



A Review on Pathology and its Contribution in Disease Surveillance

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ABSTRACT:

History of pathology has deep roots in common with other medical specialities. Since the starting of mankind there has been desire and need to know more about the causes, mechanisms and nature of diseases. Pathology test cover blood tests, and urine tests. If you are sick, many of the decisions about your care will be based on the results of your blood and pathology tests. Urine test can be used for a whole range of diagnoses. A normal sample of urine consists of urea, chloride, amino acids, phosphate, potassium, sulphate and other chemicals. Any changes in the above chemicals can be diagnosed. The basic components of blood are made up of specialized cells and fluids. Each of these components performs one or more well defined functions and each can be isolated and tested in laboratory to give vital information about person's health. For accessing information in living cells at various biological scales light microscope is used. It is used to detect and magnify very small components (blood, urine) and enlarge them by using visible light. Like light microscope semi auto analyser is also used in test of blood components and urine component. By performing various test analysis of normal and abnormal constituents of blood and urine is done. Blood is easily accessed and examined tissues of human body. Blood analysis has play an important role in diagnosis of disease. After diagnosis there is need to treat disease. In the treatment when rapid absorption of drug is required, fluid cannot be consumed orally, or when the medication to be provided is too irritating to be injected into the skin, muscles and vein i.e., Injections administered intravenously (IV), subcutaneously (SC), and intramuscularly (IM).

Key Words- Blood, Urine, Pathology, Diagnosis, Anemia.

Introduction

▪ Pathology: -

History of pathology has deep roots in common with other medical specialities. Since the starting of mankind there has been desire and need to know more about the causes, mechanisms and nature of diseases. History and evolution of pathology is traced respectively. (Prehistoric times to medieval period, human anatomy and period of gross pathology, cellular pathology, modern pathology)

The word “**pathology**” is derived from two Greek words-*pathos* (meaning suffering) and *logos* (meaning study). Pathology is thus, scientific study of changes in the structure and function of the body in disease. In other words, pathology consists of abnormalities in normal anatomy (including histology) and normal physiology owing to disease. Another commonly used term with reference to study of diseases is ‘*pathophysiology*’ (*patho*=suffering, *physiology*=study of normal function). Pathophysiology, thus, includes study of disorder function (change in physiology) and breakdown of homeostasis in disease (biochemical changes). Knowledge and understanding of pathology is necessary for all would be doctors, medical practitioners and specialist because unless they have knowledge and understanding of scientific basis of diseases and the language used in pathology laboratory reports, they would not be able to treat patient and prevent disease which are the two goals of the doctors. Any disease arises at cellular level, but we now realise that cellular disturbances arise from alterations in molecule like genes, proteins, others, etc. that influence the survival and behaviour of the cells. Thus, the foundation of pathology is understanding, the *cellular* and molecular abnormalities that give rise to diseases. Thus, the pathology provides the scientific foundation for the practice of medicine.^{[1][2][3]}

Types of pathology: -

- Anatomic pathology: The study of tissues, organs and tumours.
- Cytopathology: Changes related to the cells.
- Molecular pathology: The study of DNA and RNA sequencing, genes and genetics.

There are some important terms for pathology: -

- *Etiology*
- *Pathogenesis*

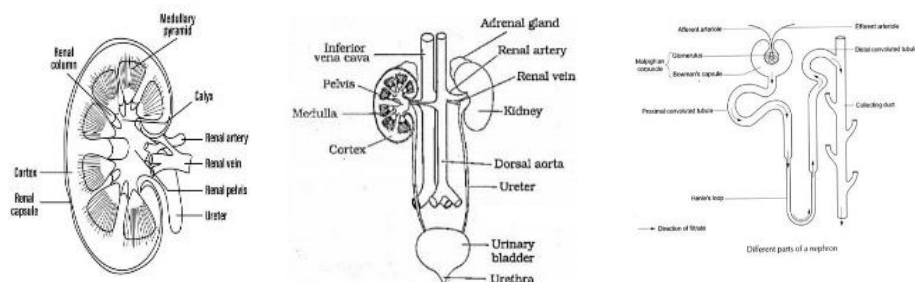
- *Morphological changes*

Etiology is origin of disease, including the underlying cause and modifying factors. Many common diseases like hypertension, diabetes, cancer are caused by the combination of inherited genetically and various environmental triggers.

Pathogenesis refers to the steps in development of disease, from first etiological trigger to the cellular and molecular changes that give rise to the specific functional and structural abnormalities which characterize any type of disease. Thus, *etiology* refers to why a disease arises and *pathogenesis* describe how disease develops.

- **Urine: -**

Urine formation is the blood-cleansing function. Blood with metabolic waste and certain essential components enter the glomerulus at high pressure and get filter. Normally, about 1,300 mL of blood (26% of cardiac output) enters into the kidneys. Kidneys excrete the waste substances along with water from the blood as urine. Urinary output in a healthy person is 1 to 1.5 L/day. In 24 hours, about 900 litre of plasma flows through the kidneys of which 150-180 L is filtered. However, more than 99% of this primitive urine is reabsorbed.^{[4][5]}



- **Blood: -**

Blood is fundamental component of living life. It is commonly used body fluid by most of the higher organisms. Approximately 4 to 5 litres of blood present in adult normal human body. Blood is denser more viscous than water and slightly sticky in nature. It is slightly alkaline and pH ranges from 7.35 to 7.45.

Blood plays an important role in the defence of the body. The study of blood is known as **Haematology**^[6]

Clinical chemistry of blood:

Blood constituents has two main parts

1. **Blood plasma**
2. **Blood corpuscles**

1. Blood plasma: -

Blood plasma is a colourless or slightly pale-yellow colour alkaline viscous fluid. A plasma contains about 90-92% of water and about 8% solutes. A plasma solute involves a following material.

- a. Plasma protein- Serum albumin, Serum globulin, Heparin.
- b. Enzymes- Fibrinogen, Prothrombin.
- c. Nutrients- Glucose, Amino acids, Fatty acids, Glycerol.
- d. Nitrogenous waste- Urea, Uric acid, Ammonia, Creatine.
- e. Gases- Oxygen, Carbon-dioxide, Nitrogen.
- f. Inorganic substances- Bicarbonates, Chlorides, Phosphates, Sulphates of sodium, Potassium, Magnesium, Calcium, etc.

2. Blood corpuscles: -

1. Red Blood Cells (Erythrocytes)
2. White Blood Cells (Leucocytes)
 - A. Granulocytes
 1. Neutrophils
 2. Acidophils

3. Basophils
- B. Agranulocytes
1. Lymphocytes
2. Monocytes

3. Platelets

1. Red Blood Cells (Erythrocytes): - Erythro + Kytos = Red cells

Red Blood Cells can be regarded as a miracle of evolution. RBCs are the most abundant cell type in human blood. RBCs are produced in bone marrow. In healthy adult, every second 2 million of newly formed RBCs enter into the circulation from bone marrow and at same time same no. is cleared.

2. White blood cells (Leucocytes): -

White Blood Cells or leukocytes derived from Greek word **leuko=white** and **cyte=cell**. The size of leucocytes 6-8µm in diameter. WBCs, are less abundant in blood than erythrocytes. The number of WBCs in blood ranging from 45 to 7000 cells per microlitre under normal conditions.

A. Granulocytes: -

1. Neutrophils: -

Elie Metchnikoff was the first to identify neutrophils. When at rest, neutrophils are transparent and spherical, but they can alter their form to fight infection. Neutrophils are 12 to 15 µm in diameter The normal range of neutrophils in healthy adult is between 2,500 to 7,000 neutrophils per microlitre of blood. **Neutropenia** is a condition where neutrophils count is too low causes repeated infection.

2. Acidophils (eosinophils): -

The term "eosinophile" was introduced by Ehrlich in 1897. Eosinophils, like neutrophils and basophils, are a type of granulocyte derived from bone marrow, distinguish by their morphological features, constituents, products, and associations with specific disease.

3. Basophils: -

Basophils were discovered by Paul Ehrlich in 1879. Basophils are the least abundant granulocyte population in the peripheral blood, contains less than 1% of leukocytes. Basophils are 12 to 15µm in diameter, have bi-lobed or s-shaped nuclei, and contain cytoplasmic specific granules size ranges from 0.5µm.

B. Agranulocytes

1. Lymphocytes: -

It shows large round nucleus it constitutes about 25-33% of total WBCs. The lymphocytes range from 7–10 µm in diameter. Lymphocytes produce antibodies which are involve in immune response of the body.

i. T lymphocytes: cells concerned with cellular immunity.

ii. B lymphocyte: cells concerned with humoral immunity.

2. Monocytes: -

Monocytes are identified as critically important for effective immune function. They are capable of ingesting bacteria and particulate matter and acts as "scavenger cells" at the site of infection. Monocytes play a role in both the inflammatory and anti-inflammatory processes that take place during an immune response. They are larger in size than leucocytes ranging 16 to 22µm. in diameter.

3. Platelets: -

Blood platelets are important element in the process of haemostasis, wound healing, inflammation, and repairing of tissues. Platelets are round and oval in shape and size of platelets ranges from 1.5 to 3µm.

▪ Anaemia: -

Anaemia happens when you do not have red blood cells or your red blood cells do not work as they should. Your red blood cells carry oxygen throughout your body. Oxygen powers your cells and gives you energy.

There are several types of anaemia: -

Iron deficiency anaemia

Vitamin deficiency anaemia

Haemolytic anaemia

Sickle cell anaemia^{[7][8][9][10]}**Disorders of White Blood Cells (WBC): -**

There are two major types of blood cell disorders are Leukocytosis and leukopenia's. In leucocytosis there is an increase in the number of white blood cells. It causes due to infection but may less common it's related to some types of cancer.

Leukocytosis: -

The condition in which the total WBC count increases is called as Leukocytosis. Mostly it is causes due to increase in no. of neutrophils, it is also causes due to increase in no. of lymphocytes, monocytes, eosinophils or basophils. It may be acute or chronic. The diagnosis of leucocytosis depends upon patients age, clinical history and morphology. Leukocytosis is mainly occurs in children and pediatric.

Leukopenia: -

The condition in which decrease in total WBC count is called as leukopenia. Mostly it is caused by the increase in granulocytes. It includes neutrophils, monocytes and lymphocytes. Leukopenia is diagnosed by blood test called as complete blood count. There are following types of leukopenia

Neutropenia: -

Decrease in no. of neutrophils is called as neutropenia. Neutropenia is considered chronic when it is persist more than 3 months and is due to decrease production or increased destruction of neutrophils. When the neutrophils no. falls below the 2500/ μ l. neutropenia is causes due to

1. Certain Infections – e.g., typhoid, Influenza, Malaria, Viral Hepatitis etc.
2. Drugs chemicals and Physical agents which decrease aplasia of bone marrow causes neutropenia

Eosinophilia: -

350-500/mm is the normal mean blood eosinophil count. Usually, when exposed to antigens, their number and activation rise, especially when the antigens are deposited in the specific tissues. An immediate hypersensitivity reaction caused by IgE or delayed hypersensitivity reaction caused by T-lymphocytes describe a response. According to their absolute amount in the bloodstream, eosinophils severity is categorised follows: -

- Mild eosinophilia: - 500 to 1500/mm³
- Moderate eosinophilia: - 1500 to 5000/mm³
- Severe eosinophilia: - greater than 5000/mm³

Basophilia: -

A high level of white blood cells can indicate an immune response in the body, which protects the body from infections and other problems. However, when a person has basophilia, the increase in the white blood cells may be due to more serious causes. Basophilia refers to an increase in the number of basophilic Leukocytosis above 100/ μ l. Basophilia is unusual and found in the following conditions: -

Chronic myeloid leukaemia

Polycythaemia Vera

Myelosclerosis

Myelosclerosis

Myxoedema^{[11][12]}

Lymphocytes and platelets, their role in health and disease: -

- **Role of lymphocytes in health and disease: -**
 - **The role of lymphocytes in the pathogenesis of asthma and COPD:**

The immune system's physiological role is to protect the body from infectious agent and potentially dangerous external substances. Pathogens and foreign antigens found in breathed air are constantly exposed to the pulmonary immune system. It is important that the cells coordinating immune response in the lung function properly in order to defend the lung from such attacks/infections identify whether an antigenic molecule is potentially harmful, and then develop the necessary defence.

- **Role of lymphocytes in gastrointestinal disease: -**

Many gastrointestinal conditions, such as food-sensitive enteropathy, inflammatory bowel disease, and chronic infantile diarrhoea, can be seen to have activated T cells in the intestinal lamina propria. According to experimental research, T cell activation in human intestinal lamina propria in vitro increases crypt cell proliferation, villous atrophy, intraepithelial lymphocyte counts, and phenotypically activates macrophages.

○ **Role of lymphocytes in alopecia areata: -**

Alopecia areata is a common hair disorder that causes unexpected hair loss. In the early acute phase of alopecia areata the normal appearance is patchy hair loss, but with time, it can progress to the loss of all scalp hair. Skin that has been affected by hair loss is smooth and natural-coloured, or occasionally somewhat pink.

○ **Role of platelets in health and disease: -**

Recent advances in platelet biology have provided greater understanding of platelet development, function, heterogeneity, genetics, signalling, and communication. The growing understanding that platelets have a functional role in the pathophysiology of a wide range of diseases, beyond the disorders of coagulation, is due to the discovery of previously unknown and newly discovered biological capacities of platelets.

○ **Platelets in Diabetes Mellitus: -**

Diabetes mellitus is a multifaceted condition that is strongly linked to both microvascular and macrovascular consequences. The pathophysiology of Diabetes mellitus is significantly influenced by platelets. Platelets from people with diabetes have dysregulated signalling pathways that cause platelet activation, which is an early stage in the development of diabetes.

○ **Platelets in cancer: -**

The multistep process of tumorigenesis necessitates coordinated alterations in the tumour microenvironment as well as the tumour cells. They penetrate into the tumour microenvironment to interact with cancer cells directly and can trigger the same proliferative pathways that are triggered by oncogenic mutations, which aids in the development and spread of illness.

○ **Platelets in neurological disorders: -**

Because platelets store and release neurotransmitters like serotonin, glutamate, and dopamine as well as express proteins related to neurons like N-methyl-D-aspartate receptors, it has been discovered that platelets and neurons share many biochemical characteristics, making them an important factor in neurological disorders.

▪ **Abnormal constituents of urine and their significance in disease: -**

Urine is a significant bodily waste that can reveal a number of health issues through physical and chemical analysis. the following list of physical characteristics

Urine in healthy people has a pale-yellow colour. Some unusual hues include:

Dark Yellow: Dehydration happens when there is a water shortage.

Yellow light: in diabetes.

Abnormal constituents of urine: - [50, 51]

Proteins: -

The presence of albumin and globulin in the urine in abnormal concentrations is known as proteinuria (albuminuria). The typical basic tests cannot identify the traces of protein (10–150 mg) seen in typical urine. Urine contains a number of pathological proteins, including Bence-Jones proteins, mucus, haemoglobin, serum albumin, and serum globulin.

Sugars: -

The amount of sugar that healthy people excrete daily is between 16 and 300 mg, which is difficult to detect with a simple test. When more than this amount is detected in urine, it is considered glycosuria. The filtered load of glucose exceeds the capacity of the tubular cells to reabsorb it, which typically happens at a glucose serum concentration of about 180 mg per dL.

Bilirubin: -

Normal urine contains no bilirubin. When there is obstructive or hepatic jaundice, bilirubin is detected in the urine. The excretion of bile salts is accompanied by bilirubinuria. In some phases of liver illness, bile salts may be excreted in the urine without bile pigment. Urine may include small amounts of bilirubin without bile salts when haemolysis is high.

Blood or blood cells: -

Blood or blood cells are not present in normal urine. In addition to being present in nephritis, blood is also discharged in the urine in kidney or urinary tract infections. Additionally, after rapid haemolysis, such as that caused by black water fever (a malarial consequence) or severe burns, free haemoglobin is discovered in the urine.

Ketone bodies: -

Ketone bodies are products of body fat metabolism. Ketone bodies are typically eliminated in urine in little amounts (less than 1 mg) every 24 hours. In many animals, excessive fat metabolism can cause a ketonuria. When acidosis and ketosis coexist, more ammonia is excreted. If your body is unable to provide enough glucose to your cells, it will instead burn fat to provide energy.^{[13][14]}

Experimental: -

- **Practice in injecting drugs by intramuscular, subcutaneous and intravenous routes.**

Injections are one of the most commonly performed medical procedures worldwide. Injections administered intravenously (IV), subcutaneously (SC), and intramuscularly (IM) are the three most used methods.

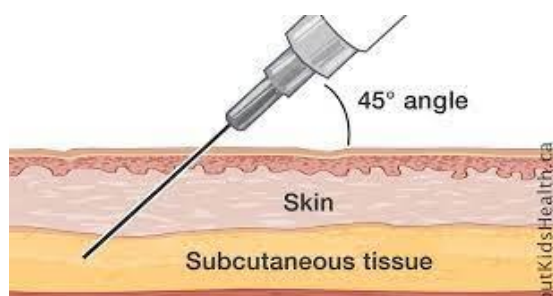
Many different factors will affect the injection site and needle size. These factors include the medication's quantity and type, as well as the patient's age and size. The right needle and syringe to use to deliver your medication will be prescribed by your doctor or pharmacist.

A. Practice in drug injecting by intramuscular route:-

An intramuscular injection is a technique for injecting medicine deep inside the muscles. This makes it possible for the drug to enter the bloodstream rapidly. An intramuscular injection can sometimes be self-administered. Self-injection may be necessary, for e.g., while taking certain medications for multiple sclerosis or rheumatoid arthritis.

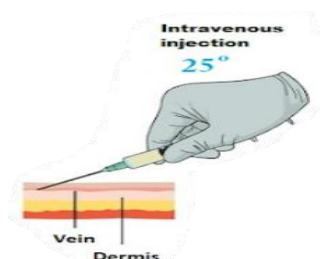
B. Practice in drug injecting by subcutaneous route: -

Subcutaneous is a type of injection which is administered under the skin. A short needle is used in this type of injection to inject a drug into the tissue layer between the skin and the muscle.



C. Practice in drugs injecting by intravenous route: -

Some medications must be administered via IV injection or infusion. This means that they are injected directly into your vein with a needle. Intravenous medication is used to control medication dosing. It gives instant result hence it is used in emergencies like heart attack, stroke and poisoning.



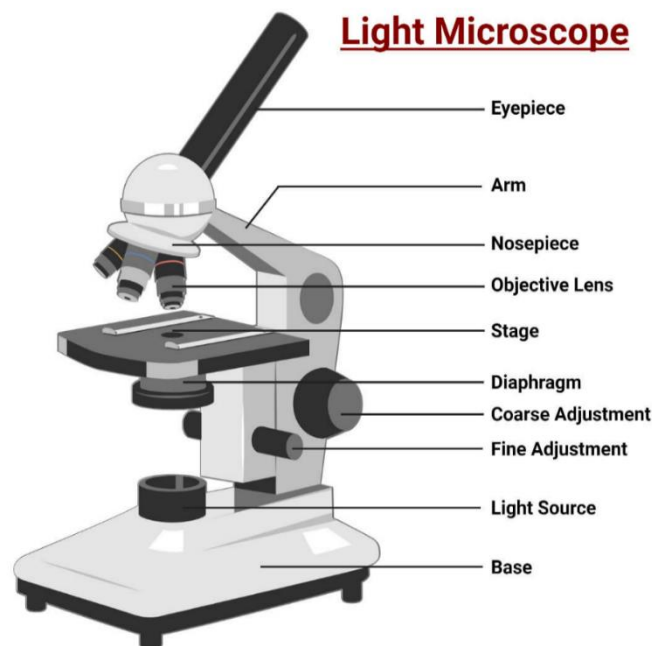
- **Withdrawal of blood sample: -**

Venous blood is typically collected from the antecubital vein or another prominent vein on the forearm. The patient is seated comfortably and asked to extend his arm. A blood pressure cuff is wrapped tightly around the upper arm. A suitable vein is chosen, and the skin over it is sterilised by rubbing spirit over it with a cottonwool pad. A sterile hypodermic needle attached to a syringe is inserted into the vein, and the blood pressure cuff is removed. The desired amount of blood is drawn into the syringe by gently withdrawing the plunger. A pad of cottonwool soaked in spirit is placed on the skin where the needle was introduced, and the needle is withdrawn.

Introduction to light microscope: -

Light microscopy is one of the least invasive techniques for accessing information in living cells at various biological scales. A prism will bend the light at an angle. The lens on receiving the light rays, focuses the rays at a specific point known as the focal point and the distance from the centre of the lens and the focal point is known as focal length. The strength of microscope is predetermined it is directly related to focal length. Light microscope is an instrument which is used to detect and magnify very small objects and enlarge them by using visible light. It has lenses to focus light

on the specimen, the specimen is placed close to the microscopic lens, magnifying it and producing a large image. There are two types of microscopes i.e., simple light microscope and compound microscope. The functioning of the light microscope is based on its ability to focus a beam of light through a specimen, which is very small and transparent to produce image. The transparency of the specimen helpful in the easy and quick penetration of light.



Principle: -

When a ray of a light passes through 1 medium to another, the ray bends at the interface causing refraction. The bending of light is called as refractive index. Refractive index is useful in determination of the direction and magnitude of the bending of the light. When refractive index is lower as glass to air it speeds up the light penetration and light bend away from the normal while when refractive index is greater for e.g., air to glass it slows down and bends towards the normal perpendicular to the surface. When an object is kept between the two mediums i.e., between water and air.^[15]

▪ **Introduction to semi auto analyser: -**

A semi-auto biochemistry analyzer is an instrument that is used in test of blood like glucose, urea, albumin, etc. It works on principle of filter photometry which is slightly different than spectrophotometry. The machine has approximately 50 pre-set parameters. You can edit those programming as you like. The semi-automatic biochemistry analyzer can store approximately 400 patient results. Semi auto analyser are faster, more precise and less boring for the analyst than the other methods.

Components of semi-auto analyser: -

1. Colour filter: -

The machine contains various coloured filters that represents different wavelength. They can be 6 to 7 in number. You may also have an extra slot (1-2) for adding additional filters if necessary. Filter wavelengths commonly used include 340 nm, 405 nm, 500 nm, 546 nm, 578 nm, 620 nm, and 670 nm.

2. Light source: -

The most common light source is halogen(6V/10W), which has a lifespan of approximately 3000 hours. The light source in some models can also be an LED light.

3. Photo detector: -

The absorbance of the sample solution is measured by the photodetector.

4. Cuvette: -

It is used to store the sample after it has been fed into semi-automatic biochemistry analyser. Inside your machine, you will find 32 μ l of quartz flow cell. If your flow ce;; becomes damaged, you can take the reading with the help of cuvettes. In this case, the analyser functions similarly to a colorimetry. However, as much as possible, use a flow cell to take the measurements.

5. Temperature controller: -

The Peltier module is the most common temperature controller found in the semi-automatic analyser. In addition, the machine has temperature settings of 25°C, 30°C, 37°C. If you require the room temperature, turn off the temperature setting.

6. Pumping system: -

The sample is drawn from the sipping tube to the flow cell by a pump. When the measurement is finished, the fluid is drawn back into the flow Thermal cell. The peristaltic pump is the most common type of pump. You might even be able to find a syringe pump for this purpose.

7. Incubator: -

You might find an incubator built in to hold a few test tubes.

8. Thermal printer: -

At the top of the machine, there is a built-in thermal printer. If you don't need it, you can disable it in the settings menu. Otherwise, set it to ON. Replace the printer paper when it runs out.

9. Fan: -

They are required to keep the temperature set and to cool the machine.

10. Display: -

It is generally LCD type display with or without touchscreen.

Principle of semi-auto analyser: -

A semi-automatic biochemistry analyzer operates on the basis of filter photometry. The light from the halogen lamp is emitted in all directions. The scattered light is then reversed by passing the radiated light through a convex lens. Following that, it passes through the sample in the flow cell/cuvette. The sample absorbs some of the light energy. While the remaining light continues to transmit. This light is then passed through a colour filter. The monochromatic light is passed through the colour filter to the photodetector. The photodetector then converts the light signal to an electrical signal, which serves as an input to the microprocessor.

Test performed by semi auto analyser: -

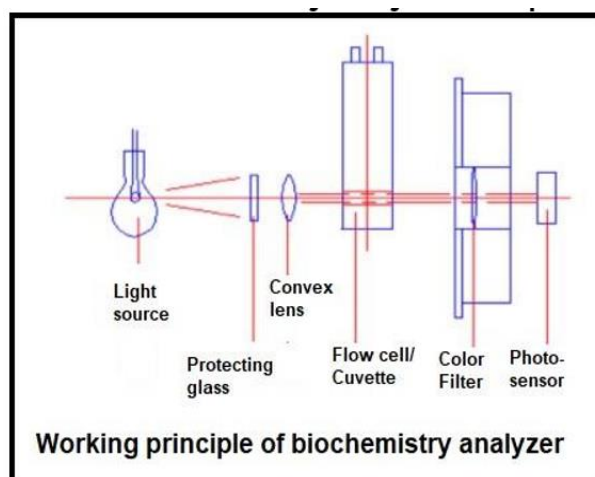
1. Estimation of urea by urease method: -

Reagents used: - Urease, glutamate, dehydrogenase (GLDH), nicotinamide adenine dinucleotide (NADH), α -ketoglutaric acid, buffers and stabilizers.

- Urea was hydrolysed in the presence of urease enzyme to produce ammonia and carbon dioxide.
- In the presence of GLDH, ammonia reacted with α -ketoglutaric acid and reduced NADH to produce glutamic acid and nicotinamide adenine dinucleotide (NAD)

The rate of urea oxidation of NADH to NAD was proportional to the urea concentration and was measured at 340nm using a semiauto analyser.

2. Estimation of total cholesterol: -



The esterified cholesterol was hydrolysed by cholesterol esterase (CHE) into free cholesterol. The free cholesterol was oxidised to produce hydrogen peroxide, which was then combined with phenol and 4 amino antipyrine by peroxidase to produce a red-coloured quinonimine dye complex. The colour intensity was proportional to the amount of cholesterol in the serum sample.

3. Estimation of triacylglycerol: -

TGs were hydrolysed by lipoprotein lipase into glycerol and free fatty acid. The glycerol formed with ATP in the presence of glycerol kinase formed glycerol 3 P, which was oxidised by glycerol phosphate oxidase to form H₂O₂, which then reacted with phenolic compound and 4-amino antipyrine via the catalytic action of peroxidase to form a red-coloured quinonimine dye complex, the intensity of which was directly proportional to the triacylglycerol present in the sample.

4. Estimation of Serum Glutamic Oxaloacetic Transaminase (SGOT): -

The amino group transfer from L-aspartate to -ketoglutarate is catalysed by SGOT (AST). The rate of the reaction was monitored using the coupling enzyme malate dehydrogenase (MDH), which converted the formed oxaloacetate to malate in the presence of NADH. NADH oxidation was measured by observing the decrease in absorbance at 340 nm.

5. Estimation of Serum Glutamic Pyruvic Transaminase (SGPT): -

The reversible transfer of an amino group from alanine to oxoglutarate is catalysed by SGPT, resulting in glutamate and pyruvate. LDH and NADH converted the pyruvate to lactate.^[16]

Analysis of constituents of blood and urine: -

▪ Analysis of normal and abnormal constituents of blood and urine: -

a. Hematologic values: -

Normal blood count: -

Haemoglobin: - 12/17 g per 100 ml

Erythrocytes: - 4.3-6.40 mill. /Cubic mm.

Haematocrit: - 42-48%

Mean corpuscular volume: - 82-95%

Mean corpuscular haemoglobin concentration: - 30-35%

Reticulocytes: - 0-2%

Neutrophils: - 2,500-6,000 per cubic mm.

Basophils: - 0-100 per cubic mm.

Eosinophils: - 0-400 per cubic mm.

Lymphocytes: - 1,000-3,000 per cubic mm.

Monocytes: - 0-800 per cubic mm.

Platelets: - 2,50,000-5,00,000 per cubic mm.

b. Thyroid function test: -

Thyroid function tests are a series of blood tests that are performed to determine how well your thyroid gland is functioning. T₃, T₃RU, T₄, and TSH tests are among those available. T₃ and thyroxine are the two primary hormones produced by the thyroid (T₄). If your thyroid gland does not generate enough of these hormones, you may experience weight gain, fatigue, and sadness. This is referred to as hypothyroidism.

If your thyroid gland produces too many hormones, you may experience weight loss, increased anxiety, tremors, and a "high." This is known as hyperthyroidism.

Preparing for the tests

You don't need to do anything special to prepare for the thyroid function tests.

The T₄ test is often referred to as the thyroxine test. A high T₄ level implies that the thyroid is hyperactive (hyperthyroidism). T₃ levels that are abnormally high are most usually associated with Grave's disease. It is an autoimmune condition that is linked to hyperthyroidism.

c. Glucose: -

The presence of detectable amounts of carbohydrates in urine is known as glycosuria. The Carbohydrate which is most commonly found in urine. is glucose.

Causes of glycosuria: Diabetes mellitus endocrine disorders such as hyperthyroidism, hyperpituitarism.

renal glycosuria-This is benign condition in which the capacity of renal tubules of reabsorb glucose is subnormal i.e., the renal threshold for glucose, which is normally about 180 mg/dl in normal individuals, is decreased in renal glycosuria.

d. Urea: -

Requirements: - Beakers, test tubes, test tube holder, spirit lamp or gas burner, phenolphthalein, urine sample

Test: -

2 ml fresh human urine sample in test tube+ pinch of soyabean/ Gram Flour+2, 3 drops of phenolphthalein, warm it in water bath for 5 minutes. Urea is present if the solution turns Pink.

Clinical significance: - The normal urine contains urea. (urea is produced in liver from amino catabolic product). It's presence in urine indicates normal functioning of excretory system. In healthy person urea is present about 60-150 mg

e. Creatinine: -

Method of folin + Wu Take 7ml of water in a test tube. Add 1 ml of serum and 1 ml of 10% sodium tungstate, mix. In above mixture, add 1ml 2/3 N H₂SO₄ and shake then stand for few minutes & filters. Take 3 test tube and transfer for the filtrate into test tube as a name them unknown, standard. & blank. let the tubes stand for to min

Principle: Serum proteins are precipitated by tungstic acid. Alkaline picrate is added to the protein-free filtrate. Creatinine is converted into orange-coloured creatinine picrate. The intensity of the colour is measured calorimetrically

Interpretation: -The normal range. of serum creatinine is 0.6-1.5 mg / 100ml. The range of creatinine in whole blood is higher. As the serum urea rises the serum creatinine also increase serum creatinine is more specific and sensitive indicator of renal dysfunction.

f. Cholesterol: -

Salkowski's Test - In a dry test tube take 2 ml of cholesterol solution. Add 2 ml Conc. H₂SO₄ shake and allow to stand. Chloroform and H₂SO₄ layers separate on standing. The acid layer shows green fluorescence & chloroform layer cherry red.

Principle: -Cholesterol is dehydrated by. H₂SO₄ and acetic anhydride to form 3,5 cholestadiene or 2, 4-cholestadiene. The cholestadienes combine to form their dimers or trimers which react with H₂SO₄ to form their sulphuric acid derivatives.

g. Alkaline phosphate: -

Procedure: Label four test tubes 'Unknown', 'Control', 'Standard' and 'Blank'. Pipette 2 ml of buffer-substrate into 'Unknown' and 'Control' and keep these tubes in an incubator at 37°C for a few minutes. Pipette 1.1 ml of buffer solution into 'Standard' and 'Blank'. Pipette 1 ml of working standard phenol solution into 'Standard' and 1 ml of water into 'Blank' Leave these two tubes at room temperature. Add 0.1 ml of serum to 'Unknown' and incubate at 37°C for exactly 15 minutes Remove 'Unknown' and 'Control' from the incubator. Add ml of sodium hydroxide and 12 ml of sodium bicarbonate to all the tubes. Add 0.1 ml of serum to 'Control'. Add 1 ml of 4-aminophenazone to all the tubes and mix thoroughly. Add 1 ml of potassium ferricyanide to all the tubes and mix thoroughly. Read 'Unknown', 'Control' and 'Standard' against 'Blank' at 520 nm or using a green filter.

Interpretation: -The normal range of serum alkaline phosphatase is 4-17 KA units/100 ml in adults and 17-33 KA units/100 ml in children. The main source of alkaline phosphatase in serum is liver in adults and bone in children. The activity is raised in hepatitis, obstructive jaundice, liver cancer, bone cancer, osteomalacia, rickets, hyperparathyroidism, Paget's disease and sometimes in the last trimester of pregnancy. A decrease in serum alkaline phosphatase occurs in achondro- plasia, cretinism, scurvy, severe anaemia, kwashiorkor and hypophosphatasia. Hypophosphatasia is a recessively inherited autosomal defect.

h. Acid phosphate: -

Estimation: - Dissolve 21 gm of crystalline, citric acid in water, adding 188 ml of 1M NaOH and make up the volume up to 500 ml with water adjust the pH up to 1. 4.9 by adding NaOH or HCl if necessary. The incubation period is 60 min.

Interpretation: -

The normal range of serum acid phosphatase is 1-5 KA units/ 100 ml. The main value of this estimation is in the diagnosis of cancer of prostate. The level is usually normal until the tumour is confined within the capsule of the prostate but once it breaks through the capsule, the serum acid phosphatase level is considerably raised. Small increases are not uncommon after rectal palpation of prostate, passage of a catheter and in acute retention of urine

i. Bilirubin: -

Requirements: Beakers, test tubes, test tube holder, urine sample, sulphur powder etc.

Test: - 3 ml of urine sample + sprinkle a pinch of dry sulphur powder on the surface. Stand for 10 to 15 minutes if sulphur powder sinks at bottom, then bilirubin is present.

Clinical significance: Bile salts in their detectable quantity are abnormal component of urine. Due to liver infections like Jaundice these bile salts are produced in larger quantity which are excreted as part of urine.

j. Serum Glutamic Pyruvic Transaminase. (SGPT) and serum glutamic-oxaloacetic transaminase (SGOT): -

SGPT - serum glutamate pyruvate transaminase (SGPT) also called as alanine amino transaminase (ALAT). SGPT is found in the liver only when there is damage to the tissues in the liver it results in increase in SGPT level. Normal SGPT levels 7 to 56 units per liter of serum. The SGPT/ SGOT valuation in the body is done by the blood test. Abnormal SGPT-SGOT levels in the blood stream have several complications such as Hepatitis, liver arrhesis, liver tissue damage, liver cancer, tumour in the liver, Hemochromatosis, Pancreatitis. SGOT and SGPT, tests are a part of the routine blood test and liver function test. some common symptoms of risk level of SGPT and SGOT are as follows: -

A constant feeling of fatigue- & tiredness, vomiting & nausea, Shortness of breath, Swelling in the leg, etc.

SGOT Serum glutamate oxaloacetate transaminase also called as aspartate amino transferase (ASAT). Generally, SGOT is found in the kidney, muscles, heart & brain but heart is richer in SCOT

SGOT level increases during liver complications, heart attacks or muscle injuries. Normal SGOT level is 5 to 40 units per litre of serum.^{[17][18][19][20]}

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