**
  - Two reliable positive results on two separate days is diagnostic for CF.
  - Because of the existence of milder variants, borderline or even near-borderline negative results may be used to diagnose CF.
  - Highly discordant sodium and chloride values may indicate technical errors.

- **Sources of error**
  - Technical errors
    - insufficient sample
    - evaporation
• contamination
• dehydration
• skin rash on the tested area may produce incorrect results.

Clinical diagnosis and symptoms

• Pulmonary
  – Shortness of breath
  – Recurrent pneumonias resistant to treatment
• GI
  – Pancreatitis
  – Large discolored stools
  – Malnourishment
  – Failure to thrive
• General abnormalities
  – Short stature
  – Late puberty
  – Infertility
    • 95% of males are azoospermic

Treatment

• Pulmonary
  – Antibiotics for appropriate infections
  – Clearance of secretions
3. GALACTOSAEMIA

Definition

- **Galactosemia** (British galactosaemia) is a rare genetic metabolic disorder that affects an individual's ability to metabolize the sugar galactose properly. **Galactosemia** follows an autosomal recessive mode of inheritance that confers a deficiency in an enzyme responsible for adequate galactose degradation.

- Classic **galactosemia** occurs when an enzyme called galactose-1-phosphate uridyltransferase (GALT) is missing or not functional.

- This liver enzyme is responsible for breaking down galactose (a sugar byproduct of lactose found in breast milk, cow's milk and other dairy foods) into glucose.

- **Galactosemia affects** the body by preventing it from breaking down galactose, a simple sugar found in lactose. ...

  Untreated **galactosemia** can also cause a **person's** white blood
cells to stop working properly, leaving them susceptible to serious infections

Duarte galactosemia

- **Duarte galactosemia** (DG or D/G galactosemia) is an inborn error of metabolism, an inherited condition in which an enzyme, galactose-1-phosphate uridyltransferase (GALT), in the body is not working well as well as usual.

- The job of this enzyme is to break down a sugar (called galactose)

**Signs and symptoms**

- Convulsions (irregular mvmt of the body)
- Irritability.
- Lethargy.
- Poor feeding (baby refuses to eat formula containing milk)
- Poor weight gain.
- Yellow skin and whites of the eyes (jaundice)
- Vomiting

**Effect of galactosemia**

- **Excess** galactose in the blood affects many parts of the body. Some of the organs that may be affected include the brain, eyes, liver and **kidneys**.
• Infants with galactosemia usually have diarrhea and vomiting within a few days of drinking milk or formula containing lactose.

some effects of untreated galactosemia

• If galactosemia is untreated, high levels of galactose cause vomiting, diarrhea, lethargy, low blood sugar, brain damage, jaundice, liver enlargement, cataracts, susceptibility to infection, and death.

Enzyme deficiency in galactosemia

• GALT (galactose 1-phosphate uridylytransferase) is responsible for hereditary galactosemia and is the most common deficiency.

• This enzyme catalyzes conversion of galactose-1-phosphate and UDP glucose to UDP galactose and glucose-1-phosphate. Individuals with GALT deficiency manifest abnormal galactose tolerance.

Hypergalactosemia

• An elevated blood galactose concentration is the result of altered metabolism of galactose due to a genetic deficiency in enzyme activity or secondary hypergalactosemia due to liver
disease (congenital hepatitis, congenital hepatic Arteriovenous malformation).

Test for galactosemia

- A **galactosemia test** is a blood or urine **test** that checks for enzymes that are needed to change galactose into glucose, a sugar that your body uses for energy.
- A person with **galactosemia** doesn't have one of these enzymes, so high levels of galactose build up in the blood or urine

Diagnosis for galactosemia

- **Diagnosis** for both classic and Duarte **galactosemia** is made usually within the first week of life by blood test from a heel prick as part of a standard newborn screening. Treatment requires the strict exclusion of lactose/galactose from the diet.

Treatment
• The disease can **only** be managed in order to help prevent complications of the condition. The **only** way to manage galactosemia is to eliminate lactose and galactose from the diet completely. However, even when galactosemia is detected and treated early, some individuals still go on to experience long-term complications

4. Phenylketonuria

**Definition**

**Phenylketonuria (PKU)** is an inborn error of metabolism that results in decreased metabolism of the amino acid phenylalanine.

• Untreated PKU can lead to intellectual disability, seizures, behavioral problems, and mental disorders. It may also result in a musty smell and lighter skin.

• Babies born to mothers who have poorly treated PKU may have heart problems, a small head, and low birth weight

• A birth defect that causes an amino acid called phenylalanine to build up in the body

It is an:

• Autosomal recessive

• Deficiency in the hepatic enzyme phenylalanine hydroxylase (PAH).
This enzyme is necessary to metabolize the amino acid phenylalanine to the amino acid tyrosine.

**Phenylalanine hydroxylase** is responsible for the conversion of phenylalanine to another amino acid, tyrosine. The enzyme works with a molecule called tetrahydrobiopterin (BH4) to carry out this chemical reaction.

**Tyrosine** can also be broken down into smaller molecules that are used to produce energy.

- The incidence of PKU is about 1 in 15,000 births
- The incidence varies widely in different human populations from 1 in 4,500 births among the population of Ireland to 1 in 13,000 births in Norway to fewer than one in 100,000 births among the population of Finland.
- Turkey, at 1 in 2600, has the highest incidence rate in the world.

PKU is caused by mutated gene for PAH

Gene located on chromosome 12.

Biochemistry of Phenylalanine

- Is an essential amino acid
  - Not manufactured in the human body and thus must be obtained from the diet.
- Is an aromatic amino acid.
  - Contains an phenyl ring side chain.
- Is in the class of large neutral amino acids.
• **Biochemistry**
  
  • Phenylalanine
    
    • Can be metabolized through the ingestion of other meats such as beef, fish, poultry
    
    • Present in breast milk of mammals
    
    • Bananas have large amounts of phenylalanine
    
    • Soya protein is high in phenylalanine
  
  • Phenylalanine is essential in the production of multiple hormones
    
    • Norepinephrine
    
    • Epinephrine
    
    • Dopamine
    
    • T3
• T4

• **Metabolism**
  
  • After the ingestion of a meal containing proteins high in phenylalanine the amino acid is transported to the liver and peripheral tissue where it undergoes conversion to tyrosine.

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**Diagnosis**

• Testing for PKU is done on routine neonatal screening in most countries

  • **HPLC is the preferred method for evaluating the level of phenylpyruvic acid in the blood.**

  • If undiagnosed or untreated may present clinically with seizures, mental retardation, albinism (excessively fair hair and skin), and a "musty odor" to the baby's sweat and urine (due to phenylacetate, one of the ketones produced).

  • Brain mass in untreated PKU patients is approximately $\frac{1}{2}$ of normal.

    • Phenylpyruvic acid inhibits the normal regulation (uptake) of other AA into the brain and thus disrupts brain development and metabolism.
Diagnosis continued

**Guthrie test: as Guthrie bacterial inhibition assay**

- A semi quantitative assay to detect elevated phenylalanine using the ability of phenylalanine to facilitate growth of B.subtilis in a culture medium in the presence of an inhibitor.
- Drop of blood from heel prick of newly born infant on 6\textsuperscript{th} or 7\textsuperscript{th} day of life.
- Blood is collected on filter paper, punch out a small disc of filter paper containing bld sample and place it on agar gel plate containing B. subtilis and β -2- thienylalanine (an inhibitor).
- However in the presence of phenylalanine (from patient serum) inhibitor is overcome and there is bacterial growth.
- The amount of growth, measured as diameter of colony is roughly proportional to the amount of phenylalanine present in newborn’s serum.

**Treatment**

- Patients must adhere to a diet low in phenylalanine and high EAA (valine, leucine, isoleucine) and tyrosine in order to have normal brain development.
A Tumor marker is a substance found in increased amounts in the blood, other body fluids and tissues that may suggest the presence of cancer.

Many cancers are associated with the abnormal production of some molecules which can be measured in plasma. These molecules are known as tumor markers.

The tumor marker that can be found in the blood at increased levels when a certain type of cancer is present, but some of them are found in urine or other body fluid.

They can be products of the cancer cells themselves, or made by the body in response to cancer or other conditions.

Tumor markers alone are rarely enough to show that cancer is present.

Most tumor markers can be made by normal cells as well as by cancer cells. Sometimes, noncancerous diseases can also cause levels of certain tumor markers to be higher than normal.
A tumor marker is a substance synthesized by the tumor or by the host response to a tumor that can be used to detect the presence of the tumor.

Tumor markers are substances that can be determined by analytical methods and have a chance or causal connection with various types of cancer.

1. ALPHA-FETOPROTEIN

Definition

Alpha-Fetoprotein is a normal fetal serum protein synthesized by the liver, yolk sac, and gastrointestinal tract that shares sequence homology with albumin.

It is a major component of fetal plasma, reaching a peak concentration of 3 mg/ml at 12 weeks of gestation.

Following birth, it clears rapidly from the circulation, having a half life of 3.5 days, and its concentration in adult serum is less than 20 ng/ml.

AFP is of importance in diagnosing hepatocellular carcinoma and may be useful in screening procedures.

AFP elevation is more common in areas where hepatocellular carcinoma is endemic, such as Africa and in patients who are HBsAg positive.

AFP is a marker for hepatocellular and germ cell (non seminoma) carcinoma.
It is a glycoprotein produced in large amounts during fetal life and is homologous to albumin.

In healthy adults, **less than 10 µg/L** of AFP is found in the circulation.

AFP is elevated in normal pregnancy, benign liver disease (hepatitis, cirrhosis), as well as in cancer.

Valuable in screening for hepatocellular carcinoma in high risk populations

AFP is elevated in testicular germ cell tumors.

A definitive positive marker value is highly sensitive in indicating **response to treatment**.

The AFP is less frequently elevated in other malignancies such as pancreatic cancers, gastric cancers, colonic cancers, and bronchogenic cancers.

This elevation was not necessarily associated with liver metastases

The AFP is rarely elevated in healthy persons, and a rise is seen in only a few disease states.

Elevation occurs in certain liver diseases, especially acute viral or drug induced hepatitis and conditions associated with hepatic regeneration.

In general, the elevations are under 500 ng/ml and do not denote hepatocellular carcinoma.

Thus, AFP is a useful marker in hepatocellular carcinoma and germ cell tumors, the only conditions associated with extreme elevations greater than 500 ng/ml.

In both tumors it has value in diagnosis and monitoring of therapy.
In the former, which is one of the most common tumors worldwide, AFP may be of use in screening............

2.CARCINOEMBRYONIC ANTIGEN (CEA)

Definition

CEA: Carcinoembryonic antigen (CEA) is a protein found in many types of cells but associated with tumors and the developing fetus. The normal range is $<2.5 \text{ ng/ml}$ in an adult non-smoker and $<5.0 \text{ ng/ml}$ in a smoker. The CEA was one of the first oncofetal antigens to be described and exploited clinically.

It is a complex glycoprotein of molecular weight 20,000, that is associated with the plasma membrane of tumor cells, from which it may be released into the blood.

Although CEA was first identified in colon cancer, an abnormal CEA blood level is specific neither for colon cancer nor for malignancy in general.

Elevated CEA levels are found in a variety of cancers other than colonic, including pancreatic, gastric, lung, and breast.

It is also detected in benign conditions including cirrhosis, inflammatory bowel disease, chronic lung disease, and pancreatitis.

The CEA was found to be elevated in up to 19 percent of smokers and in 3 percent of a healthy control population.

As a screening test, the CEA is inadequate.

Since cancer prevalence in a healthy population is low, an elevated CEA has an unacceptably low positive predictive value, with excess false positives.
Measurements of plasma CEA concentration do not have the sensitivity or specificity required for use as a screening test, and should never be used in isolation to establish a diagnosis for cancer.

Plasma CEA concentration measurements should be used in conjunction with clinical and radiological evidence.

The CEA has been suggested as having prognostic value for patients with colon cancer.

Preoperative CEA values have been positively correlated with stage and negatively correlated with disease free survival.

The main indication for CEA concentration measurement is in monitoring for recurrence of disease.

3. HUMAN CHORIONIC GONADOTROPIN

Definition

Human chorionic gonadotropin (hCG)/Beta Human chorionic gonadotropin is a hormone (a glycoprotein of 2 subunits A&B).

It is produced during pregnancy that is made by the developing placenta after conception, and later by the placental component syncytiotrophoblast.

However this Human chorionic gonadotropin is a tumor maker produced by some cancerous cells. Therefore it is used in cancer diagnosis.

They are also higher in some people with mediastinal germ cell. Germ cell tumors that develop outside the ovary or testicle are very rare tumors. Doctors call them extragonadal germ cell tumors. The mediastinum is the most common place for extragonadal tumors to develop.
It starts in the same cells as germ cell tumors of the testicles and ovaries.

Gestational trophoblastic disease which is a disease of pregnancy-related tumors. They appear when cells in the womb start to grow out of control (trophoblasts) to form the placenta during pregnancy.

And germ cell tumors which are a neoplasm derived from germ cells that can be cancerous.

**USE OF HCG IN SCREENING TESTS**

The HCG is a good marker of in early detecting of gestational trophoblastic disease and germ cell tumors.

The human chorionic gonadotropin (hCG) test is done to check for the hormone hCG in blood or urine. Some hCG tests measure the exact amount. Some just check to see if the hormone is present. Or it can be done as part of a screening test for birth defects.

**USE OF HCG IN DIAGNOSIS**

HCG is used in diagnosis of tumor and follow-up care after treatment of ectopic pregnancy when it is performed serially.

It can help figure out if a cancer is likely.

And if a cancer is already widespread when it is found, tumor markers can help figure out where it started.

However the HCG is not good in diagnosing because it is not specific; it is also elevated in breast, lung and gastrointestinal cancers.
As the disease progresses, the HCG level in blood is increased due to the presence of syncytriophoblasts.

An elevated blood level of HCG will also raise suspicion of cancer in certain situations.

Also low levels of HCG in ectopic pregnancy.

But it is hard to define the HCG normal level because there are different ways to test for this marker and each has its own normal value.

HCG may be useful, once treatment is complete and there is no sign of cancer in the body, in detecting recurrence of cancer.

**EVALUATION OF DRUG TREATMENT RESPONSE**

Levels of HCG can be followed over time to see how well treatment is working.

If the tumor marker level in the blood goes down, it is almost always a sign of the effective treatment.

On the other hand, if the marker level goes up, then the cancer is not responding and the treatment may need to be changed.

The results of HCG have to be skewed for further evaluation.
4. CALCITONIN

Definition

✓ Calcitonin, also called thyrocalcitonin, is a polypeptide hormone with 32 AA and a molecular mass of about 3.4 kD and is synthesized and secreted by thyroid parafollicular cells (C cells) of the thyroid gland.

✓ Also secreted from cells originating from the neural crest.

✓ Its actions are antagonist to those of the parathyroid hormone, inhibiting the release of calcium from bone and increasing the renal excretion of calcium.

✓ Normally, calcitonin is secreted in response to increased serum calcium.

✓ It inhibits the release of calcium by the bone and thus lowers the serum calcium concentration.

✓ The half-life of calcitonin is about 12 minutes.

✓ MTC (Medullary thyroid carcinoma) is a rare cancer that starts in the parafollicular C cells; blood levels of this hormone are often greater than 100 pg/ml.

✓ Normal calcitonin are below 5 to 12 pg/ml (pictograms per milliliter).

✓ An elevated serum calcitonin level is a highly sensitive marker for medullary thyroid carcinoma (MTC) that can be used for screening, differential diagnosis, prognostic assessment, follow-up monitoring, and assessment of treatment response.
An elevated level of HCT (Human carcitonin) is associated with medullar thyroid cancer with or without metastases.

**Calcitonin as a Screening Test**

- Calcitonin is the most useful in screening of asymptomatic familial medullar carcinoma of the thyroid gland, autosomal dominant disorder.
- Asymptomatic family members of the affected individuals benefit from the screening with the computer tomography because the basal levels of calcitonin are increased in such populations.
- There are many difficulties associated with using calcitonin as a screening tool, in part related to its lack of perfect specificity or sensitivity with regard to MTC.
- In addition, serum calcitonin can be elevated in a variety of systemic conditions, including: chronic renal insufficiency, patients with hypergastrinemia, smoking, and in patients with neuroendocrine tumors of the lung, pancreas, prostate and in leukemia.

Briefly, CT screening of MTC is a highly sensitive test for early diagnosis of MTC.

Calcitonin may be used diagnostically as a tumor marker for medullary thyroid cancer (MTC).

It may even be used on biopsy samples from suspicious lesions (e.g., swollen lymph nodes) to establish whether they are metastasis of the original cancer.

Normal range of calcitonin is below 5 to 12 pg/ml.
Cutoffs (reference value) for calcitonin to distinguish cases with medullary thyroid cancer have been suggested to be as follows, with a higher value increasing the medullary thyroid cancer:

- Females: 5 ng/L or pg/mL
- Males: 12 ng/L or pg/mL
- Children under 6 months of age: 40 ng/L or pg/mL
- Children between 6 months and 3 years of age: 15 ng/L or pg/mL.

Use of Calcitonin in detection of MTC recurrence

Tumor markers are also used to look for cancer that may have come back (recur) after treatment.

If a cancer recurrence is detected, surgery to remove the disease is usually the best option.

Patients who have very high calcitonin levels after removal of the thyroid may have metastatic disease in the liver or bones.

Recurrent disease develops in approximately 50% of patients with MTC. Calcitonin levels are very sensitive ways for detecting either residual or recurrent disease.

When the postoperative calcitonin level is elevated, a careful metastatic evaluation must be performed prior to proceeding with operative exploration.
Use of Calcitonin in evaluating response to drug treatment regime

Measurement of serum calcitonin is important in the follow up of patients with medullar thyroid carcinoma (MTC) and reliably reflects the presence of the disease.

Measurement of serum calcitonin levels can also be used to assess the response of MTC to novel systemic treatments (e.g. tyrosine kinase inhibition).

A decrease in calcitonin concentrations during such treatment might, however, is related to drug-mediated inhibition of calcitonin synthesis and/or secretion.

Nonetheless, such reductions do indicate that the drug has successfully targeted the tumor cells.

Calcitonin testing is used primarily in the postoperative follow-up of patients with MTC.

Finally, measurement of serum calcitonin levels is also widely used as an index of the response of MTC to systemic treatment.
Use of calcitonin in monitoring disease progression

Calcitonin is serum tumor markers used to monitor the progression of MTC.

Calcitonin is directly proportional to MTC tumor mass, in most circumstances.

Additionally, there is evidence that a rapid rate of progression of calcitonin concentration correlates with increased aggressiveness of MTC.

Also calcitonin level helps us to monitor the progression of MTC in pre-operation and post-operation.

The pre-operative calcitonin levels correlate with tumor size and disease stage:

- Calcitonin levels < 100 pg/ml is associated with a median tumor size of 3 mm.
- Calcitonin levels > 1000 pg/ml correlated with a median tumor diameter of 2.5 cm
- Nodal metastasis first can be observed at basal calcitonin levels of 10-40 pg/ml (normal range, <10 pg/ml).
- Distant metastasis and extrathyroidal growth begin appearing in patients with calcitonin levels of 150-400 pg/ml.

5. CATHECOLAMINE
**Definition**

Catecholamines are hormones produced by the adrenal glands. They are found on top of the kidneys. They are released into the blood during times of physical or emotional stress.

The major catecholamines are dopamine, norepinephrine, and epinephrine (which used to be called adrenalin).

Urine or a blood test can be done to measure the level of catecholamine in the body.

The adrenal glands make large amounts of catecholamines as a reaction to stress.

The main catecholamines are epinephrine (adrenaline), norepinephrine (noradrenaline), and dopamine.

They break down into vanillylmandelic acid (VMA) and metanephrine, which are passed in the urine.

Catecholamines increase heart rate, blood pressure, breathing rate, muscle strength, and mental alertness.

They also lower the amount of blood which is going to the skin and increase blood going to the major organs, such as the brain, heart, and kidneys.

The increased amount can cause high blood pressure, excessive sweating, headaches, fast heartbeats (palpitations), and tremors.

**DIAGNOSIS**
A catecholamine test is done to diagnose a tumor in the adrenal glands called a pheochromocytoma in symptomatic patients. It also may be ordered to monitor the effectiveness of treatment when a pheochromocytoma is discovered and removed, to monitor for recurrence.

The plasma test is most useful when the patient has persistent hypertension or is currently experiencing an episode of hypertension. This is because the hormones do not linger in the blood.

**PLASMA TEST**

The plasma test is most useful when the patient has persistent hypertension or is currently experiencing an episode of hypertension. This is because the hormones do not linger in the blood;

**URINE TEST**

Urine catecholamine testing measures the total amount of catecholamines released in 24 hours. Since the hormone levels may fluctuate significantly during this period, the urine test may detect excess production that is missed with the blood test.

Plasma and urine tests may be ordered together or separately to look for excessive amounts of both catecholamines and their metabolites.
Since these tests are affected by drugs, foods, and stresses, there will be a certain number of false positives. For this reason, catecholamine testing is not recommended as a screen for the general public.

Doctors will frequently investigate a positive result by evaluating a patient’s stresses, work to alter or minimize any influences, and then repeat the test to confirm the original findings.

Occasionally, the tests may be ordered on an asymptomatic person if an adrenal or neuroendocrine tumor is detected during a scan that is done for another purpose or if the patient has a strong personal or family history of pheochromocytoma.

This is a 24 hour urine sample.

You may be asked to avoid the following foods and fluids for 2 to 3 days before having this test because they may cause false positive results:

1. Caffeine: such as coffee, tea, cocoa, and chocolate
2. Amines: These are found in bananas, walnuts, avocados, fava beans, cheese, beer, and red wine.
3. Any foods or fluids with vanilla
4. Licorice(root):mostly used as sweetener in candies and beverages.
5. Aspirin

Drink plenty of fluids during the 24-hour time period to avoid dehydration.
For this test, you must urinate into a special bag or container every time you use the bathroom for 24-hour period.

You start collecting your urine in the morning. When you first get up, empty your bladder but do not save this urine. Write down the time that you urinated to mark the beginning of your 24-hour collection period.

Write down the time that you urinated to mark the beginning of your 24-hour collection period.

For the next 24 hours, collect all your urine.

Your doctor or lab will usually provide you with a large container that holds about (4 L).

The container has a small amount of preservative in it.

Urinate into a small, clean container and then pour the urine into the large container.

Do not touch the inside of the container with your fingers.

Keep the large container in the refrigerator for the 24 hours.

Empty your bladder for the final time at or just before the end of the 24-hour period. Add this urine to the large container and record the time.
Normal Results

All of the catecholamine are broken down into inactive substances that appear in the urine:

a. Dopamine becomes homovanillic acid (HVA)

b. Norepinephrine becomes normetanephrine and vanillylmandelic acid (VMA)

c. Epinephrine becomes metanephrine and VMA

BLOOD

- Epinephrine: 0-900 picograms/ml (pg/ml)
- Norepinephrine: 0-600 pg/ml

Higher-than-normal levels of blood catecholamines may suggest:

- Acute anxiety
- Ganglioneuroblastoma (very rare)
- Ganglioneuroma (very rare)
- Neuroblastoma (rare)
- Pheochromocytoma (rare)
- Severe stress
The following normal values are the amount of the substance found in the urine over a 24-hour period:

1. Dopamine: 65 - 400 micrograms (mcg)/24 hours.
2. Metanephrine: 24 - 96 mcg/24 hours (some laboratories give the range as 140 - 785 mcg/24-hours).
3. Normetanephrine: 75 - 375 mcg/24 hours
4. VMA: 2 - 7 milligrams (mg)/24 hours

**Test result**

Since the catecholamine test is sensitive to many outside influences and pheochromocytomas are rare, a doctor may see more false positives with this test than true positives.

If a symptomatic patient has large amounts of catecholamines in her blood and urine, further investigation is indicated.

Serious illnesses and stresses can cause moderate to large temporary increases in catecholamine levels

Doctors must evaluate the patient as a whole - her physical condition, emotional state, medications, and diet

When interfering substances and conditions are found and resolved, the doctor will frequently re-test the patient to determine whether the catecholamines are still elevated.

The doctor may also order blood and/or urine metanephrine testing to help confirm his findings and imaging tests, such as an MRI, to help find the tumor(s).
If levels are elevated in a patient who has had a previous pheochromocytoma, then it is likely that either treatment was not fully effective or that the tumor is recurring.

If the concentrations of catecholamines are normal in both the plasma and urine, then it is unlikely that a patient has a pheochromocytoma.

Pheochromocytomas do not necessarily produce catecholamines at a constant rate.

However, if the patient has not had a recent paroxysm of hypertension, their plasma and urine concentrations of catecholamines could be at normal or near.

6.PROSTATE SPECIFIC ANTIGEN

Definition
Prostate-specific antigen (PSA) is a substance made by cells in the prostate gland (both normal cells and cancer cells).

It is produced exclusively by the epithelial cells of the acini and ducts of the prostate gland. It is specific for prostate tissue.

PSA functions to liquefy seminal fluid and has been widely used as a clinical marker for the diagnosis and staging of prostate cancer.

It was generally believed that a “normal” PSA level was anything between 0 and 4 microg/l.

PSA exists into two molecular forms in blood circulation.

The majority of PSA is complexed with protease inhibitor alpha1 antichymotrypsin (ACT) (100kD) or with alpha2 macroglobulin (A2M) and minor component of free PSA (28.43Kd).

Most immunoassays measure both free and ACT-Complexed PSA but not A2M-PSA.

The half life of PSA is ranged from 2 to 3 days.

PSA is useful tumor marker for prostate cancer. It is used to detect stage and monitor treatment of prostate cancer.

**PSA as screening test for prostate cancer**
Because it is specific for prostate tissue not for prostate cancer, PSA testing itself is not effective in screening or detecting the early prostate cancer. It is requested together with other tests, like digital rectal examination.

Localized prostate cancer generally does not usually cause any clinical symptoms. Men usually feel perfectly well.

Prostate biopsy to detect prostate cancer is commonly initiated by an abnormal prostate.

To improve the ability of PSA testing to detect early prostate cancer, several approaches have been suggested:

- One approach is to use age adjusted reference intervals:
  
  0 to 2.5 microg/l for men aged from 40 to 49,
  
  0 to 3.5 microg/l for men aged from 50 to 59,
  
  0 to 4.5 microg/l for those aged from 60 to 69 and
  
  0 to 6.5 microg/l for men aged from 70 to 79.

PSA levels are normally higher in older men than in younger men, even when there is no cancer.
Another approach is to use PSA density (i.e. divide PSA concentration by the prostate volume, determined by transrectal ultrasonography)

The PSA density (PSAD) is sometimes used for men with large prostate glands to try to adjust for this. A higher PSA density (PSAD) indicates a greater likelihood of cancer.

➢ The third approach, is use of PSA velocity (that is the rate of PSA increase as function of time).

Normally, PSA levels go up slowly with age.

Some research has found that these levels go up faster if a man has cancer, but studies have not shown that the PSA velocity is more helpful than the PSA level itself in finding prostate cancer.

PSA as diagnostic test for prostate cancer
When prostate cancer develops, the PSA level usually goes above 4 microg/l.

Still, a level below 4 does not guarantee that a man doesn't have cancer – about 15% of men with a PSA below 4 will have prostate cancer on biopsy.

Men with a borderline PSA level between 4 and 10 have about 1 in 4 chance of having prostate cancer.

If the PSA is more than 10, the chance of having prostate cancer is more than 50%.

**PSA in monitoring disease progression**

The surgery removes all prostate tissue. Because PSA is produced exclusively by the prostate tissue; after radical prostatectomy, the PSA level should fall below the detection limit of the assay.

This drop may require 2 to 3 weeks because of the 2 to 3 days half life of PSA.

If the half life is longer than normal, a residual tumor is assumed to be present.

PSA levels should be measured every 3 months during the first year after surgery, every 4 months in the second year and every six months thereafter.

**PSA in detecting recurrence of cancer**
A recurrence means that the prostate cancer has returned after initial treatment. PSA determination is used to detect disease recurrence after treatment.

Serum PSA should decrease and remain at undetectable levels after local treatment such as radical prostatectomy.

Following initial therapy, a PSA increase is a strong indication recurrence of prostate cancer.

**PSA in evaluating response to drug treatment regime.**

PSA is not only increased in prostate cancer.

**Other conditions which can increase PSA level include:**

- benign prostatic hyperplasia (BPH), an enlarged prostate condition common in older men.
- The level of PSA may also be high in men who have an infection or inflammation of the prostate (prostatitis).
- Sexual activity (ejaculation) may also briefly raise PSA levels.
- Because of those conditions, research have shown that some patients present an increased level of PSA but doctors still challenged with negative biopsies (no detection of prostate cancer).
- For this reason, measuring the change in PSA levels after treatment with drugs may help diagnose aggressive prostate cancer in patients who have consistently abnormal PSA readings despite negative biopsies.
These drugs when used, reduce the level of PSA greater in Men found to have prostate disease than those who have prostate cancer.

Use of these drugs for PSA treatment can help to differentiate prostate cancer from benign prostate disease in patients who are difficult to diagnose.

7. CANCER ANTIGENS 125
CA-125 (cancer antigen 125, carcinoma antigen 125, or carbohydrate antigen 125) also known as mucin 16 or MUC16 is a protein that in humans is encoded by the MUC16 gene. MUC16 is a member of the mucin family glycoproteins. CA-125 has found application as a tumor marker or biomarker that may be elevated in the blood of some patients with specific types of cancers, or other conditions that are benign.

CA 125 is an antigen present on 80% of non mucinous ovarian carcinomas. It circulates in the serum of patients with ovarian carcinoma and was therefore investigated for possible use as a marker.

CA 125 is often elevated in patients with ovarian cancer, its level following the patient's clinical course.

With surgical resection or chemotherapy, the level correlates with patient response.

The CA 125 is elevated in other cancers including endometrial, pancreatic, lung, breast, and colon cancer, and in menstruation, pregnancy, endometriosis, and other gynecologic and non gynecologic conditions.

CA 125 is a glycoprotein normally expressed in coelomic epithelium during fetal development. This epithelium lines body cavities and envelopes the ovaries.

Elevated CA 125 values most often are associated with epithelial ovarian cancer, although levels also can be increased in other malignancies.
CA 125 levels are elevated in about 85% of women with ovarian cancer, but in only 50% of those with stage I disease.

Higher levels are associated with increasing bulk of disease and are highest in tumors of the ovaries.

Multiple benign disorders also are associated with CA 125 elevations.

In the largest study, CA 125 levels were monitored in all patients annually for three years, and elevated values prompted ultrasound imaging examinations.

Randomized trials are being conducted to assess the CA 125 in ovarian cancer.

Annual ultrasound examination and CA 125 screening have been advocated for women with hereditary ovarian cancer syndromes.

CA 125 has been used as an adjunct in the diagnosis of pelvic masses.

In postmenopausal women with asymptomatic palpable pelvic masses,

CA 125 levels higher than 65 U/mL have a positive predictive value of 98% for ovarian cancer.

Because premenopausal women have more benign causes of elevated CA 125 levels, testing for the marker is less useful in this population.

Currently, ovarian cancer is treated with maximal surgical reduction, which leaves minimal clinical or radiographic disease.
Because studies have demonstrated concordance of CA 125 levels with disease activity, oncologists rely on CA 125 levels to guide therapeutic decisions.

After definitive treatment of ovarian cancer, CA 125 levels should be obtained every three months for two years, and with decreasing frequency thereafter.

Elevated CA 125 levels during follow-up nearly always indicate ovarian cancer recurrence.
LIPID

• Definition

  • An oily organic compound insoluble in water but soluble in organic solvents; essential structural component of living cells.

Lipids

Function

• Phospholipids are the essential building blocks of living cells. They allow the separation of soluble and insoluble materials

• Cell Wall structure and function
  • Cholesterol allow for cellular rigidity and permeability.

• Endocrine function
  • Cholesterol is essential in the formation of over 20 essential hormones.

• Transport and Storage of energy

• With the aid of lipoproteins lipids are transported from liver to tissue for storage and oxidation to ATP.

• Neurologic development

• The myelin insulation around neural axons and glial cells, which is mostly lipid, brings the fat content of an animal brain to about 60%.
Maternal high lipid intake is necessary for the development of the neonatal brain.

Clinical significance

Lipid malnutrition

- Reduction in polyunsaturated fatty acids
- Resulting in abnormal hemostasis and cellular communication.
- Reduction in cholesterol
- Precursors in cholesterol synthesis are reduced and thus cell wall dysfunction and reduction of sterol hormones.
- Reduced transport of lipid soluble vitamins (tocopherols, betacarotens) and lipid soluble drugs in low density, very low density and high density lipoproteins.
- Maternal and early childhood lipid malnutrition is associated with reduced in neurologic function in later adult life.

Hyperlipidemia

- Hyperlipidemia is known to cause early mortality and morbidity.
- Hyperlipidemia is believed to be essential in the formation of atherosclerosis plaques that result in:
  - Heart attack
  - Stroke
  - Peripheral Vascular Disease
  - Premature dementia
Lipid Nomenclature

• As previously noted the definition of a lipid is An oily organic compound insoluble in water but soluble in organic solvents; essential structural component of living cells.
• The pathophysiologic study of lipids requires the understanding of terms that relate to the formation, transport and function of lipids.

Lipid Vocabulary

• Phospholipids
  • Class of lipids that are the major component of cell walls.
    • Form a bilayer that surround and protect cells
    • Can form single layer spheres that allow for the transport of insoluble or non-polar lipids in the aqueous environment of the body.
• Non-polar
  • Non-polar molecule: A molecule in which the electrons are shared equally between the nuclei. As a result, the distribution of charge is even and the force of attraction between different molecules is small.
  • Examples of household non-polar compounds include fats, oil and petrol/gasoline. Most non-polar molecules are water insoluble.
• Polar
  
  • Polarity refers to the unequal sharing of electrons in a molecule.
  
  • Usually due to the asymmetric shape of a molecule
• Hybrids (Amphipathic)
  • Many biological macromolecules can have both polar and non-polar components.
    • It is this property that allows lipids to function as transport molecules in an aqueous or watery environment.
    • The phospholipids we discussed earlier are a perfect example.
    • Cholesterol is also a hybrid molecule.
Apolipoproteins

- Apolipoproteins are proteins that bind to lipids
- Enzyme cofactors (C-II for lipoprotein lipase and A-I for lecithin)
- Lipid transport proteins
- Ligands for interaction with lipoprotein receptors in tissues (apoE for LDL-receptors, apoA-I for HDL receptors)

Lipoproteins

- A lipoprotein is a biochemical assembly that contains both proteins and lipids.
  - Enzymes, transporters, structural proteins, antigens, and toxins are lipoproteins.
  - LDL, HDL, IDL, VLDL are all transport lipoproteins that are responsible for the transport of cholesterol and triglycerides to and from tissues stores.
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**Cholesterol**

- Cholesterol is a waxy steroid metabolite found in the cell membranes and transported in the blood of all animals.
- It is formed from the Amphipathic sterol molecule.

![Cholesterol molecule](image)

**Synthesis**

- When intracellular sterol levels are low, genes for the LDL receptor and HMG-CoA reductase are upregulated.
  - In the absence of sterol molecules SREBP (Sterol Regulatory Element Binding Protein) are released from the Endoplasmic reticulum.
• They are transported to nuclear receptors where they regulate the formation of HMG-CoA reductase and LDL receptors.

• This in turn increases the uptake of cholesterol from the circulation and the synthesis of cholesterol.

**Regulation**

• Biosynthesis of cholesterol is directly regulated by the cholesterol levels present.

• When total intake of cholesterol is reduced the rate limiting enzyme HMG-coA reductase is up regulated to begin the formation of endogenous cholesterol production.

• When total intake of cholesterol is increased HMG-coA reductase is inhibited.

• Drugs that inhibit HMG-coA reductase result in the reduction of total body cholesterol.
Cholesterol Function

• Cholesterol is required to build and maintains membranes

• Regulates membrane rigidity over a large range of physical conditions.

• Reduces the permeability of the membrane to protons and sodium ions.

• Function intracellular as a transport molecule

Is the precursor to

• Vitamin D
• Aldosterone
• Progesterone
• Estrogens
• Testosterone
• Cortisol
• Progestin
• Is converted in the liver to bile which is necessary for the absorption of fat soluble vitamins

• A
• D
• E
• K
**Production**

- The body makes about 1 gram of cholesterol per day.
- 25% of the total cholesterol production occurs in the liver with the remaining 75% split between the intestines, ovaries and testes, and adrenal glands.
- Total body cholesterol synthesis is about 1 g (1,000 mg) per day.

**Intake**

- Typical daily additional dietary intake is about 200 mg.
- Animal fats are the primary source of cholesterol in the diet.
- Eggs, cheese, beef, pork, poultry, shrimp and human breast milk contain large amounts of cholesterol.
- Cholesterol is not present in plant based foods unless it was artificially added.

**Bile**

- Bile or gall is a dark green to yellowish brown fluid, produced by the liver of most vertebrates, that aids the digestion of lipids in the small intestine.
- In humans, bile is produced continuously by the liver (liver bile), and stored and concentrated in the gallbladder (gallbladder bile).
- After eating, this stored bile is discharged into the duodenum. The composition of gallbladder bile is 97% water, 0.7% bile
salts, 0.2% bilirubin, 0.51% fats (cholesterol, fatty acids and lecithin)

**Triacylglycerol (Triglycerides)**

**Synthesis**
- Triglycerides are formed from a single molecule of glycerol, combined with three fatty acids on each of the OH groups, and make up most of fats digested by humans. Ester bonds form between each fatty acid and the glycerol molecule.

**Function**
- The primary function of triglycerides is the storage of energy.
- Triglycerides provide 9 kcal/gram compared to glycogen’s 4 kcal/gram.
  - This makes triglycerides twice as efficient in storing energy.
  - In addition the anhydrous nature of triglycerides allow them to be stored more efficiently than carbohydrates.

**Absorption**
- Triglycerides cannot be absorbed by the intestines and thus must be broken down to their basic parts to be absorbed.
• Once broken down they are actively transported across the intestinal membrane.
• Once inside the enterocytes the individual components are pieced back together and packaged with cholesterol and proteins to make the exogenously formed chylomicron.

Lipoproteins

Chylomicrons
• Are formed from exogenous or ingested cholesterol, proteins and triglycerides
• Chylomicrons are the largest and least dense lipoproteins in circulation.

Structure
• They are composed of 85% triglycerides and only a very small amount of cholesterol.
• Apolipoproteins present
  • APOB48
  • APOE
  • APOC2
Stages of Chylomicron metabolism

- **Nascent (new or immature)**
  - These particles are light and fluffy and are the least dense of all lipoproteins.
  - Produced by the enterocytes of the small intestines
  - They are released by exocytosis into the lymphatic vessels originating in the small intestines.
  - They are dumped into the blood stream at the communication between the thoracic duct and the subclavian vein.

- **Mature chylomicrons**
  - During circulation the chylomicrons interact with HDL to accumulate to other apolipoproteins and is converted to mature chylomicrons
    - APOE and APOC2 are added in circulation
  - APOC2 allows the mature chylomicron to interact with the capillary lipoprotein lipase which releases free fatty acids, allowing for storage and future use as energy.
Chylomicron remnant

• Once triglyceride stores are distributed, the chylomicron returns APOC2 to the HDL (but keeps APOE), and, thus, becomes a chylomicron remnant.

• At this point the CM remnant returns to the liver where the remaining APOB48 and APOE are used to identify and remove the particle from circulation.

VLDL

• Very-low density lipoprotein

  • Is made in the liver and is considered the endogenous form of triglyceride packaging and transportation.

  • Made up of 50% triglycerides

  • Primary apolipoprotein

    • APOB100

    • APOE

    • APOC2

Synthesis

• APOB100 is synthesized intracellularly and in the presence of adequate levels of endogenously manufactured or recycled triglycerides is used to manufacture VLDL.
- VLDL is then excreted into the hepatic circulation and meets with HDL and undergoes transformation similar to chylomicrons Circulation and metabolism

**Nascent VLDL**

- Meets HDL in the circulation and acquires APOC2 and APOE.
- The VLDL is now considered mature VLDL.

**Mature VLDL**

- VLDL interacts with Capillary LPL via the APOC2 and gives up triglycerides
  VLDL for storage or energy production. HDL also transfers cholesteryl esters to the VLDL in exchange for phospholipids and triglycerides via cholesterylester transfer protein (CETP). As more and more triglycerides are removed from the VLDL because of the action of LPL and CETP enzymes, the composition of the molecule changes.
  
  - As the molecule becomes smaller due to the loss of triglycerides.
    - At this point the VLDL becomes Intermediate Density Lipoprotein (IDL)
      - 50% of IDL is recognized by the liver by the APOB100 molecule and are removed from the circulation and disassembled.
The remaining 50% of IDLs continue to lose triglycerides and eventually their cholesterol content exceeds their triglyceride content become LDL.

**LDL**

- **Low Density Lipoprotein**
  - Is the primary transporter of cholesterol to peripheral tissue.
  - Is the end product of VLDL metabolism
    - After VLDL interacts with capillary LPL the resulting by product is IDL.
    - As noted above, 50% of IDL remains in circulation where the majority of its remaining triglycerides are stripped resulting in the formation of LDL.

**Regulation**

- As noted previously when the intracellular environment is deficient in cholesterol the LDL receptor is up-regulated to increase the capture and absorption of cholesterol into the cell.
- The introduction of cholesterol into the cell results in the down-regulation of the LDL receptor.
**LDL**

**Subtypes**

- LDL particles can be separated on the basis of size and density into several different subclasses.
- A predominance of small LDL is referred to as the pattern B phenotype.
- Pattern B phenotype is one manifestation of what has been termed the Atherogenic Lipid Profile, a dominant inherited condition which also includes low levels of HDL-C, raised triglycerides, and insulin resistance.
- The presence of the pattern B phenotype increases the risk for clinical coronary heart disease by several fold.

**Atherosclerosis of Subtype B**

- The endothelium of the small and large vessels of the circulator system allow for particles smaller than 260 Angstroms to enter.
  - Subtype A LDL particles are greater than 260A in size and thus will not pass through the endothelium.
  - Subtypes in the B range are small enough to diffuse through the endothelium and there begin to form plaques resulting in atherosclerosis.
• **Oxidation (Loss of electrons)**
  • In pathologic states such as diabetes, smoking, metabolic syndrome, excessive intake of trans fatty acids LDL particles can be stripped of electrons.
    • This results in the binding of oxidized LDL to endothelium followed by cellular destruction.
    • This damage results in the recruitment of macrophages which results in further inflammation, thickening and damage to the vascular wall.

**HDL**

• **Structure**
  • HDL is the smallest of the lipoproteins
  • It contains APOA1 and APOA2.
    • In the genetic absence of APOA1 there is a significant reduction or absence of HDL resulting in pathologic disease.

**Function**

• HDL is responsible for the transfer of cholesterol from peripheral tissue back to the liver for recycling or excretion.

**Metabolism**

• Synthesized from lipid-poor APOA1 that is released from the liver as an empty flat disc. (Like a flat balloon)
• As APOA1 interacts with peripheral cells.

• The cholesterol is converted to cholesterol esters (with long-chain fatty acids linked to the hydroxyl group, are much less polar than free cholesterol and appear to be the preferred form for transport in plasma and as a biologically inert storage (de-toxification) form.) by LCAT (lecithin-cholesterol acyltransferase).
  • APOA1 is necessary for the interaction of HDL with LCAT.

• Cholesterol esters are then returned to the liver either by direct uptake of the HDL particle by scavenger receptor B1 (SR-B1)
  • As noted before HDL interacts with circulating VLDL and trades triglycerides for cholesterol esters through the action of CEPT (Cholesterol ester transfer protein).

Clinical Correlation

• The primary general correlation between lipids and disease is Atherosclerosis.
  • Atherosclerosis (also known as Arteriosclerotic Vascular Disease or ASVD) is the condition in which an artery wall thickens as the result of a build-up of fatty materials such as cholesterol.
Atherosclerosis

• Coronary heart disease (CHD), also called coronary artery disease (CAD), is a condition in which plaque builds up inside the coronary arteries. These arteries supply oxygen-rich blood to your heart muscle.

• Plaque is made up of fat, cholesterol, calcium, and other substances found in the blood. When plaque builds up in the arteries, the condition is called atherosclerosis. The buildup of plaque occurs over many years.

• Over time, plaque hardens and narrows your coronary arteries. This limits the flow of oxygen-rich blood to your heart muscle.

• Eventually, an area of plaque can rupture (break open). This causes a blood clot to form on the surface of the plaque. If the
clot becomes large enough, it can mostly or completely block blood flow through a coronary artery.

• If the flow of oxygen-rich blood to your heart muscle is reduced or blocked, angina or a heart attack may occur.

• Angina is chest pain or discomfort. It may feel like pressure or squeezing in your chest. The pain also may occur in your shoulders, arms, neck, jaw, or back. Angina pain may even feel like indigestion.

• A heart attack occurs if the flow of oxygen-rich blood to a section of heart muscle suddenly becomes blocked. If blood flow is not restored quickly, the section of heart muscle begins to die.

• Without quick treatment, a heart attack can lead to serious problems and even death.

• Over time, CHD (coronary heart disease) can weaken the heart muscle and lead to heart failure and arrhythmias.

• Heart failure is a condition in which your heart can't pump enough blood to meet your body’s needs.

• Arrhythmias are problems with the rate or rhythm of the heartbeat.

Causes

• The landmark study, suggest that LDL is the primary culprit of heart disease.
• Atherosclerosis is believed to develop from low-density lipoprotein molecules (LDL) becoming oxidized (ldl-ox) by free radicals.

• When oxidized LDL comes in contact with an artery wall, a series of reactions occur to repair the damage to the artery wall caused by oxidized LDL.

• The body's immune system responds to the damage to the artery wall caused by oxidized LDL by sending macrophages and T-lymphocytes to absorb the oxidized-LDL forming specialized foam cells.

• Unfortunately, these white blood cells are not able to process the oxidized-LDL, and ultimately grow then rupture, depositing a greater amount of oxidized cholesterol into the artery wall.

• Eventually, the artery becomes inflamed. The cholesterol plaque causes the muscle cells to enlarge and form a hard cover over the affected area. This hard cover is what causes a narrowing of the artery, reduces the blood flow and increases blood pressure.

Initially it appeared that LDL was the primary agent responsible for the most common cause of heart disease.

• Another analysis has found that other than high LDL-c are independently associated with heart disease.

  • Triglycerides are now known to be an independent risk factor for CAD (coronary artery disease).

  • Low HDL is now considered and independent risk factor in the pathogenesis of CAD.

  • Lp(a) has recently emerged as a risk factor for CAD.
Lp(a)

- Lipoprotein(a) is a unique particle essentially composed of an LDL particle and an additional adhesive protein designated apoprotein(a) APO(a).
- The adhesive properties of apo(a) are the cause for the selective retention of lipoprotein(a) in the vascular walls.

Causes of Hyperlipidemia

- **LPL deficiency or dysfunction**
  - LPL (capillary Lipoprotein lipase) is an enzyme required for the removal of triglycerides from chylomicrons and VLDL.
  - Causes
    - Genetic mutation resulting in reduced function or absent enzyme
      - Familial LPL deficiency
    - Loss of function due to over expression of inhibitors
      - Poorly controlled diabetes
      - LPL is dependent on insulin for activation and function
• In poorly controlled diabetes there is either reduced concentrations of insulin or the enzyme is resistant to the insulin present.

• APOC2 deficiency
  • Without APOC2 VLDL nor chylomicrons are unable to interact with LPL to release their triglyceride loads for storage.
    • This results in a syndrome clinically identical to LPL deficiency with high plasma triglycerides.

LPL deficiency or dysfunction

• Insulin resistance
  • In diabetes LPL is either genetically resistant to the insulin present or there is inadequate concentrations of insulin.
    • This results in incomplete breakdown of chylomicrons and VLDL resulting increased circulation of triglycerides.

Familial LPL deficiency
Familial Hypercholesterolemia

- FH is the result of genetically abnormal clearance of LDL at the liver or peripheral tissue.
- Absent LDL receptors in both the liver and peripheral tissue.

- Class I: LDLR is not synthesized at all.
- Class II: LDLR is not properly transported from the endoplasmic reticulum to the Golgi apparatus for expression on the cell surface.
- Class III: LDLR does not properly bind LDL on the cell surface because of a defect in either apolipoprotein B100.
• **Deficiency of APOB**
  
  • APOB is necessary for the binding of LDL to the LDL receptor. When APOB is missing the resulting clinical syndrome is nearly identical to absent or deficient LDL receptor.

  • Class IV: LDLR bound to LDL does not properly cluster in clathrin-coated pits for receptor-mediated endocytosis.

  • Class V: LDLR is not recycled back to the cell surface.

  • Results in total cholesterol levels over 7.8 mmol/L (300 mg/dL) and significant increase in LDL to levels of 6.5 mmol/L (250 mg/dL).

  • Results in significantly increased risk for heart disease.

  • Results in Xanthomas and Xanthelasma of the face.
Combined Hyperlipidemia

- **Two subtypes**
  - Acquired
    - Often the results of diabetes or metabolic syndrome
      - Increased triglycerides (as VLDL)
      - Increased LDL
      - Decreased HDL
  - Familial
    - Unknown genetic cause at this time
    - Follows a pattern of autosomal dominant
- Occurs in 1/100 individuals

**Disorders of HDL**

- **Familial Hypoalphalipoproteinemia**
  - Autosomal dominant
  - HDL reduced to less than 30 mg/dL
  - Increase risk for CAD

- **APOA1 deficiency**
  - APOA1 is needed for the enzyme LCAT to interact with HDL.
  - In order for the formation of HDL to proceed adequate amounts of the precursor APOA1 must be present.
  - Causes premature CAD (coronary artery disease)

- **Cholesterol Ester Transfer Protein Deficiency**
  - CETP allows for the transfer (or trade) of triglycerides and cholesterol from VLDL and chylomicrons to HDL.
    - When CETP is absent HDL cannot accept triglycerides from VLDL or chylomicrons.

- **LCAT deficiency**
  - Very rare but autosomal recessive
  - Lack of LCAT prevents the esterification of cholesterol to cholesterol esters
• This stops the reverse cholesterol transport function of HDL.

• This results in the accumulation of lipids throughout the body.
  • Premature vascular disease

**Tangier Disease**

• Autosomal recessive
• Decreased HDL
• Early vascular disease
• Defective ABCA1 transporters resulting in a greatly reduced ability to transport cholesterol out of their cells, which leads to an accumulation of cholesterol in many body tissues
  • Classic appearance includes orange tonsils

Tangier tonsils                                  Tangier hands

**Hypolipidemias**
• Familial Hypobetalipoproteinemia
  • Autosomal dominant
  • Total cholesterol levels less than 50 mg/dL
  • Low risk of vascular disease
  • Missing or defective apoB
    • Results in the decreased formation of liver lipoproteins
      • LDL, VLDL, IDL
    • In severe cases patients cannot create chylomicrons in the intestines and do not absorb fat soluble vitamins.

TEST PROCEDURES

Patient Preparation for lipid/Lipoprotein Testing

➢ Lipid/lipoprotein analyses maybe affected by a number of pre-analytical variables.
➢ Maintain customary lifestyle for 3 days prior to testing.
➢ Abstain from alcohol for preceding 24 hours.
➢ Fasting for at least 12 hours before sampling recommended (required for TG, apo B and calculated LDL-C).

➢ Remain sitting for at least 15 mins prior to sampling to avoid haemoconcentration resulting in falsely elevated lipid/lipoprotein.

➢ Phlebotomist should minimize tourniquet use.

➢ Testing normally done on serum or EDTA plasma; [] in EDTA plasma are 4.7% lower than serum due to osmotic shifts.

➢ For long term sample stability, EDTA may reduce oxidation and enzymatic cleavage of lipoproteins by chelating metal ions.

➢ Heparinized samples may be acceptable; however it activates lipoprotein lipase.

➢ Due to intra-individual variability and analytical variation, it is recommended that lipid/lipoprotein testing be performed on more than one occasion when evaluating a patient.

Appearance of plasma (serum) samples with elevation in lipoprotein fractions.

➢ Plasma or serum sample that appears milky is lipaemic.

➢ Physical appearance of a fasting plasma or serum sample after standing undisturbed at 4°C for 16 hrs may be informative in lipid/lipoprotein testing.

➢ Increased chylomicrons- creamy layer on top.

➢ Increased VLDL and or IDL- remains turbid or milky throughout.
Increased LDL-no turbidity, may have orange discoloration
Increased HDL-normal, clear.

CHAPTER IV
GENERAL PRINCIPLES OF TOXICOLOGY

The study of poisons

Poisons are chemical/physical agents that produce adverse responses in biological organisms

Any substance can be toxic if introduced in a dose capable of disturbing the normal physiological homeostasis of the exposed body

OTHER TERMS

Toxicants: are toxic substances from chemicals

Toxins: are poisonous substance produced within living cells or organisms

-venom
Toxicology

The science of poisons that studies toxic substances with respect to their:

- sources
- properties
- mechanism of toxicity
- toxic effects
- Detection
- clinical manifestations
- management

Sources

- Chemical sources: the commonest source
  E.g: drugs, corrosives
  - Plant source: e.g: hashish, cocaine
  - Animal source: the least but most serious source.
  venomous animals such as scorpions, spiders, snakes, wasps
Venomous & poisonous animals

Venomous & poisonous animals deliver or inject venom into other organisms, using a specialized apparatus of some kinds (usually fangs or a stinger). The venom is produced in a gland attached to this apparatus.

Poisonous animals do not deliver their toxins directly. The entire body, or large parts of it, may contain the poisonous substance. These organisms may be harmful when eaten or touched.

SITES OF TOXIC ACTIONS

Local (non specific):
Wherever the poison contacts the biological system it starts its harmful effect. It does not require specific site or receptor to elicit its effects such as toxicity by acids or alkalis.

Remote (systemic):
The poison affects a system far from its portal of entry.

Local & remote
The poison has the capacity of acting locally and systematically. Oxalic acid is an example of these poisons.
Duration and frequency of exposure

**Acute**: application of a single or short term (less than a day)dosing of a substance to cause toxicity.

**Sub acute**: toxicity is expressed after repeated applications for a duration less than half-life expectancy of the time.

Chronic: expression of toxic symptoms only after repeated exposure to a chemical in doses regularly applied to the organisms for a time greater than half of its life expectancy.

Chronicity index

The ratio of the acute to chronic.

Compounds with strong cumulative properties have larger chronicity index.

Types of toxic mechanisms

Direct: the poison itself can cause toxic effects as in corrosives.

Indirect: toxicity results from the interaction of the poison with the biological activity within biological system.

- Binding to cell membrane to change in their function or structure thus affecting their normality.
- Interference with enzymatic actions.
- Formation of metabolites which are more toxic than the parent poison.
-Effects on DNA.

**Classification of toxic agents**

1. According to the target organ they are acting on it (hepatotoxic, nephrotoxic).
2. According to their use (food additive, drug, pesticide)
3. According to their source (animal or plant)
4. According to their effects (carcinogen, mutagen)
5. According to their physical state (gas, liquid)
6. According to their chemistry (amine, hydrocarbon)
7. According to their poisoning potentiality (extremely toxic, slight toxic, etc)
8. According to their biochemical mechanism of action (alkalating agent, AchE inhibitor).

**Factors affecting action of poison**
A. factors related to the poison

1. Dose: A basic principle in toxicology
   - Dose is the amount of chemical that comes in contact with the body or gets inside the body.
   - The increase of dose will increase the severity of toxicity.

2. Physical status: Gaseous state is more toxic than liquid state than the solid state.

3. Purity: This depends on the impurity of the poison; if the impurities are more toxic than the poison, the toxicity will be more and vice versa.

B. Factors related to the individual:

- Age
- Health
- Sensitivity
- Sex

C. Factors related to mode of exposure:

- Inhalation
- Ingestion
- Skin contact

D. Factors related to environment
Temperature, pressure, humidity, radiation can cause alterations on poisons status

**Forensic Toxicology**

Forensic toxicology is concerned with the detection and estimation of poison for legal purposes.

- tissues and body fluids obtained at autopsy
- blood, urine, or gastric material obtained from a living person

Poisoning as a cause of death can be proven only with toxicologic analyses that demonstrate the presence of the poison in the tissues or body fluids.

Presence of poisons can be demonstrated only by chemical methods of isolation and identification.

If toxicological analyses are avoided, death may be ascribed to poisoning without definite proof.

**Analytical toxicology**
Deals with the detection, identification and quantification of poisons.

**Samples required for toxicological analysis**

- **Blood**
  The best place at autopsy is from femoral and iliac veins, the axillary veins in consequence.

  No samples from:
  1. Jugular veins: may be contaminated by reflux from upper thorax
  2. General body cavity: highly contaminated by intestinal contents
  3. Heart or great vessels in chest: postmortem diffusion of drugs and alcohol from the stomach or aspirated vomit contaminate these sites.

- **Urine**
  20-30ml urine in sterile container without preservatives

- **Faeces**
  Used in heavy metals as arsenic, lead, mercury

- **Vomit and stomach contents**

- **Organs**
  - The most common organ saved for analysis is liver
  - Bile can be helpful in morphine and chlorpromazine
  - Lungs in some cases of solvents
Hair and nails
- Heavy metal poisoning
- Recently, prolonged use of opiates

METHODS OF ANALYSIS

Qualitative methods

A. COLOR TEST
This is a rapid, easily performed, qualitative, screening test, but not specific method.
Can be used as rapid test.

Examples
1. Ferric chloride test for salicylates (pink-purple)
2. Zwikker test for barbiturates (purple color)
3. Formaldehyde-sulfuric acid test for Benzodiazepines (orange)
4. Mandalin Test for opioid (brown color)

B. CHEMICAL TEST
Reinsch Test is an initial indicator to detect the presence of one or more of the following heavy metals in a biological sample and is often used by toxicologists where poisoning by such metals is suspected:

- Antimony
- Arsenic
- Bismuth
- Selenium
- Thallium
- Mercury

**Procedure**

Dissolve suspect body fluid or tissue in a hydrochloric acid solution.

Insert a copper strip into the solution.

The appearance of a silvery coating on the copper may indicate **Mercury**. A dark coating indicative of the presence of **one of the other metals**.
QUANTITATIVE METHODS

A. CHROMATOLOGY

1. Thin-Layer Chromatography (TLC)

Mobile phase (a mixture of organic solvents such as chloroform and methanol) is run across a stationary phase (silica gel spread on a glass plate).

2. Gas chromatography - Mass spectrometry

Stationary phase is a liquid and the mobile phase (a carrier gas) is an inert gas such as helium or nitrogen.

3. High-Performance Liquid Chromatography (HPLC):

In HPLC the stationary phase is column packed with solid particles and the mobile phase is a liquid solvent.

1. THIN-LAYER CHROMATOGRAPHY

In TLC a mobile phase (a mixture of organic solvents such as chloroform, and methanol) is run across a stationary phase (silica gel spread on a glass plate).

The samples to be analyzed are spotted near the bottom portion of the plate and allowed to dry. Then the plate is placed upright into a chamber, with the bottom of the plate (where the sample has been spotted) in contact with the mobile phase. The
Mobile phase will then draw up across the plate by capillary action.

As the solvent moves past the samples, the components of the samples will migrate, with the speed of migration dependent upon the relative affinity of the components for the mobile phase compared to the stationary phase.

When the leading edge of the solvent reaches the top of the plate, it is removed from the solvent and allowed to dry. The location of the sample components can then be visualized.

STAHL provided methods for 264 stains or dyes that can be applied for the required component such as ninhydrin will react with amphetamine to give pink color.

Alternatively, a fluorescent dye can be incorporated in the solid phase, so that ultraviolet light can reveal the sample components as dark spots against the bright background.

The results of TLC can be quantified by using the retention factor (Rf) which is the ratio of the distance that a sample component moves to the distance that the leading edge of the solvent moves.

\[ Rf = \frac{\text{Sample distance movement}}{\text{Solvent distance movement}} \]

The results of TLC can be quantified by using the retention factor (Rf) which is the ratio of the distance that a sample component moves to the distance that the leading edge of the solvent moves.

Rf = Sample distance movement / solvent distance movement

High-performance liquid chromatography
In HPLC, the stationary phase is a column packed with solid particles and the mobile phase is a liquid solvent.

As the mobile phase is pumped through the column, the sample is injected. A detector then identifies the components as they exit the column. Components are identified by their retention time (the length of time they take to pass through the column). And the results are compared with standards.

A basic HPLC instrument has seven primary components

1. Solvent reservoir
2. Pump
3. Injector
Component of HPLC instrument

1. **Solvent reservoir**: Mobile phase solvents are contained in a glass reservoir.

2. **Pump**: The pump aspirates the mobile phase from the solvent reservoir and forces it through the systems column and detector.

3. **Injection systems**: A liquot of sample is introduced into a liquid chromatograph through the sample injector.

4. **Column**: Material such as silica, alumina, charcoal, and organic polymers are used as stationary supports for HPLC.

5. **Detector**: The function of a detector is to detect analytes as they elute from the chromatographic column. Usually the ultraviolet, visible light, and diode array absorbance detectors are used in the HPLC systems.

6. **System and data control microprocessor technology**: This technology can fulfill both process control and data processing functions. As a process controller, the microprocessor controls various parameters such as:
   
   - mobile phase composition
   - mobile phase flow rate
   - column-back pressure
   - column temperature
-detector temperature
-sample injection
-d detector selection
-d detector operation
- various steps of system operation.

CHAPTER V
PATHO-CHEMICAL ASPECT OF DRUG TOXICOLOGY

V. 1. Therapeutic Drug Monitoring
- The word *therapeutic* is an adjective that describes drug.
- TD produces healing or curative effect when undesirable physiological or psychological is present.

**Drug:** chemical used to selectively pertube specific tissues or specific functions of these tissues in organism

### Drug

- A drug is any substance (other than food that provides nutritional support) that, when inhaled, injected, consumed, absorbed via a patch on the skin, or dissolved under the tongue causes a physiological change in the body

#### Chemical considered drug if it has:

- selectivity to site of action or organ
- reversibility in its action and
- production of beneficial or therapeutic effect

- **Monitoring:** process of constant determination of the quantity of drug required to produce a predetermined desirable effect. (cessation of pain)
- All drugs cease to be therapeutic and become toxic.
- **TDM**: is a branch of clinical chemistry and clinical pharmacology that specializes in the measurement of medication concentrations in blood.

Its main focus is on drugs with a narrow therapeutic window.

- However, for a few drugs, measurement of drug levels in blood is essential to ensure a therapeutic effect without toxicity.

**Measurement of plasma or blood drug levels is required for:**

- drugs with narrow therapeutic range, e.g. lithium (0.6-1.0 mmol/L)
- for drugs which in overdose may produce symptoms similar to those of disease being treated, e.g. phenytoin
- Where drug may produce abnormalities in hepatic or renal.
- Where drug absorption may vary with dose or other.
- Establish a dose regimen when therapy has just begun or when it needs to be changed.
- To check for toxicity, if several drugs are being given.
  - However regular monitoring of patients on drugs is not usually required once patient has been stabilized on a dose of drug that has produced the desired clinical effect.

**Drug Action:**

- Mechanism of action- elicit a biological result.
• Response from drug dependant on dose administered, but only to a certain patient.

At some patient, drug dose will not increase the biological response.

Terms use in TDM:

• **Min. effective** (*MEC*) : lowest of drug in blood that will produce the desired effect.

• **Min. toxic** (*MTC*): lowest of drug in blood that will produce an adverse response.

• **Therapeutic index** (also referred to as **therapeutic ratio**) is a comparison of the amount of a therapeutic agent that causes the **therapeutic** effect to the amount that causes toxicity.

![Graph showing the relationship between dose and response](image)

*Other terms used in TDM*

**Trough**: lowest of drug in blood

**Peak** is the highest of drug measured in blood.

**Half-life** (*t₁/₂*) of drug indicates the time required for elimination
Why therapeutic drug monitoring?

- Therapeutic drug monitoring can guide the clinician to provide effective and safe drug therapy in the individual patient using serum drug concentration.

Why should drug level be monitored?

- Certain drugs may have a narrow therapeutic range in concentrations above the upper limit of the range, the drug can be toxic.

In concentrations below the lower limit of the range, the drug can be ineffective. Not all patients have the same response at similar doses.
Therapeutic range/therapeutic window

- The therapeutic range/therapeutic window is the concentration range of drug in plasma where the drug has been shown to be efficacious without causing toxic effects in most people.

Therapeutic Drug Monitoring

DRUG DISPOSITION:

- A drug follows a certain route from its initial exposure in body to production of pharmacological response.
• Drug must undergo series of steps for it to be removed from body.

The steps are **absorption, distribution, biotransformation** and **excretion**

Administered Drug

\[
\downarrow
\]

Absorption

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\downarrow
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Blood

\[
\downarrow
\]

Protein bound ↔ free drug

\[
\downarrow
\]

Distribution

\[
\downarrow
\]

Tissues

\[
\downarrow
\]

Pharmacological Action

\[
\downarrow
\]

\[
\downarrow
\]

Biotransformation
Excretion

Process of pharmacokinetics

Absorption:

• Process of drug uptake into body enters blood.
• Different site of absorption (stomach, intestines)
• Drugs administered by oral, rectal, or sublingual routes absorbed in GIT.
• Transported to liver by hepatic portal vein.
• Undergo first pass effect
• Drug on its 1st pass thru liver is substantially metabolized before reaching the systemic circulation.
• 1st pass effects of particular drugs have to be taken into account when determining dose of drug required to produce desired response.
• Drugs that are injected are said to be administered parenterally.

Parenteral admin includes intravenous, intramuscular, intradermal (skin) and subcutaneous (beneath the skin).

• Other routes of admin are inhalation of gaseous drugs (respiratory).

Distribution:

• -Drug must be transported to site of action.
• -Distributed to all organs and fluid compartments through blood.
**Biotransformation (metabolism)**

Occurs in mainly liver,

**second sites include:**

- lungs
  - Kidneys
  - skin
  - brain and
  - GIT.

**Metabolism may affect drug in one of 3 ways**

- Increase its activity (activation)
- Decrease its activity (inactivation)
- Have no effect on its activity

**Two major metabolic pathways: phase I and II.**

**Phase I:** reactions metabolize lipophilic drugs to more polar forms for renal excretion, through oxidative, reduction, processes.

- e.g. hydroxylation, deamination, sulphoxidation, in which small groups are added or removed from drug.
Phase II reactions:

- Involves conjugation of drugs with compounds like glutathione, sulfonic acid, glucoronic acid or amino acids particularly glycine to facilitate their elimination.

Excretion

- Water soluble drugs or conjugated drugs through metabolism eliminated from body through urine.

- Acidic urine facilitates elimination of basic drugs and vice versa

When should blood sample be taken?

- When single dose is taken for 1st time, plasma concentration will rise rapidly and then decline.

- Blood samples often withdrawn just before dose of drug is taken.
• In all cases, time of blood sampling and of last dose of drug must be noted on the request form, including drugs being monitored.

• **Clinical status of patient**, when sample was collected, dosage, route of administration etc.

• To ensure correct specimen collection and accurate patient results requires frequent open communication between lab physicians, nurses and pharmacists.

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**Interpretation of drug levels**

• If the blood has been taken at the appropriate time, the plasma level can be compared with published therapeutic ranges.

• These published therapeutic ranges indicate the range of plasma drug level which in the majority of the population have been shown to provide the desired therapeutic effect without a high risk of toxicity.

• PTR(*Prospective thematic review*) indicate the range of plasma drug levels which in the majority of the population have been shown to provide the desired therapeutic effect without a high risk of toxicity.

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**Clinical information**

There is no allowance for possible effects of hepatic or renal disease in individuals or interactions from prescribing combinations.
• E.g: In patients with renal impairment, drugs will be eliminated from circulation at slower rate than normal.

• **Alternatively**, in patients taking drugs that result in the induction of hepatic drug-metabolized systems, the clearance of other drugs being taken simultaneously might be enhanced.

**Free and bound drugs:**

• Most drugs circulate bound to plasma proteins.

• However, pharmacological response, is determined by tissue concn which in turn is related to plasma bound drug concn.

• Free drug concn is difficult to measure hence, TDM relies on measurements of plasma total drug concn.

**ANALYTICAL TECHNIQUES:**

• HPLC and GLC used have the advantage of analyzing several different drugs simultaneously **as well as** separating closely related compounds and parent drugs and metabolites.

• Not routinely used by labs because of high cost and labor intensive nature of instrumentation.

• Automated immunoassays have been developed.

• Chromatographic and immunoassays measure total drug concentration
e.g: protein bound and free.

TDM depends on measurements of plasma total drug concentration

**PHENYTOIN**

**Specific Drugs Anticonvulsants**

1. **Phenytoin**

   - The phenytoin test is used to measure and monitor the amount of phenytoin in the blood and to determine whether drug concentration are in the therapeutic range. It may be ordered
every few days when a person first begins taking phenytoin to help adjust the dose to the desired blood level.

• Most widely prescribed antiepileptic drug but also used for cardiac dysrhythmias.
• Therapeutic concentration is 10 to 20 µg/mL
• Has undesirable side effects, include neurotoxicity.

**TDM required in new patients**, where there is unexpected loss of control, or when other drugs that interact with phenytoin are added.

• **At concentration less than 10 µg/mL**, half life is one day, but increase as the drug concentration increases.

2. **LITHIUM**

• Used in treatment of manic depressive illness (bipolar personality disorder).

• **Therapeutic action is thought to involve its ability** to substitute for Na+ and K+ ions in cellular transport and to decrease catecholamine activity.
• Because Li+ can decrease Na+ and K+ levels, patients treated concomitantly with Li+ and other drugs affecting ion loss, such as diuretics, must be frequently dosed, and receive ion supplementation, and be carefully monitored.

• Has short half life, plasma conc determined 12hrs after.

• Administered as Li carbonate.

• Has short half life (10-35hrs) and plasma levels should be determined 12hrs after.

• TDM is essential because drug is toxic, producing a range of symptoms including polyuria, hypothyroidism, muscle twitching and rigidity and in severe cases, renal failure and coma.

• Patients with plasma Li+ concentration above 1.4mmol/L are at risk of oliguria and acute renal failure.

TDM may also be necessary to monitor compliance

DIGOXIN

• Cardiac glycosides, which are a class of organic compound that increase the output force of the heart and decrease its rate of contractions

• Used in treatment of congestive heart failure.
• **Has little clinical effect** at plasma concentration **below 1nmol/L**.

• Toxicity occurs at above 3.8nmol/L and manifests as cardiac arrhythmias and vomiting.

• Digoxin results should always be interpreted together with plasma K+ concentration, **since** hypokalemia potentiates cardiac digoxin toxicity.

• Thus, toxic effects of the drug may occur in hypokalaemic patients who have a plasma digoxin within the therapeutic range.

• K+ is monitored and is often supplemented in patients on treatment with digoxin

• Digoxin has a low therapeutic range (1.0-2.6nmol/L)

• Digoxin concentrates in cardiac tissue.

• The **therapeutic levels** are determined 8hrs after dosing.

**THEOPHYLLINE**

• Is a central nervous system stimulant, respiratory stimulant, and cardiac stimulant, used to prevent or treat bronchoconstriction in children or elderly who can not use inhaler.
• Therapeutic range in adults (10-20µg/L) and in neonates 5-10µg/L.

• **Half life** may **vary** depending on such factors as age, but is normally 8hrs in adults and 30hrs in neonates.

• Drug commonly causes **minor side effects** such as nausea and headache even at concentration within therapeutic range.

• **Serious toxicity** leading to cardiac arrhythmia can occur with plasma levels above 20 µg/L.

**CYCLOSPORIN**

• Used to prevent graft rejection following transplantation.

• Nephrotoxic with signs that might mimic rejection in patients with renal transplants.

• Tacrolimus is a relatively new immunosuppressive drug that has replaced cyclosporin in some transplant centers.

• Less toxic

• TDM still required to ensure efficacy

**AMINOGLYCOSIDE ANTIBIOTICS**

• Streptomycin, gentamycin, kanamycin, tobramycin and neomycin.
• Contain amino sugars in glycosidic linkages
• Treatment of gram –ve infections
• Bind to bacterial ribosome, causing inhibition of protein synthesis.
• Intravenous or intramuscular injection.
• Polar compounds, do not cross cell membranes and are rapidly excreted by normal kidney.
• Very short half-life (2-3hrs) if renal function is normal.
• Gentamycin is nephrotoxic and ototoxic (involving auditory) and vestibular(balance) may be permanent
• TDM is important in patients with Rheumatoid factor who receive the drug for more than 7 days, or those on high loading doses for serious infection

V.2. CHEMICAL TOXICOLOGY
• **Toxicology** is the study of poisonous substances, their actions on the living organism, their detection by lab and other methods and measures taken to counter their biologic effects.

• Poisoning is one of the commonest causes of emergency admission to hospital

• Accidental, intentional more common.

• Unfortunately, few of these drugs commonly taken in overdose have specific clinical signs

**Lab can confirm toxicity.**

• When patient is unconscious, lab can be asked to perform drug screen to identify which drugs and poisons may have been taken.

**Treatment:**

• A few drugs have a specific antidote.

• Patient is treated conservatively until drug has been eliminated from the body.
• If there is impaired renal, it may be necessary to use haemodialysis to eliminate the drug from the body and in such cases measurement of plasma levels is important.
• Online computer databases now readily available to aid the doctor with advice regarding both diagnosis and treatment of poisoned patient.

Specific drugs and poisons:

Paracetamol
• Overdose with paracetamol is common.
• Large dose may produce symptoms of depressed consciousness and metabolic acidosis.
• In patients presenting after 20hrs biochemical evidence of liver dysfunction may be apparent.
• Approximately 8% of ingested paracetamol is converted in the liver to a toxic metabolite N-acetyl- p-benzoquinoneimine (NAPQI), which is usually detoxified by conjugation with glutathione
• In overdose with paracetamol, hepatic glutathione stores become depleted, and NAPQI binds irreversibly within the hepatocyte resulting in necrosis.
• Can also result in renal damage.
• Increased NAPQI production occurs in patients with a chronic alcohol problem and in patients taking drugs such as
phenytoin and phenobarbitone; these patients are at risk of hepatotoxicity at lower doses of paracetamol.

• If paracetamol overdose is diagnosed quickly, a specific treatment is available (intravenous N-acetylcysteine or oral methionine).

• Decision to treat is based on plasma paracetamol conc related to the time from overdose, (4 hrs from time of ingestion).

• Treatments show most benefits if done within 12 hours of the overdose.

• Because effective treatment is available for paracetamol poisoning if diagnosed within 12 hrs of ingestion, it is necessary to measure plasma paracetamol in:

• Patients who have taken paracetamol.

• Patients who have taken unidentified tablets.

• (suspected of drug poisoning)

• Gastric lavage, better known as stomach pumping, may be considered if the amount ingested is potentially life-threatening.

Laboratory diagnosis

Acetaminophen (paracetamol)/Salicylate test

• Patients often present with nausea, vomiting and increased rate of respiration.
• Dehydration due to vomiting is severe.
• Acid-base disturbances are common, usually a mixed respiratory alkalosis, and metabolic acidosis, but if vomiting is severe, a metabolic alkalosis can develop.
• Diagnosis is confirmed measuring plasma salicylate concentration/Acetaminophen.
• Blood gases may also be indicated.

SALICYLATE TEST PRINCIPLE

• The Acetaminophen (Paracetamol)/Salicylate Test is a rapid immunoassay, in which acetaminophen/salicylate-protein conjugates compete with acetaminophen/salicylate that may be present in the specimen.

• The test device contains a membrane strip that has been pre-coated with acetaminophen-protein and salicylate-protein conjugates on the test band regions. Colored anti-acetaminophen/salicylate antibody-colloidal gold conjugates are placed on a pad at the end of the membrane. In the absence of drug in test specimen, the colored antibody gold particles move along with the sample solution by capillary action across the membrane to the test band regions forming visible lines.

• Therefore, formation of a visible precipitant in the specific test line occurs when the test specimen is negative for that particular drug.
• When a **drug is present** in test specimen, it competes with the **drug conjugate** on the test band region for the limited antibody binding sites.

• When an adequate amount of drug is present, it will fill the limited antibody binding sites and prevent the formation of red complex on the test line.

• Therefore, absence of the color band on the test region indicates a positive result for that particular drug.

• In patients with plasma salicylate conc above 3.6mmol/L, **treatment** with repeated oral administration of activated charcoal and intravenous infusion of sodium bicarbonate is often used to increase the excretion of the drug in urine.

• Haemodialysis may also be required in the severely poisoned patient, or if there is renal impairment.


**ETHANOL**

The term alcohol originally referred to the primary alcohol ethanol (ethyl alcohol), which is used as a drug and is the main alcohol present in alcoholic beverages

• Alcohols detected include ethanol, methanol, isopropanol, ethylene glycol.
• Blood alcohol concentration (BAC) is expressed in g/dL or mg/dl.

• Acute effects of over-indulgence in ethanol sometimes lead to admission to hospital.

• **Preferred sample** for ethanol analysis is whole blood preserved with sodium fluoride.

• Ethanol may be measured on site in breath samples using breath alcohol analyzers.

Ethanol can also be measured by kit methods in saliva

**Methanol and ethylene glycol**

• Urgent measurement of plasma conc of these substances is required if poisoning is suspected.

• Methanol metabolized to formaldehyde and formic acid, while ethylene glycol is metabolized to a number of products including glycoaldehyde glyoxylic and oxalic acids, toxic leading to metabolic acidosis.
• Severe **methanol poisoning** leads to permanent visual impairment or complete blindness.

• Hypocalcaemia occurs with ethylene glycol poisoning.

• Treatment consists of giving ethanol to prevent metabolism of methanol to toxic metabolites and haemodialysis.

**IRON**

• Iron overdose has been one of the leading causes of death caused by toxicological agents in children younger than 6 years.

• **Iron** is used as a pediatric or prenatal vitamin supplement and for treatment of anemia.

• Iron is particularly tempting to young children because it appears similar to candy.

• Patients with anemia that require frequent blood transfusions also are at risk for developing chronic iron toxicity.

• Iron overload may develop chronically as well, especially in patients requiring multiple transfusions of red blood cells. This condition develops in patients with sickle cell disease, thalassemia, and myelodysplastic syndromes.

**Pathophysiology**

• **Iron toxicity** can be classified as corrosive or cellular.
• **Corrosive toxicity:** Iron is an extremely corrosive substance to the GI tract.

• **It acts on the mucosal tissues** and can manifest with nausea, vomiting, abdominal pain, hematemesis (vomiting of blood from UGI tract), and diarrhea; patients may become hypovolemic because of significant fluid and blood loss.

• **Cellular toxicity:** The absorption of excessive quantities of ingested iron results in systemic iron toxicity.

• **Severe overdose** causes impaired oxidative phosphorylation and mitochondrial dysfunction, which can result in cellular death.

• The liver is one of the organs most affected by iron toxicity, but other organs such as the heart, kidneys, lungs, and the hematologic systems also may be impaired.

**CARBON MONOXIDE (CO)**

• Tasteless, odorless and colourless

• Produced by incomplete combustion of organic matter.

• Automobile exhaust, improperly vented gas heating systems and fires.

• CO has an increased affinity for Hb molecules about 250 X greater than that for O2
Exposure to CO, results in oxyHb being converted to carboxyHb.

- Reduces delivery of O2 to tissues leading to hypoxia and anoxia.

**CarboxyHb levels of 10% to 20% leads to:**
- Shortness of breath
- Slight headache
- Progressing to irritability
- Fainting
- Impairment of judgment at 40-50% and coma,
- Respiratory failure and death at 70-80%.

**REFERENCES**