2. (1)

Mineral metabolism:

Our body contains 29 different elements. minerals constitute about 4% of body weight. These minerals are essential for a number of metabolic processes like blood coagulation, muscle contraction and enzyme action.

classification of mineral elements:

1. Principal elements (Macro minerals):
   Calcium, magnesium, Sodium, Potassium, phosphorous, sulphur and chlorine.

2. Trace elements: (Micro minerals).
   (a) Essential trace elements: iron, Iodine, zinc, copper, cobalt & fluorine.
   (b) Possibly essential trace elements:
   Nickel, Vanadium, tin & Silicon.
   (c) Non-essential trace elements:
   Aluminium, Cadmium, arsenic, lead, & mercury.

Functions of Individuals minerals:

① **Sodium:** maintainance of acid, base balance, maintenance osmotic Pressure

② **Potassium:** maintainance of acid, base balance contraction of muscles.

③ **Calcium:** bone & teeth formation, blood coagulation.

④ **Phosphorous:** Synthesis of DNA & RNA. synthesis of Phospho lipids.

⑤ **Sulphur:** component of biotin, cystine and methionine.

⑥ **Iron:** for the synthesis of hemoglobin cytochrome synthesis

⑦ **Iodine:** Synthesis of thyroid hormone.

⑧ **Zinc:** component of carbonic anhydrase, for insulin synthesis and release.

⑨ **copper:** constituent of peroxidase and catalase, helps in incorporation of iron in haemoglobin.

⑩ **cobalt:** For the synthesis of vitamin B12.

Sodium:

Sodium is the principal cation of extracellular fluid. It is present in the body of Nacl & NaHCO3

Function:

1. It helps in maintaining acid-base balance.
2. It maintains the osmotic pressure of body fluids.
3. It preserves the normal irritability of muscles.
4. With other ions, it maintains the permeability of cells.

Source: The main source is salt used in cooking.

Hypernatremia: It is an increase in plasma sodium concentration. It occurs in:-

1. Dehydration as occurs in severe sweating.
2. Diabetes insipidus.
3. Excessive intravenous [illegible] of saline.

Hyponatremia:

It is a decrease in plasma sodium level. It occurs on:-

① Diuretic medication.

② kidney diseases.

③ Severe vomitting & diarrhoea.

④ Severe burns.

---

diabetes insipidus → a rare form of diabetes resulting from a deficiency of vasopressin. (the pituitary hormone that regulates the kidneys).

The symptoms of hyperkalemia are bradycardia, depression, mental confusion and muscle weakness.

Hypokalemia:

It is a decrease in serum potassium level. It occurs in,

1. Cushing's syndrome.
2. Severe vomitting & diarrhoea.
3. Prolonged use of diuretics.

Symptoms: Tachycardia, muscle weakness, irritability & paralysis.

Chlorine:

It is present in the body as chloride ion. It has wide distribution in the body. Highest conc. is present in CSF.

Functions:

1. Maintaining acid-base balance (as chloride shift).
2. Necessary for HCl secretion in gastric juice.

Deficiency:

1. Profuse vomitting caused by pyloric obstruction loss of chlorides leads to

Potassium: (K^+)

It is the major intracellular cation. It is widely distributed in body tissues and body fluids. A high concentration is present in nerve tissue, cells, muscles and blood.

Functions:

1. It helps in maintaining acid-base balance.
2. It influences muscular activity.
3. It plays an important role in cardiac function.
4. It is essential for conduction of nerve impulses.
5. Enzyme like Pyruvate kinase require K^+ as a co-factor.

Hyper kalemia:

It is an increase in serum Potassium level. It occurs in:

1. Renal failure.
2. Severe dehydration.
3. Excess administration of I.V Potassium.

The symptoms of hyperkalemia are bradycardia, depression, mental confusion and muscle weakness.

Hypokalemia:

It is a decrease in serum Potassium level. It occurs in,

1. Cushing's syndrome.
2. Severe vomitting & diarrhoea.
3. Prolonged use of diuretics.

Symptoms: Tachycardia, muscle weakness, irritability & paralysis.

Chloride:

It is present in the body as chloride ion. It has wide distribution in the body. Highest conc. is present in CSF.

Functions:

1. maintaining acid-base balance (as chloride shift).
2. necessary for HCl secretion in gastric juice.

Calcium: Ca+

Of all the minerals calcium is present in large amounts in the body. It constitutes 2.1% of body weight. A normal adult has 1200 grams of calcium in the body.

Normal value → 100-300 mg/day.

Physiological functions:

1. Calcium is necessary for the formation and growth of bones & teeth.
2. It is essential for coagulation of blood.
3. It is essential for transmission of nerve impulses.
4. It is also necessary for muscle contraction.
5. It helps in ~~maintaining~~ maintaining acid balance & water balance.
6. It activates a number of enzyme.

Sources: milk, cheese, ~~&~~ & vegetables.

Blood calcium: In blood calcium is present only in the plasma. The plasma conc. of calcium is 9 to 11 mg.

Plasma calcium exists in three forms

1. Ionised or diffusible form.
2. unionised or non diffusible form.
3. unionised complexed with citrate.

Except unionised (or non-diffusible) form, the other two forms can diffuse through cell membranes and capillaries.

Factors maintaining plasma calcium:

1. Parathyroid hormone: It maintains plasma calcium by mobilising calcium from bone.
2. Vit-D: It increases the absorption of calcium from intestine.
3. Plasma Protein: About 40% plasma calcium is bound to plasma proteins (mainly albumin). A decrease in plasma protein may decrease plasma calcium level.
4. Plasma phosphate: Increase in plasma phosphate level produces a decrease in plasma calcium level. The reverse is also true. Thus there is an inverse relationship between plasma level of calcium & phosphate.
5. Calcitonin: Increases in plasma calcium level stimulates the release of calcitonin. Calcitonin in turn induces the deposition of calcium in the bone. Thus calcitonin regulates plasma calcium level.

Diseases related to calcium metabolism.

(1) Tetany: It is a manifestation of hypo-calcemia as occurs in hypoparathyroidism. There is increased neuromuscular excitability in tetany. This leads to:

1. fibrillation & twitchings of muscles.
2. muscular Spasm.
3. epilepsy like convulsions.

(2) Osteoporosis: It is a metabolic disorder. It occurs as a result of calcium deficiency. It also occurs in hypo Parathyroidism and deficiency of vitamin D. In osteoporosis calcification of bones is defective. Bones become spongy and brittle. So spontaneous fracture of bones is very common. It is treated by calcium supplemented diet & vitamin D.

(3) Rickets: It occurs in children due to the deficiency of calcium. Also it occurs in deficiency of vitamin D.

Features: (1) softness & deformities of bones (like bow-legs & pigeon chest)

(2) delay in closure of fontanelle.

(3) delay in tooth formation.

(4) Osteomalacia: It is a calcium deficiency disease which occurs in adults. It also occurs due to deficiency of vitamin D.

Features:

(1) deformities & easy fracture of bones.
(2) bending of vertebrae and bow-legs
(3) muscle weakness & pain.

20/8/15

Pharm D I year

Lipid Profile test: (150 - 220 mg/dl)

~~Lipid Proteins~~ Total cholesterol

The total cholesterol was estimated by the method of Zlatkis et al. (1953)

Sample treated with ferric chloride – acetic acid reagent to Precipitate the Protein. The Protein free filtrate containing cholesterol ferric chloride is treated with conc. H_2SO_4. The reaction involves in the 3-OH-end part of the cholesterol molecule, it is first dehydrated to form cholesta – 3-5 diene. and then oxidised by sulphuric acid to dimer to two molecules together as bis – cholestra 3-5 diene, this material is sulphonated by sulphuric acid to red coloured disulfonic acid in the presence of ferric ion as catalyst (Salkowski's reaction) The color developed was read at 560 nm using suitable standard and a reagent blank.

Reagent:

Ferric chloride – 0.05% in acetic acid (w/v)

re 2. Conc. H_2SO_4
od 3. Standard – Cholesterol 200 mg/dl

7. Procedure:

To 0.1 ml of the serum/lipid extract 4.9 ml of ferric chloride – acetic acid reagent was added. To that 3.0 ml of conc. H_2SO_4 was added. The color developed was read after 20 min at 560 nm against at a reagent blank. A set of standards was also performed in the similar manner.

Values were expressed as mg/dL serum and mg/100g tissue.

HDL – cholesterol: (Normal Value → 45–65 mg/dl)

HDL – Cholestrol fraction was separated by the precipitation techniques of Burnstein et al (1970) and the cholesterol content was determined by the method of Zlatkis et al (1957)

Reagent:

Heparin – manganese chloride – 3.167g manganese chloride was added to 1.0 ml solution of heparin containing 20,000 units the mixture was made upto 8.0 ml with distilled water.

Triglycerides:

were estimated by the method of Foster et al., 1973.

Reagent:

Isopropanol, Alumina (activity Grade I for chromatography) was washed with water and dried in an oven overnight.

Saponifying agent.
Sodium metaperiodate reagent.
Acetyl acetone reagent.
Standard triolein solution.

Procedure: To an aliquot of dried lipid extract, 4ml isopropanol was added, mixed well and 100mg washed alumina was added. This was placed in a mechanical rotor for 15 minutes and then centrifuged. To 2ml supernatant, 0.6ml Potassium hydroxide was added and incubated at 60-70°C for 15min; cooled and 1ml of Sodium meta-periodate solution and 0.5ml acetyl acetone reagent were added. It was then mixed and incubated at 50°C for 30 minutes. A series of Standards of concentration 8-40µg triolein were treated similarly along with a blank containing only the reagent. cooled and read at 405 nm against blank. The triglyceride was expressed as

Procedure:-

To 1.0ml of serum added 0.1ml of heparin-manganese chloride reagent and mixed. The solution was allowed to stand at 4°C for 30min and then centrifuged in a refrigerator at 1800 g for 30min. The supernatant represented the HDL fraction. An aliquot of supernatant was used for cholesterol estimation.

The values were expressed as mg/dl

VLDL-cholesterol:

VLDL-cholesterol was calculated using the following equation. (Friedwald et al, 1972)

$$VLDL-C = TG/5$$

The values were expressed as mg/dl

LDL-cholesterol → (80-175 mg/dl) normal value

LDL-C was calculated using the following equation.

LDL-C = Total cholesterol - (HDL-C + VLDL-C)

→ Same.

(X) DL-Decidite).

Each organ of the body has to perform its biochemical functions to keep the body, as a whole, in a healthy state. This is possible only when the cells of the organ are intact in structure and function. Any abnormality in the tissue, caused by exogenous or endogenous factors, will seriously impair the organ function which, in turn, influences the health of the organism.

Based on the functional capabilities of the organs, ***specific biochemical investigations*** have been developed ***in the laboratory***, to assess their function. In this chapter, the biochemical investigations to assess the functioning of liver, kidney, stomach and pancreas are discussed. The tests to evaluate the function of endocrine organs are discussed elsewhere (***Chapter 19***).

LIVER FUNCTION TESTS

Liver performs several diversified functions. It is the central organ of body's metabolism.

Major functions of liver

1. **Metabolic functions** : Liver actively participates in carbohydrate, lipid, protein, mineral and vitamin metabolisms.

2. **Excretory functions :** Bile pigments, bile salts and cholesterol are excreted in the bile into intestine.

3. **Protective functions and detoxification :** Kupffer cells of liver perform phagocytosis to eliminate foreign compounds. Ammonia is detoxified to urea. Liver is responsible for the metabolism of xenobiotics (detoxification).

4. **Hematological functions** : Liver participates in the formation of blood (particularly in the embryo), synthesis of plasma proteins (including blood clotting factors) and destruction of erythrocytes.

5. **Storage functions :** Glycogen, vitamins A, D and B_{12} and trace element iron are stored in liver.

Causes of liver damage

Hepatocellular damage may occur due to viruses (hepatitis A virus, hepatitis B virus), toxins (carbon tetrachloride, alfatoxin, alcohol), hepatocellular carcinoma, autoimmune hepatitis etc.

Tests to assess liver function

The liver function tests (LFT) are the biochemical investigations to assess the capacity of the liver to carry out any of the functions it performs. LFT will help to detect the abnormalities and the extent of liver damage.

Two important facts should be borne in mind while carrying out LFT.

1. Liver is a large-size factory of safety. Therefore, it can perform many of its functions almost normally, despite the damage.

2. Selection of the right test is important in LFT. This is due to the fact that since liver participates in several functions, the function that is measured in LFT may not be the one that is adversely affected.

The major liver function tests may be classified as follows

1. Tests based on ***excretory function***—Measurement of bile pigments, bile salts, bromosulphthalein.

2. Tests based on ***serum enzymes*** derived from liver—Determination of transaminases, alkaline phosphatase, 5'-nucleotidase, γ-glutamyl-transpeptidase.

3. Tests based on ***metabolic capacity***—Galactose tolerance, antipyrine clearance.

4. Tests based on ***synthetic functions***—Prothrombin time, serum albumin.

5. Tests based on ***detoxification***—Hippuric acid synthesis.

This above list, contains the most important biochemical investigations to assess LFT. Among these, the commonly used tests are described in the following pages.

Markers of liver function

The important liver functions and the common plasma/serum markers for the impaired functions are listed in Table 28.1. The most important markers namely, bilirubin, enzymes, albumin, prothrombin time and drug metabolism with special reference to jaundice and other liver diseases are described.

Table 28.1 A list of liver (hepatic) functions and the common markers to monitor the impaired functions

Hepatic function	Common plasma/serum markers for impaired function
Heme catabolism	↑Bilirubin
Enzymes	↑Alanine transaminase ↑Aspartate transaminase ↑γ-Glutamyltranspeptidase
Protein synthesis	↓Albumin ↑Prothrombin time
Protein catabolism	↑Urea ↑Ammonia
Lipid metabolism	↑Cholesterol ↑Triglycerides
Drug metabolism	↑Half-lives of drugs
Bile acid metabolism	↑Bile acids

BILIRUBIN

Bilirubin is a bile pigment, and is the excretory end product of heme degradation. It is conjugated in the liver to form bilirubin diglucuronide, and excreted in bile. The details of bilirubin metabolism are discussed elsewhere (Chapter 18).

Serum bilirubin

The normal concentration of serum bilirubin is in the range of **0.2-1.0 mg/dl**. Of this, the conjugated bilirubin (diglucuronide 75%, monoglucuronide 25%) is 0.2-0.4 mg/dl, while the unconjugated bilirubin is 0.2-0.6 mg/dl.

Icterus index

This is a simple test to measure the **yellow colour of serum due to bilirubin**. It is rather crude and almost outdated. However, it is still useful for a rapid assessment of neonatal jaundice.

van den Bergh reaction

This is a specific reaction to identify the increase in serum bilirubin (above the reference level). Normal serum gives a negative van den Bergh reaction.

Mechanism of the reaction : van den Bergh reagent is a mixture of equal volumes of sulfanilic acid (in dilute HCl) and sodium nitrite. The principle of the reaction is that diazotised sulfanilic acid (in the above mixture) reacts with bilirubin to form a purple coloured azobilirubin.

Direct and indirect reactions : Bilirubin as such is insoluble in water while the conjugated bilirubin is soluble. van den Bergh reagent reacts with ***conjugated bilirubin*** and gives a purple colour immediately (normally within 30 seconds). This is referred to as a ***direct positive*** van den Bergh reaction. Addition of methanol (or alcohol) dissolves the ***unconjugated bilirubin*** which then gives the van den Bergh reaction (normally within 30 minutes) positive and this is referred to as ***indirect positive***. If the serum contains both unconjugated and conjugated bilirubin in high concentration, the purple colour is produced immediately (direct positive) which is further intensified by the addition of alcohol (indirect positive). This type of reaction is known as ***biphasic***.

van den Bergh reaction and jaundice : This reaction is highly useful in understanding the nature of jaundice. This is due to the fact that the type of jaundice is characterized by increased serum concentration of unconjugated bilirubin (hemolytic), conjugated bilirubin (obstructive) or both of them (hepatic). Therefore, the response of van den Bergh reaction can differentiate the jaundice as follows

Indirect positive	—	Hemolytic jaundice
Direct positive	—	Obstructive jaundice
Biphasic	—	Hepatic jaundice.

Bilirubin in urine

The conjugated bilirubin, being water soluble, is excreted in urine. This is in contrast to unconjugated bilirubin which is not excreted. Bilirubin in urine can be detected by Fouchet's test or Gmelin's test.

Bromosulphthalein (BSP) test

Bromosulphthalein is a ***dye*** used ***to assess*** the ***excretory function of liver***. It is a non-toxic compound and almost exclusively excreted by the liver (through bile). BSP is administered intravenously (5 mg/kg body weight) and its serum concentration is measured at 45 min and at 2 hrs. In normal individuals, less than 5% of the dye is retained at the end of 45 min. Any impairment in liver function causes an ***increased retention of the dye***. This test is quite sensitive to assess liver abnormality with particular reference to excretory function.

SERUM ENZYMES DERIVED FROM LIVER

Liver cells contain several enzymes which may be released into the circulation in liver damage. Measurement of selected enzymes in serum is often used to assess the liver function. It must, however, be noted that there is no single enzyme that is absolutely specific to liver alone. Despite this fact, serum enzymes provide valuable information for LFT. Some of these enzymes are discussed hereunder.

Transaminases or aminotransferases

The activities of two enzymes—namely serum glutamate pyruvate transaminase (SGPT; recently called as ***alanine transaminase***—ALT) and serum glutamate oxaloacetate transaminase (SGOT; recently known as ***aspartate transaminase***—AST) —are widely used to assess the liver function. ALT is a cytoplasmic enzyme while AST is found in both cytoplasm and mitochondria. The activity of these enzymes is low in normal serum (ALT 5-40 IU/l; AST 5-45 IU/l). Serum **ALT** and AST are increased in liver damage. However, alanine transaminase is ***more sensitive*** and reliable for the assessment of LFT.

The normal ***AST/ALT ratio*** is around **0.8**. This ratio is increased (>2) in myocardial infarction, alcoholic hepatitis, and cirrhosis. AST/ALT ratio is decreased (i.e. ALT higher) in acute hepatocellular damage and cholestasis.

Alkaline phosphatase

Alkaline phosphatase (ALP) is mainly derived from bone and liver (the cells lining the bile canaliculi). A rise in serum ALP (normal 1-13 KA units/dl), usually associated with elevated serum bilirubin is an indicator of biliary obstruction (**obstructive/posthepatic jaundice**). ALP is also elevated in cirrhosis of liver and hepatic tumors.

Liver is not the sole source of alkaline phosphatase. Therefore, its measurement has to be carefully viewed (along with others) before arriving at any conclusion. The liver and bone isoenzymes of ALP can be separated by electrophoresis.

γ-Glutamyl transpeptidase

This is a microsomal enzyme widely distributed in body tissues, including liver. Measurement of γ-glutamyl transpeptidase (GGT) activity provides a sensitive index to asses liver abnormality. The activity of this enzyme almost parallels that of transaminases in hepatic damage. Serum GGT is highly elevated (normal 5-40 IU/l) in **biliary obstruction** and **alcoholism**. Further, several drugs (e.g. phenytoin) induce (liver synthesis) and increase this enzyme in circulation.

5 -Nucleotidase

The serum activity of 5'-nucleotidase (normal 2-15 U/l) is elevated in hepatobiliary disease and this parallels ALP. The advantage with 5'-nucleotidase is that it is not altered in bone diseae (as is the case with ALP).

Other enzymes

Serum isocitrate dehydrogenase and isoenzymes of lactate dehydrogenase (LDH_5 and LDH_4) are also useful in LFT.

Enzyme combinations

Very often, a combination of serum enzyme estimations (instead of a single one) is used for a better understanding of liver functions. For instance, a large increase in transaminases (particularly ALT) relative to a small increase in alkaline phosphatase indicates hepatocellular damage. On the other hand, a small increase in transaminases and a large increase of alkaline phosphatase shows biliary obstruction.

JAUNDICE

Jaundice (*French* : jaune—yellow) is characterized by **yellow coloration of sclera** (of eyes) and skin. This is due to the elevated serum **bilirubin** level, usually **beyond 2 mg/dl** (normal < 1 mg/dl).

The metabolism of heme to produce bilirubin and its conjugated derivatives and the types of jaundice have already been described. The reader must refer this (**Chapter 10**) now. The biochemical changes and the related parameters for the differential diagnosis of the three types of jaundice (hemolytic, obstructive and hepatic) are given in **Table 20.2**.

In the **Fig.20.1**, the normal and abnormal bilirubin metabolism (along with the associated

Table 20.2 Biochemical changes for the differential diagnosis of three types of jaundice

Parameter	Hemolytic jaundice (prehepatic jaundice)	Obstructive jaundice (posthepatic jaundice)	Hepatic jaundice (intrahepatic jaundice)
Serum bilirubin	Unconjugated bilirubin ↑	Conjugated bilirubin ↑	Both ↑
van den Bergh reaction	Indirect positive	Direct positive	Biphasic
Serum enzymes	ALT, AST and ALP →	ALP ↑↑, ALT and AST marginal ↑	ALT and AST ↑↑, ALP marginal ↑
Bilirubin in urine	Not excreted	Excreted	Excreted
Urobilinogen in urine	Excretion ↑	→ or ↓	→ or ↓

ALT : Alanine transaminase; AST : Aspartate transaminase; ALP : Alkaline phosphatase; ↑ : Increase; ↓ : Decrease; → : Normal

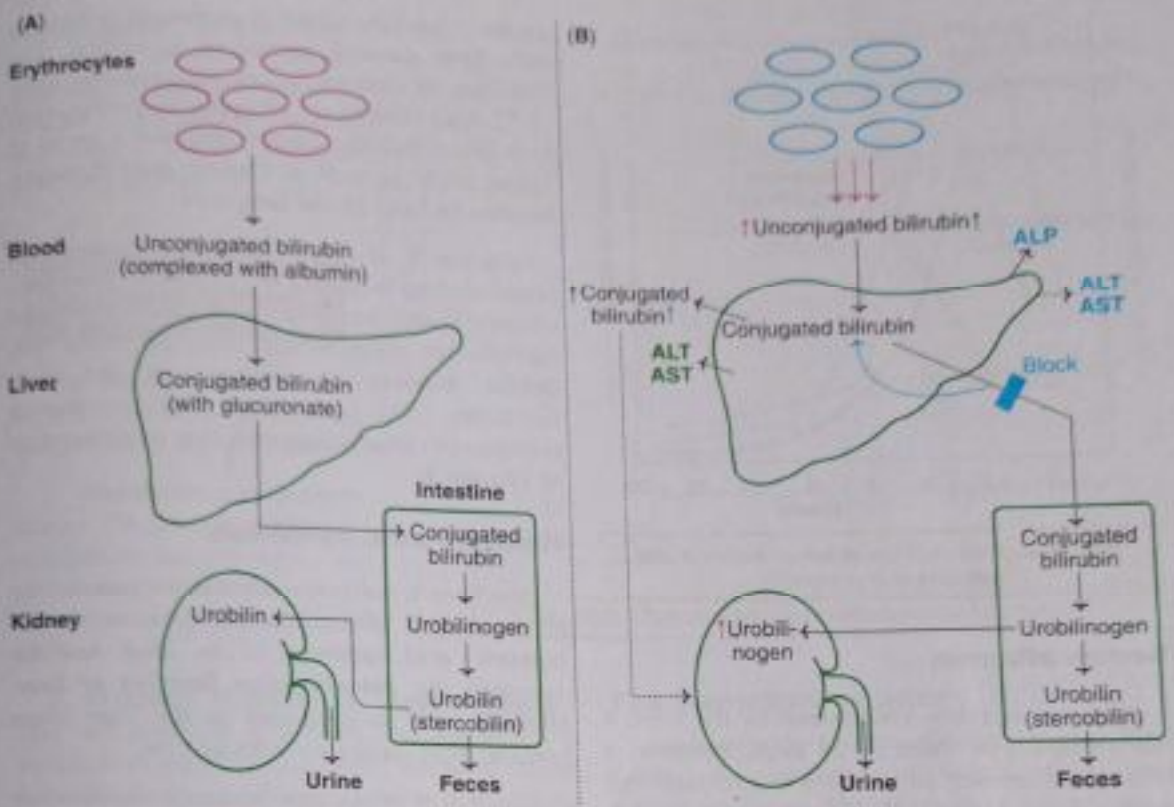


*Fig. 20.1 : Normal and abnormal bilirubin metabolism (**A**) Normal bilirubin metabolism (**B**) Alterations in bilirubin metabolism along with enzymes in three types of jaundice (**Note :** Colours indicate major changes; Red–changes in hemolytic jaundice; Green–changes in hepatic jaundice; Blue–changes in obstructive jaundice; Dotted lines indicate minor pathways; ALT–Alanine transaminase, AST–Aspartate transaminase; ALP–Alkaline phosphatase).*

enzyme changes) are depicted. The major changes in the 3 types of jaundice are listed below

Hemolytic jaundice : Elevated serum unconjugated bilirubin, and increased urinary excretion of urobilinogen.

Obstructive jaundice : Elevated serum conjugated bilirubin and increased activities of alkaline phosphatase (ALP), alanine transaminase (ALT) and aspartate transaminase (AST).

Hepatic jaundice : Elevated serum unconjugated and conjugated bilirubin, and increased activities of ALT and AST.

The pattern of rise in the serum alanine transaminase, aspartate transaminase and bilirubin in acute viral hepatitis is depicted in ***Fig.21.2***. It may be noted that the transaminase activities (more predominantly ALT) are elevated much before the bilirubin starts increasing.

Galactose tolerance

Galactose is a monosaccharide, almost exclusively metabolized by the liver. The liver function can be assessed by measuring the utilization of galactose. This is referred to galactose tolerance test. The subject is given intravenous administration of galactose (about 300 mg/kg body weight). Blood is drawn at 10 minute intervals for the next 2 hours and galactose estimated. In the normal individuals, the ***half-life of galactose*** is about 10-15 minutes. This is markedly ***elevated*** in hepatocellular ***damage*** (infective hepatitis, cirrhosis).

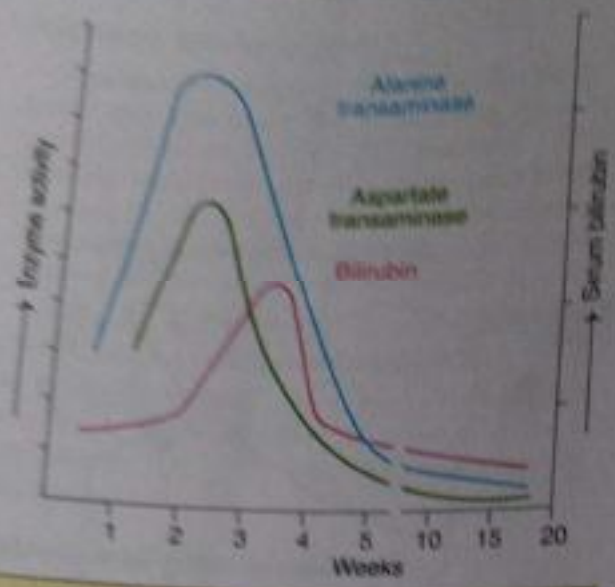


Fig. 20.2 : Pattern of rise in serum enzymes and bilirubin in viral hepatitis.

Serum albumin

Albumin is solely synthesized by the liver. It has a half-life of about 20-25 days, therefore, it is a good marker to assess chronic (and not acute) liver damage. Low serum albumin is commonly observed in patients with severe liver damage. It must, however, be noted that the serum albumin concentration is also ***decreased*** due to other factors such as ***malnutrition***.

Functional impairment of liver is frequently associated with increased synthesis of globulins. Cirrhosis of the liver causes a ***reversal*** of albumin/globulin ratio (***A/G ratio***). Serum electrophoresis of proteins reveals increased albumin and decreased γ-globulin concentration. This, however, may not have much diagnostic importance since several diseases are associated with altered electrophoretic pattern of serum proteins.

Prothrombin time

The liver synthesizes all the factors concerned with blood clotting. A decrease in the concentration of plasma clotting factors is found in the impairment of liver function. This can be assessed in the laboratory by measuring prothrombin time which is ***prolonged in*** patients with severe liver damage. The half-lives of clotting factors are relatively short (5-72 hrs.), therefore, changes in prothrombin time occur quickly. Hence, this test is useful to assess acute as well as chronic liver damages, besides its help in the prognosis.

Vitamin K is required for the synthesis of blood clotting factors II, VII, IX and X. Therefore, vitamin K deficiency can also cause prolonged prothrombin time which must be ruled out before drawing conclusions on the liver functions. This is done by measuring prothrombin time before and after administration of vitamin K.

Hippuric acid synthesis

The liver is the major site for the metabolism of xenobiotics (detoxification). Measurement of hippuric acid synthesis is an ideal ***test for assessing the detoxification function of liver***. Hippuric acid is produced in the liver when benzoic acid combines with glycine.

About 6 g of sodium benzoate dissolved in (about 250 ml) water, is orally given to the subject, after a light breakfast (usually 2 hrs later) and after emptying the bladder. Urine collections are made for the next 4 hours and the amount of hippuric acid excreted is estimated. Theoretically, 6 g of sodium benzoate should yield 7.5 g of hippuric acid. In the healthy persons, about 60% of sodium benzoate (equivalent to 4.5 g hippuric acid) is excreted in urine. A ***reduction*** in hippuric acid excretion (particularly < 3 g) ***indicates hepatic damage***.

Choice of liver functions tests

The choice of biochemical tests to measure liver functions mostly depends on the purpose of the investigation. The clinical history of the subject is often a guiding factor in this regard. A single test in isolation may have a little diagnostic value.

Frequently, a ***combination of laboratory investigations are employed in LFT***. These include serum bilirubin (conjugated and

unconjugated), alanine transaminase, aspartate transaminase, alkaline phosphatase, γ-glutamyl transpeptidase and proteins (albumin, globulins).

KIDNEY (RENAL) FUNCTION TESTS

The kidneys are the vital organs of the body, performing the following major functions.

1. **Maintenance of homeostasis :** The kidneys are largely responsible for the regulation of water, electrolyte and acid-base balance in the body.

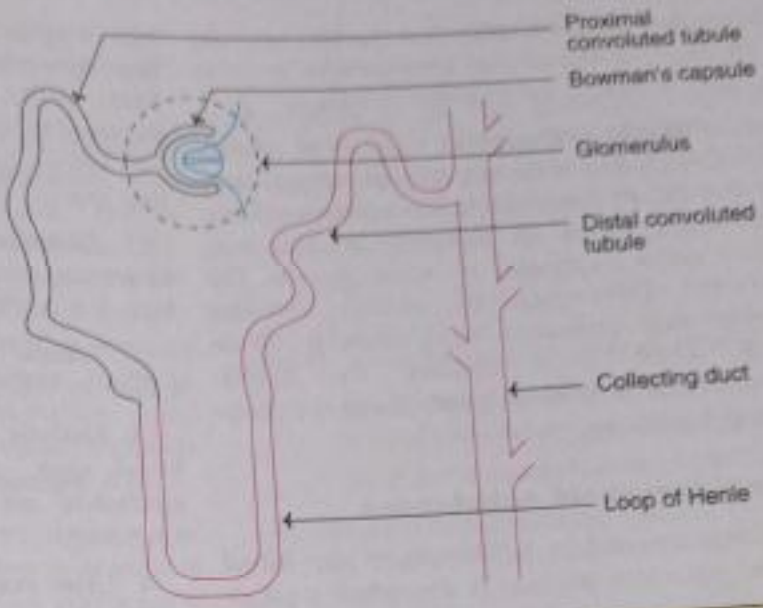


Fig. 20.3 : Diagrammatic representation of a nephron.

2. **Excretion of metabolic waste products :** The end products of protein and nucleic acid metabolism are eliminated from the body. These include urea, creatinine, creatine, uric acid, sulfate and phosphate.

3. **Retention of substances vital to body :** The kidneys reabsorb and retain several substances of biochemical importance in the body e.g. glucose, amino acids etc.

4. **Hormonal functions :** The kidneys also function as endocrine organs by producing hormones.

- **Erythropoietin,** a peptide hormone, stimulates hemoglobin synthesis and formation of erythrocytes.
- 1,25-Dihydroxycholecalciferol ***(calcitriol)*** – the biochemically active form of vitamin D – is finally produced in the kidney. It regulates calcium absorption from the gut.
- **Renin,** a proteolytic enzyme liberated by kidney, stimulates the formation of angiotensin II which, in turn, leads to aldosterone production. Angiotensin II and aldosterone are the hormones involved in the regulation of electrolyte balance.

The formation of urine

Nephron is the functional unit of kidney. Each kidney is composed of approximately one million nephrons. The structure of a nephron, as depicted in ***Fig.20.3***, consists of a Bowman's capsule (with blood capillaries), proximal convoluted tubule (PCT), loop of Henle, distal convoluted tubule (DCT) and collecting tubule.

The blood supply to kidneys is relatively large. About 1200 ml of blood (650 ml plasma) passes through the kidneys, every minute. From this, about ***120-125 ml is filtered per minute by the kidneys*** and this is referred to as ***glomerular filtration rate*** (GFR). With a normal GFR (120-125 ml/min), the glomerular filtrate formed in an adult is about 175-180 litres per day, out of which only 1.5 litres is excreted as urine. Thus, more than 99% of the glomerular filtrate is reabsorbed by the kidneys.

The process of urine formation basically involves two steps—glomerular filtration and tubular reabsorption.

1. **Glomerular filtration :** This is a passive process that results in the formation of ultrafiltrate of blood. All the (unbound) constituents of plasma, with a molecular weigh

less than about 70,000, are passed into the filtrate. Therefore, the glomerular filtrate is almost similar in composition to plasma.

2. **Tubular reabsorption :** The renal tubules (PCT, DCT and collecting tubules) retain water and most of the soluble constituents of the glomerular filtrate by reabsorption. This may occur either by passive or active process. The excreted urine has an entirely different composition compared to glomerular filtrate from which it is derived. The normal composition of urine is given elsewhere (***Refer inside backcover***).

Renal threshold substances

There are certain substances in the blood whose excretion in urine is dependent on their concentration. Such substances are referred to as renal threshold substances. At the normal concentration in the blood, they are completely reabsorbed by the kidneys, with a result that their excretion in urine is almost negligible.

The renal threshold of ***a substance*** is defined as its ***concentration in blood (or plasma) beyond which*** it is ***excreted into urine***. The renal threshold for glucose is 180 mg/dl; for ketone bodies 3 mg/dl; for calcium 10 mg/dl and for bicarbonate 30 mEq/l. While calculating the renal threshold of a particular compound, it is assumed that both the kidneys are optimally functioning, without any abnormality. But this is not always true—in which case the renal threshold is altered. For instance, ***renal glycosuria*** is associated with reduced threshold for glucose due to its diminished tubular reabsorption.

The term ***tubular maximum*** (***Tm***) is used to indicate the maximum capacity of the kidneys to absorb a particular substance. For instance, tubular maximum for glucose (TmG) is 350 mg/min.

Tests to assess renal function

In view of the important and sensitive functions the kidney performs (described already), it is essential that the abnormalities (renal damages), if any, must be detected at the earliest. Several tests are employed in the laboratory to assess kidney (renal) function. It must however, be remembered that about two-thirds of the renal tissue must be functionally damaged to show any abnormality by these tests. The kidney function tests may be divided into four groups.

1. **Glomerular function tests :** All the clearance tests (inulin, creatinine, urea) are included in this group.

2. **Tubular function tests :** Urine concentration or dilution test, urine acidfication test.

3. **Analysis of blood/serum :** Estimation of blood urea, serum creatinine, protein and electrolyte are often useful to assess renal function.

4. **Urine examination :** Simple routine examination of urine for volume, pH, specific gravity, osmolality and presence of certain abnormal constituents (proteins, blood, ketone bodies, glucose etc.) also helps, of course to a limited degree, to assess kidney functioning.

Some of the important renal function tests are discussed in the following pages.

CLEARANCE TESTS

The clearance tests, measuring the ***glomerular filtration rate (GFR)*** are the most useful in assessing the renal function. The excretion of a substance can be expressed quantitatively by using the concept of clearance.

Clearance, in general, is defined as the ***volume of plasma that would be completely cleared of a substance per minute***. In other words, ***clearance of a substance refers to the milliliters of plasma which contains the amount of that substance excreted by kidney per minute.*** Clearance (C), expressed as ml/minute, can be calculated by using the formula

$$C = \frac{U \times V}{P}$$

where U = Concentration of the substance in urine.

V = Volume of urine in ml excreted per minute.

P = Concentration of the substance in

Care should be taken to express the concentrations of plasma and urine in the same units (mmol/l or mg/dl).

The clearance of a given substance is determined by its mode of excretion. The maximum rate at which the plasma can be cleared of any substance is equal to the GFR. This can be easily calculated by measuring the clearance of a plasma compound which is freely filtered by the glomerulus and is neither absorbed nor secreted in the tubule. ***Inulin*** (a plant carbohydrate, composed of fructose units) and ^{51}Cr-EDTA satisfy this criteria. Inulin is intravenously administered ***to measure GFR***.

In practice, however, measurement of clearance for the substances already present in the blood is preferred. The two compounds, namely ***creatinine*** and ***urea***, are commonly employed for this purpose. Creatinine clearance (~145 ml/min) is marginally higher than the GFR as it is secreted by the tubules. On the other hand, urea clearance (~75 ml/min) is less than the GFR, since it is partially reabsorbed by the tubules.

Diodrast (diiodopyridone acetic acid) is used as a contrast medium to take urinary tract X-rays. Diodrast and ***para amino hippuric acid (PAH)*** are peculiar substances as they are entirely excreted by a single passage of blood through the kidneys. It is partly filtered by the glomerulus and mostly excreted by the tubules. PAH has a clearance of about 700 ml/min (or 1,200 ml, if expressed as blood). Thus clearance of PAH represents the ***renal plasma flow***.

Creatinine clearance test

Creatinine is an excretory product derived from creatine phosphate (largely present in muscle). The excretion of creatinine is rather constant and is not influenced by body metabolism or dietary factors. As already stated, creatinine is filtered by the glomeruli and only marginally secreted by the tubules. The value of creatinine clearance is close to GFR, hence its measurement is a sensitive and good approach to assess the renal glomerular function. Creatinine clearance may be defined as ***the volume (ml) of plasma that would be completely cleared of creatinine per minute***.

Procedure : In the traditional method, creatinine content of a 24 hr urine collection and the plasma concentration in this period are estimated. The creatinine clearance (C) can be calculated as follows :

$$C = \frac{U \times V}{P}$$

where U = Urine concentration of creatinine
V = Urine output in ml/min (24 hr urine volume divided by 24 × 60)
P = Plasma concentration of creatinine.

As already stated, creatinine concentration in urine and plasma should be expressed in the same units (mg/dl or mmol/l).

Modified procedure : Instead of a 24 hr urine collection, the procedure is modified to collect urine for 1 hr, after giving water. The volume of urine is recorded. Creatinine contents in plasma and urine are estimated. The creatinine clearance can be calculated by using the formula referred above.

Reference values : The normal range of creatinine clearance is around ***120-145 ml/min***. These values are slightly lower in women. In recent years, creatinine clearance is expressed in terms of body surface area.

Diagnostic importance : A ***decrease in creatinine clearance*** value (< 75% normal) serves as ***sensitive indicator of a decreased GFR***, due to renal damage. This test is useful for an early detection of impairment in kidney function, often before the clinical manifestations are seen.

Urea clearance test

Urea is the end product of protein metabolism. After being filtered by the glomeruli, it is partially reabsorbed by the renal tubules. Hence, urea clearance is less than the GFR and, further, it is influenced by the protein content of the diet. For these reasons, ***urea clearance is not as sensitive as creatinine clearance*** for assessing renal function. Despite this fact, several laboratories traditionally use this test.

Urea clearance is defined as the volume (ml) of plasma that would be completely cleared of urea per minute. It is calculated by the formula

$$C_m = \frac{U \times V}{P}$$

where C_m = Maximum urea clearance
U = Urea concentration in urine (mg/ml)
V = Urine excreted per minute in ml
P = Urea concentration in plasma (mg/ml).

The above calculation is applicable if the ***output of urine is more than 2 ml per minute.*** This is referred to as ***maximum urea clearance*** and the normal value is around 75 ml/min.

Standard urea clearance : It is observed that the urea clearance drastically changes when the volume of urine is less than 2 ml/min. This is known as ***standard urea clearance (C_s)*** and the normal value is around 54 ml/min. It is calculated by a modified formula

$$C_s = \frac{U \times \sqrt{V}}{P}$$

Diagnostic importance : A urea clearance value below 75% of the normal is viewed seriously, since it is an indicator of renal damage. Blood urea level as such is found to increase only when the clearance falls below 50% normal. As already stated, creatinine clearance is a better indicator of renal function.

Urine concentration test

This is a test to assess the renal tubular function. It is a simple test and involves the accurate measurement of specific gravity which depends on the concentration of solutes in urine. A specific gravity of 1.020 in the early morning urine sample is considered to be normal.

Several measures are employed to concentrate urine and measure the specific gravity. These include overnight water deprivation and administration of antidiuretic hormone. If the specific gravity of urine is above 1.020 for at least one of the samples collected, the tubular function is considered to be normal.

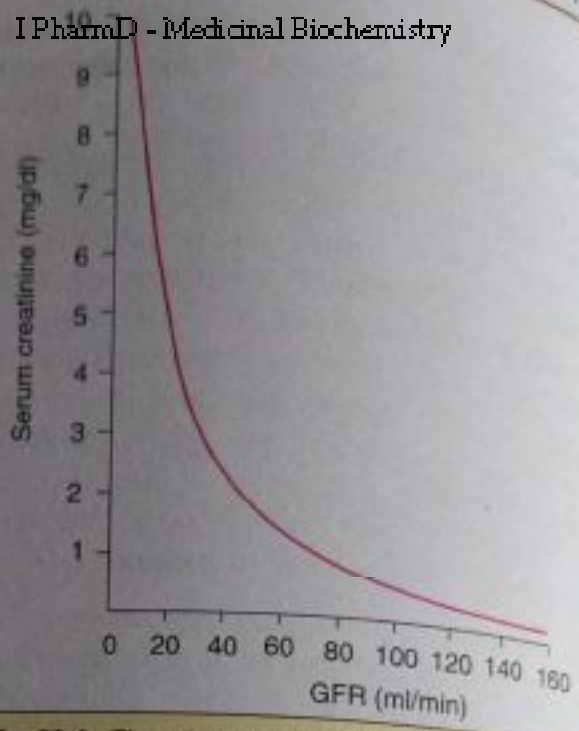


Fig. 20.4 : The relationship between glomerular filtration rate (GFR) and serum creatinine concentration.

Osmolality and specific gravity : The osmolality of urine is variable. In normal individuals, it may range from 500-1,200 milliosmoles/kg. The plasma osmolality is around 300 milliosmoles/kg. The normal ratio of the osmolality between urine and plasma is around 2-4. It is found that the urine (without any protein or high molecular weight substance) with an osmolality of 800 mosm/kg has a specific gravity of 1.020. Therefore, measurement of urine osmolality will also help to assess tubular function.

Analysis of blood (or serum)

Estimation of serum creatinine and blood urea are often used to assess the overall kidney function, although these tests are less sensitive than the clearance tests. ***Serum creatinine*** is a ***better*** indicator than urea in this regard. The diagnostic importance of urea and creatinine estimations are discussed elsewhere (***Refer Chapter 15***).

The relationship between GFR and serum creatinine levels is depicted in ***Fig.20.4***. It is observed that the GFR must fall to about 50% of its normal value before a significant increase in serum creatinine occurs. Therefore, a normal

serum creatinine level does not necessarily mean that all is well with the kidney. It is estimated that a loss of 50% of the functions of nephrons leads to (approximate) doubling of serum creatinine concentration.

Cystatin C is a ***protein marker of kidney function*** (serum reference range 0.8–1.2 μg/dl), and is more sensitive than creatinine. Even minor changes in GFR in the early stages of chronic kidney diseases are associated with increased cystatin C.

Urine examination

The routine urine examination is undoubtedly a guiding factor for renal function. The volume of urine excreted, its pH, specific gravity, osmolality, the concentration of abnormal constituents (such as proteins, ketone bodies, glucose and blood) may help to have some preliminary knowledge of kidney function. More information on urine laboratory tests is given in the ***appendix***.

Choice of renal function tests

In general, the assessment of kidney function starts with the routine urine examination, followed by serum creatinine and/or blood urea estimations and, finally, the specific tests to measure the tubular and glomerular functions (clearance tests).

GASTRIC FUNCTION TESTS

The stomach is a major organ of digestion and performs the following functions

1. Stomach is a reservoir of ingested foodstuffs.

2. It has a great churning ability which promotes digestion.

3. Stomach elaborates HCl and proteases (pepsin) which are responsible for the initiation of digestive process.

4. The products obtained in the stomach (peptides, amino acids) stimulate the release of pancreatic juice and bile.

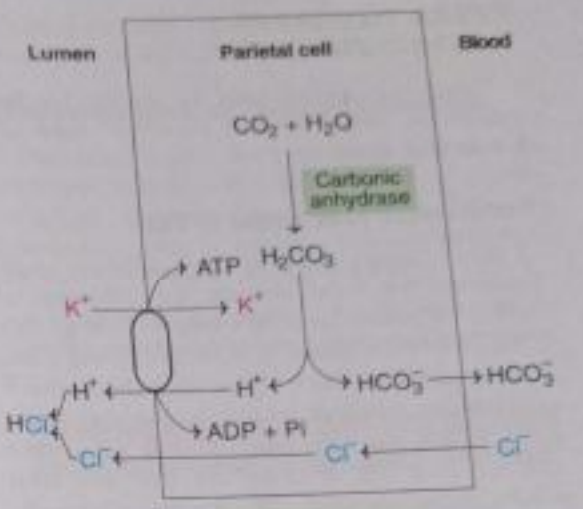


Fig. 20.5 : Mechanism of HCl secretion (⬭—represents K^+ activated ATPase).

Secretion of gastric HCl

The parietal (oxyntic) cells of gastric glands produce HCl. The pH in the gastric lumen is as low as 0.8 (against the blood pH 7.4). Therefore, the protons are transported against the concentration gradient by an active process.

A unique enzyme—namely K^+ activated ATPase—present in the parietal cells is connected with the mechanism of HCl secretion (***Fig.20.5***). The process involves an exchange of H^+ ions (of the parietal cells) for K^+ ions (of the lumen). This is coupled with the consumption of energy, supplied by ATP. The H^+ are continuously generated in the parietal cells by the dissociation of carbonic acid which, in turn, is produced from CO_2. The bicarbonate ions (HCO_3^-), liberated from the carbonic acid (H_2CO_3) dissociation, enter the blood in exchange for Cl^- ions. The latter diffuse into the gastric lumen to form HCl. Gastrin—a peptide hormone of gastrointestinal tract—stimulates HCl secretion.

Following a meal, there is a slight ***elevation in the plasma bicarbonate concentration*** which is linked to the gastric HCl secretion. This is referred to as ***alkaline tide***.

TESTS TO ASSESS GASTRIC FUNCTION

There are several tests for gastric function evaluation, some of the important ones are briefly discussed.

Fractional test meal (FTM)

This is rather old and not used these days. Fractional test meal involves the collection of stomach contents by ***Ryle's tube*** in fasting. This is followed by a gastric stimulation, giving a test meal (rice gruel, black coffee etc.) The stomach contents are aspirated by Ryle's tube at different time periods (usually every 15 min for 2 hrs.) The samples are analysed for free and total acidity in the laboratory. The results are normally represented by a graph.

Alcohol test meal

In this case, the test meal in the form of 100 ml of 7% alcohol is administered. The response to alcohol test meal is more rapid, and the test time can be reduced to $1\frac{1}{2}$ hour. Clear specimens can be collected by this test, and the free acidity levels are relatively higher compared to FTM.

Pentagastrin stimulation test

Pentagastrin is a synthetic peptide which stimulates the gastric secretion in a manner similar to the natural gastrin. The test procedure adapted is as follows

The stomach contents are aspirated by Ryle's tube in a fasting condition. This is referred to as residual juice. The gastric juice elaborated for the next one hour is collected and pooled which represents the basal secretion. Pentagastrin (6 mg/kg body weight) is now given to stimulate gastric secretion. The gastric juice is collected at 15 minute intervals for one hour. This represents the maximum secretion.

Each sample of the gastric secretion collected is measured for acidity by titrating the samples with N/10 NaOH to pH 7.4. The end point may be detected by an indicator (phenol red) or a pH meter.

Basal acid output (BAO) refers to the acid output (millimol per hour) under the basal conditions i.e. basal secretion.

Maximal acid output (MAO) represents the acid output (millimol per hour) after the gastric stimulation by pentagastrin i.e. maximum secretion.

In normal individuals, the BAO is 4-10 mmol/hr while the MAO is 20-50 mmol/hr.

Augmented histamine test meal

Histamine is a powerful stimulant of gastric secretion. The basal gastric secretion is collected for one hour. Histamine (0.04 mg/kg body weight) is administered subcutaneously and the gastric contents are aspirated for the next one hour (at 15 minute intervals). The acid content is measured in all these samples.

Insulin test meal

This is also known as ***Hollander's test.*** It is mainly done to assess the completeness of vagotomy (vagal resection). Insulin (0.1 unit/kg body weight) is administered intravenously which causes hypoglycemia (blood glucose about 40 mg/dl), usually within 30 minutes, in normal persons.

If the vagotomy operation is successful, insulin administration does not cause any increase in the acid output, compared to the basal level. This test has to be carefully perfomed, since hypoglycemia is dangerous.

Tubeless gastric analysis

In the traditional methods of gastric analysis, a tube is invariably passed into the stomach to collect the gastric juice. This causes inconvenience to the subject. Recently, some tests involving tubeless gastric analysis have been developed. Such tests, however, are mostly useful for preliminary screening.

The principle of tubeless gastric analysis involves administration of a cation exchange resin that gets quantitatively exchanged with the H^+ ions of the gastric juice. The resin is then excreted into urine which can be estimated for an indirect measure of gastric acidity (concentration of H^+ ions).

Diagnex blue containing ***azure-A-resin*** is employed in the tubeless gastric analysis.

Abnormalities of gastric function

Increased gastric HCl secretion is found in Zollinger-Ellison syndrome (a tumor of gastrin secreting cells of the pancreas), chronic duodenal ulcer, gastric cell hyperplasia, excessive histamine production etc.

A decrease in gastric HCl is observed in gastritis, gastric carcinoma, pernicious anemia etc.

OTHER ORGAN FUNCTION TESTS

PANCREATIC FUNCTION TESTS

The pancreas is a specialized organ with exocrine and endocrine functions. The endocrine functions are discussed under the topic diabetes mellitus (***Chapter 36***).

The exocrine functions involve the synthesis of pancreatic juice containing several enzymes (for the digestion of foodstuffs) and bicarbonate. The major enzymes of pancreatic juice are trypsin, chymotrypsin, elastase, carboxypeptidase, amylase and lipase.

Pancreatic enzymes in serum : *Serum amylase* and ***lipase measurements*** are commonly employed to assess the pancreatic function. Both these enzyme activities are elevated in acute pancreatitis, obstruction in the intestine and/or pancreatic duct.

THYROID FUNCTION TESTS

Thyroid gland produces two principal hormones—thyroxine (T_4) and triiodothyronine which regulate the metabolic rate of the body. The laboratory tests employed for the diagnosis of thyroid function are described in the ***Chapter 19*** on hormones.

Define & classify antacid
15-16 IPC. Assay, use of CaCO3 8m 2017

Antacid

These Drugs used for neutralizing excess acid in the stomach.

Acid neutralizing capacity of antacid ?

classification:-

1. Systemic antacid
2. Non systemic antacid

I. Systemic antacid

They are used to reduce the acidity of blood.

eg: Sodium bicarborate inj
Sodium citrate.

II. Non-systemic antacid.

They are used to reduce gastric acidity.

eg Aluminium hydroxide.
magnesium trisilicate.
Magnesium hydroxide.

(1) Sodium Bicarbonate.

$NaHCO_3$.

$$2NaOH + CO_2 \longrightarrow Na_2CO_3 + H_2O$$

$$Na_2CO_3 + H_2O + CO_2 \longrightarrow 2NaHCO_3.$$

Assay.

$S + H_2O \rightarrow$ Titrated with H_2

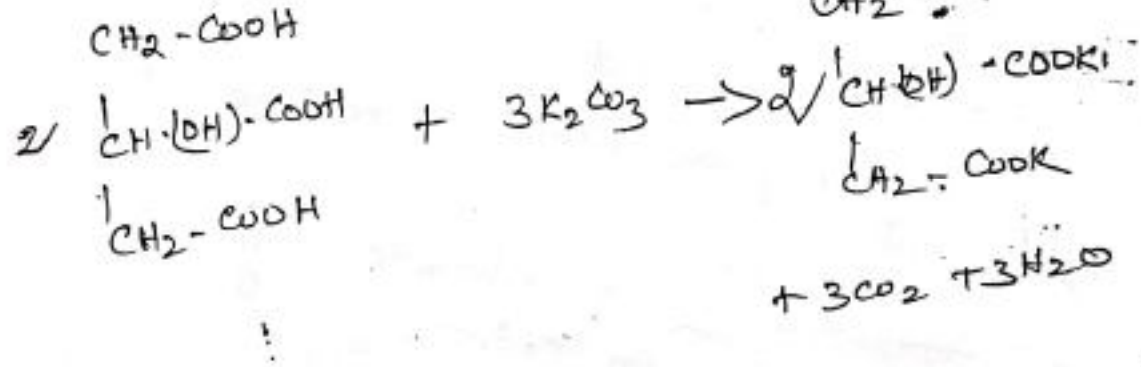
using methyl orange as indicator.

Use

Systemic acidosis.

(2) Potassium citrate.

PPn



Assay

$S \xrightarrow{\Delta}$ residue → Dissolved in H_2O ⇒

HCl → filter the residue → excess of acid

Titrated with NaOH using methyl orange as indicator.

Use. Systemic alkalinizer

Expectorant.

(3) Aluminium hydroxide gel

PR

$$AlCl_3 + 3NaOH + H_2O \rightarrow 3NaCl + Al(OH)_3$$

Assay

S + HCl $\xrightarrow{\Delta}$ add EDTA ⇒ Neutralizing by adding 1N NaOH + hexamine (to maintain alkaline pH) · Excess of EDTA titrated ē std lead nitrate - xylenol orange as indicat

Use Antacid.

(4) Magnesium hydroxide

6m - 2014

PR

$$MgCO_3 \xrightarrow{\Delta} MgO + CO_2$$
$$MgO + H_2O \rightarrow Mg(OH)_2 \downarrow$$

Assay

S + dil HCl + NH_3 - NH_4Cl solution → Titrated with 0.05M EDTA using mordant black T mixture as indicator.

Use Antacid,
Laxative.

(5) Magnesium trisilicate.

$2.MgO\ 3SiO_2\ 3H_2O$.

This compound having Magnesium Oxide + Silicon Oxide.

PPn

Sodium silicate treat with magnesium Sulphate → mg. trisilicate is ppt.
The ppt is filtered, washed and dried.

Assay

MgO

Complexometric titration.

S + HCl + NaOH → Titrated ē

EDTA using mureoxide as indicator.

(6) Silicondioxide (SiO_2)

S + H_2SO_4 → filtered → insoluble SiO_2 is washed until free from sulphate and ignited for 5 min, cooled and weighed

Use. Antacid.

(17) Magnesium Carbonate

PP2

$MgSO_4 + Na_2CO_3 \rightarrow MgCO_3 + Na_2SO_4$

Assay Complexometric

S + HCl + NaOH → Titrated ē EDTA using murexide as indicator.

Use Antacid
Laxative.

2017 5m + 3m definition & classification of antacid

(18) Calcium Carbonate

PP2

$CaCl_2 + Na_2CO_3 \rightarrow CaCO_3 + 2NaCl$

Assay. S + HCl + NaOH → Titrated ē EDTA using murexide + Naphthol green mixture as indicator,

Use Antacid.

Aluminium phosphate 18
Magnesium oxide 20

(4)

(8) Poisamuth Carbonate

PP$_n$

I $2Bi + 8HNO_3 \rightarrow 2Bi(NO_3)_3 + 2NO + 4H_2O$.

II $4Bi(NO_3)_3 + 6Na_2CO_3 + H_2O \rightarrow [(BiO_2)_2CO_3] + 12NaNO_3 + 4CO_2$

Assay Gravimetric method

· S → ignited → weighed · Poisamuth oxide.

N. IL. T · 90%.

Use used in diarrhoea and dysentry.

Magaldrate

$Al_2H_{14}Mg_4O_{14} \cdot 2H_2O$.

Preparation

Aluminium hydroxide + $Mg(OH)_2$

28 to 39% MgO + 17 to 25% Aluminium Oxide

Assay. MgO ⇒ Complexometric titration

S + HCl + NaOH → Titrated ε EDTA using murexide as an indicator.

Aluminium oxide

S + HCl → added EDTA → Neutralizing adding in Nacetate + hexamine → excess of EDTA Titrated ε Lead Nitrate xylenol orange as an indicator.

Use. Antacid.