Urine Analysis

8

8A. ANALYSIS OF NORMAL CONSTITUENTS OF URINE

INTRODUCTION

Urine is the ultra filtrate of plasma formed when the blood perfuse the two kidneys. Glomerulus filters plasma and the volume of glomerular filtrate amount to 180 L in 24 hours for an adult. Tubules of the kidney modify the glomerular filtrate by reabsorption and secretion of water and solutes to produce final urine volume of 1-2 L per day. Glomerular filtration rate is about 120 ml per minute. Thus, the kidneys retain essential substances and excrete waste products from the body. By this process it also helps in maintaining the acid base balance.

Clinical laboratory analysis of urine can provide information of kidney dysfunction (e.g. Nephrotic syndrome, glomerular nephritis) and about certain systemic diseases (e.g. Phenyl ketonuria, Diabetes mellitus) in an individual.

SPECIMEN COLLECTION

For getting correct analytical results , care must be taken in the collection of urine and transportation of it to the laboratory. Urine should be collected in clean sterile containers with a tightly fitting lid to avoid spillage, evaporation and contamination. Specimen containers should be labeled with name, age, date and time of collection.

The best urine specimen is the first voided urine in the morning since it is the most concentrated urine. Mid stream specimen is to be collected.

PHYSICAL EXAMINATION OF URINE (TABLE 8A-1)

Physical examination of urine is to be carried out prior to routine analysis which includes assessment of **volume, appearance, odor, color, pH and specific gravity**. Careful interpretation of these physical properties gives us a lot of information regarding various types of illnesses.

1. Volume: Normal adults excrete about 750 to 2000 ml of urine. It is influenced by fluid and salt intake, perspiration, respiration and functional status of cardiovascular and renal systems.

Oliguria: A decreased urine output is called oliguria.

Causes of Oliguria

– Prerenal causes
  - low blood pressure, shock, bleeding, fluid deprivation

– Renal causes
  • acute tubular necrosis, poisons causing renal damage, renal vascular disease
– Post renal causes
  • calculi, tumors compressing urinary tract from within or outside, prostate enlargement

Polyuria

An increased output of urine is referred to as polyuria.

Causes of Polyuria

– Conditions leading to excretion of a large amount of solutes along with isoosmotic amount of water
– Excessive salt intake, diabetes mellitus
– Deficiency of antidiuretic hormone
– Excessive fluid intake
– Intake of diuretics

2. Appearance: Normal urine is clear (transparent).

Causes of Cloudiness

Urine may become cloudy due to the presence of amorphous **phosphates** which will disappear or due to **urates** in urine. The cloudiness caused by phosphate appear and that due to urates disappear upon heating.

– Pus cell (white blood cells) clears on filtering
– Bacteria or fungi cleared by centrifugation
– Colloidal suspension of fat (as in chyluria) which cannot be cleared off by usual filtering or centrifugation.

3. Odor: Normally fresh urine has a faint aromatic smell.

– Upon standing, strong ammoniacal odor develop due to formation of ammonia by the decomposition of urea.
– Presence of ketone bodies (acetone and acetoacetic acid)in urine produces a fruity odor

4. Color: Normal color of urine varies from colorless to deep yellow. The color of urine is conferred by urochromes and urobilin. The intensity of the color varies with degree of dilution – dilute urine is pale yellow and concentrated urine is deep yellow.

Change in color of urine is observed in different clinical conditions - a few examples are given below:

• Deep or brownish yellow – bile pigments (jaundice)
• Red color – Intact red cells, free hemoglobin or myoglobin
• Black color – alcaptonuria, melanuria

5. pH: In a healthy person the pH of the urine varies from 4.6 to 8 depending on many factors like dietary intake and metabolic activities. Most often the urine pH is acidic around 6.0 due to the presence of sulfates, phosphates, chlorides and nonvolatile organic acids.

Vegetarian diet produces alkaline urine. On keeping urine, it becomes alkaline due to the formation of ammonia by the decomposition of urea.

Measurement of pH

– ***Litmus paper*** – In acid urine blue litmus turns red and in alkaline urine red litmus turns blue.
– ***pH paper*** which has a wide range of colors from 4.5 to 7.5.
– ***Dip sticks*** uses a combination of indicators methyl red and bromophenol blue which give a range of different colors from orange to green to blue as the pH rises from pH 5.0 to pH 9.0.

Points to Ponder: Extremely acidic or alkaline urine suggest the possibility of poorly collected urine.

6. Specific gravity: Specific gravity of urine serves to assess the concentration ability of the kidneys.

Increased specific gravity more than 1.030 seen in

- Dehydration
- Diabetes mellitus
- Congestive heart failure
- Proteinuria
- Adrenal insufficiency

Decreased specific gravity seen in

- Hypothermia
- Diuretic therapy

Fixed specific gravity: The specific gravity of urine is identical to the glomerular filtrate around 1.010. It is seen in patients with chronic kidney disease (CKD).

Presence of substances with high molecular weight substances like proteins and glucose in the urine impart much higher specific gravity than due to the excessive excretion of crystalloids.

Measurement of specific gravity is done by

- **Urinometer** (see it in the section - spotters)
- **Refractometer** used in higher laboratories

DEMONSTRATION OF INORGANIC CONSTITUENTS OF URINE (TABLE 8A-2)

The main inorganic constituents of urine are Na^+, K^+, Ca^+, Mg^{++}, NH_4^+, Cl^-, phosphates and sulfates.

- Chief inorganic constituent of urine is **chloride**. It is derived from salts of the diet. Rate of excretion of chloride in urine **10 -15 g per day**. It's content in urine is increased in addison's disease in which there is aldosterone deficiency so that reabsorption of sodium and chloride are defective and so get excreted in urine.
- The **sulfates** of urine derived from sulfur containing amino acids. Rate of excretion of inorganic sulfates in urine **0.8-1 g/day**.
- **Calcium** is excreted at the rate of **0.1-0.3 g per day**.
- Phosphates derived from inorganic phosphates in the diet – phosphoproteins, nucleoproteins and phospholipids. It is excreted at the rate of **1 g per day**.

1. Test for Chloride

Procedure: Acidify 2 ml of urine with 2 drops of concentrated HNO_3 and add 2 ml of silver nitrate solution.

Observation: White precipitate.

Inference: A white precipitate of silver chloride (AgCl) forms. Nitric acid prevents precipitation of salts other than chloride like silver urates and silver phosphates.

2. Test for Sulfates

Procedure: Acidify 3 ml of urine with 2-4 drops of concentrated HCl and add 1 ml of barium chloride solution.

Observation: White precipitate.

Inference: A white precipitate of barium sulfate. HCl prevents precipitation of phosphates.

3,4. Test for Calcium and Phosphates (Fig. 8A-1)

Procedure:

- Take 10 -12 ml of urine in a boiling tube. Add 3 ml of strong ammonia solution and boil till white precipitates of calcium and magnesium are formed.
- Filter through a filter paper placed in a funnel placed over a test tube.
- Wash the precipitate thus collected in the filter paper by just pouring a few ml of water through the filter paper.
- Then take the funnel with the filter paper in situ, and place it over another test tube.

- Add 3 ml hot acetic acid through the filter paper placed over the test tube so that precipitate in the filter paper get dissolved in hot acetic acid and will be collected in the test tube underneath.
- Divide it into 2 parts
- ***To detect calcium:*** To one part add 1 ml of potassium oxalate
- ***To detect phosphates:*** To the other part add a drop of concentrated HNO_3 and a few drops of ammonium molybdate solution. Boil.

Observation: white precipitate forms in the test is meant for **calcium**.

Fine lemon yellow (canary yellow) precipitate forms in the tube is meant for detecting **phosphates**.

Inference: Calcium forms a white precipitate of calcium oxalate on addition of potassium oxalate.

On boiling with ammonium hydroxide phosphates of calcium and magnesium are precipitated. These are then filtered and redissolved in hot acetic acid.

Phosphates react with ammonium molybdate to form canary yellow colored ammonium phosphomolybdate in the presence of HNO_3.

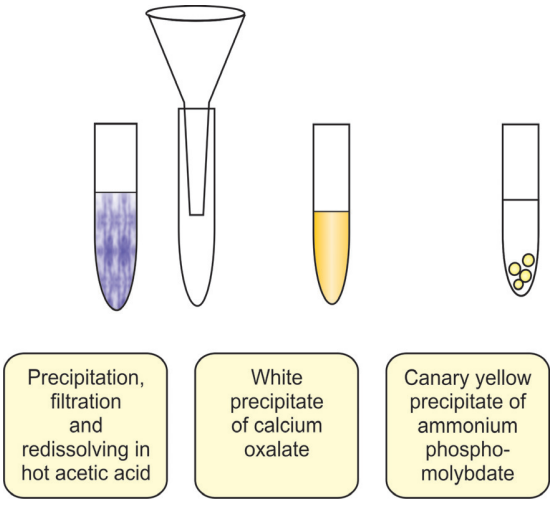

Fig. 8A-1: Test for calcium and phosphate

5. Test for Ammonia

Procedure: To 10 ml of urine add a drop of phenolphthalein and make just alkaline by adding 0.1 N NaOH in drops. Hold a glass rod dipped in phenolphthalein at the mouth of the test tube and heat the contents of the tube.

Observation: The phenolphthalein indicator at the tip of the glass rod turns pink.

Inference: Ammonium salts give off ammonia in alkaline medium and the ammonia vapors emerging from the tube turn the phenolphthalein indicator to show pink color since ammonia is alkaline (Color range of phenolphthalein – colorless to pink; pH range - 8.3-10).

ORGANIC CONSTITUENTS OF URINE

Important organic constituents in urine are urea, uric acid, ethereal sulfates, creatinine, organic sulfates, urinary pigments.

1. Urea: The amino acids released as a result of protein breakdown are transdeaminated to release ammonia. The toxic ammonia is converted to less toxic urea in the liver. Urea in the blood is denoted as blood urea nitrogen (BUN). Urea is filtered at the glomerulus and 40-50% of the filtered urea is reabsorbed by the proximal renal tubules.

Causes of increased urea content in urine

1. High protein diet
2. Conditions leading to increased tissue break down (increased protein catabolism), e.g. fever, diabetes mellitus, adrenal cortical hyperactivity.

Causes of decreased urea content in urine

Liver diseases: In affections of liver synthetic function is disturbed and urea is formed in low amounts.

BUN in blood: 5-17 mg% (1.8-6.1 mmol/L)

Urea content in urine in healthy subjects – **7-16 g/day**.

2. Uric Acid: It is the catabolite of dietary or tissue purine nucleotides. Plasma levels of uric acid is variable and it is higher in males than in females. It is completely filterable and it is reabsorbed at the PCT and secreted at DCT.

Uric acid content in urine is raised in

1. High purine diet
2. Conditions where there is increased tissue turn over without any impairment of kidney function –leukemia and other malignancies
3. Gout
4. Cortisone therapy

Uric acid level in blood

Males: 3.6 – 7.7 mg% (214 – 458 µmol/L)

Females: 2.5 – 6.8 mg% (149 – 405 µmol/L)

Uric acid content in urine: 300 – 800 mg/day on an average diet.

3. Creatinine: The compound creatine phosphate is formed from liver, kidneys and pancreas and is carried by the blood to other tissues, especially utilized in muscles and brain. About 1-2% of creatine in muscle undergo spontaneous conversion to form cyclical anhydride of creatine, the creatinine. Creatinine is filtered by the glomerulus and a small amount of creatinine is reabsorbed in the proximal convoluted tubule and secreted in the distal convoluted tubule in small amounts. Hence the measurement of creatinine excretion can be used to assess the glomerular filtration function of the kineys. The amount of creatinine formed in the body at a point of time depends on age, sex and muscle mass and to a minor extent on the content of creatine in the diet (meat muscle rich in creatine). But daily variations of creatinine levels in blood and excretion in urine are very minimal. Because of this fact, creatinine levels are useful to assess the kidney function.

Creatinine level in blood

Males: 0.6 – 1.0 mg% (57 – 92 µmol/L)

Females: 2.5 – 6.8 mg% (50 – 81 µmol/L)

Creatinine content in urine: On an average diet 1-2 g/day (nearer to higher limit in males and to lower limit in females)

Raised creatinine levels in blood: Seen in renal failure, nephritis.

High creatinine levels in urine seen in:

- Myopathies
- Fever
- Muscle injuries

4. Organic Sulfates: Urinary sulfates are of three types:

(i) Inorganic sulfates which come from metabolism of sulphur containing amino acids.

(ii) Ethereal or organic sulfates: This constitute 10% of total sulfates excreted in urine. The different ethereal sulfates seen in human urine are conjugated phenols, phenol sulfuric acid, p-cresol sulfuric acid, skatoxyl sulfuric acid and indoxyl sulfuric acid (indican). Altogether the excretion rate is **0.04 to 0.1 g per day**. In health the ratio of sum of ethereal and neutral sulfates to inorganic sulfate is about 1: 10.

Formation of ethereal sulfates: Phenols are produced during putrefaction of protein material in the intestine. Phenol then reaches liver where it is conjugated to form phenol potassium sulfate and excreted as such in urine. Indole and skatole get oxidized to indoxyl and skatoxyl respectively and conjugated and then excreted in urine. Action of intestinal bacteria on tryptophan leads to the formation of indoxyl sulphuric acid and it is excreted in the urine as potassium salt (indican) (Fig. 8A-2). Excretion rate of ***indican*** alone give

rough estimate of intestinal putrefaction. Excretion rate of indican in normal individuals **10-20 mg/day**. Excretory rate increases with high meat diet and decreases with high carbohydrate diet.

Pathological increase seen with intestinal obstruction which causes stagnation of intestinal contents and putrefaction there upon and in situations where there is bacterial decomposition of body proteins – gangrene, putrid pus formation.

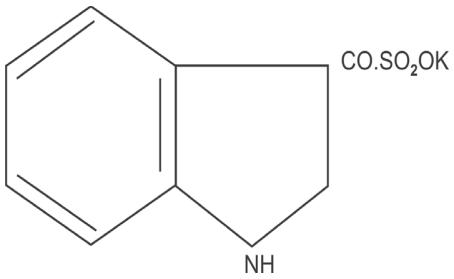

Fig. 8A-2: Indoxyl sulphuric acid

(iii) Neutral sufates: It is produced from endogenous sources and its rate of excretion do not change with diet. Generally sulfur in these compounds is in unoxidized or neutral state. The compounds coming under this category sre cystine, methyl mercaptan, ethyl sulfide, thiocyanates, taurine derivatives. Neutral sulfur content of normal human urine is 5-25% of total sulfur content **(0.08 – 0.16 g/day)**. Its content in urine is raised in cystinuria.

5. Urobilinogen: After the life span of 120 days red blood cells undergo lysis and hemoglobin is released in the reticuloendothelial system (spleen, bone marrow, kupffer cells in liver). Bilirubin released is transported by albumin to the liver and there get conjugated with glucuronic acid to form bilirubin glucuronide (conjugated bilirubin) which then passes to the intestine via common bile duct.

In the intestine, the conjugated bilirubin is reduced by intestinal bacteria to urobilinogen – a term used to include d-urobilinogen, mesobilirubinogen and stercobilinogen. The greater part of urobilinogen thus formed is excreted in the feces as faecal urobilinogen. The rest of the urobilinogen is reabsorbed into the portal circulation and reaches liver. The greater part of this fraction is re excreted by the liver in the bile as urobilinogen. A small part enters the systemic circulation and is excreted in the urine as urine "urobilinogen".

DEMONSTRATION OF ORGANIC CONSTITUENTS OF URINE (TABLE 8A-3)

1. Test for Urea

1. Alkaline hypobromite.
2. Specific urease test (see Chapter 4—Reactions of Urea).

2. Test for Uric Acid

1. Benedict's uric acid test
2. Schiff's test
3. Murexide test (see Chapter 6—Uric Acid).

3. Test for Creatinine

Jaffe' test (see Chapter 5—Reactions of Creatinine)

4. Test for Ethereal Sulfates

Procedure: To 5 ml of urine add 2 ml barium chloride and 2 ml hydrochloric acid. Mix well and filter. Divide the filtrate into two tubes. Boil the contents in one tube. Carefully look for the turbidity developing in the tubes.

Principle: Hot HCl hydrolyzes ethereal sulfate to inorganic sulfate which then gives precipitate with barium chloride.

5. Test for Urobilinogen—Ehrlich's Test

Procedure: To 5 ml urine add 1 ml Ehrlich reagent, mix well and keep for 5 minutes.

Observation: Red color develops.

Principle: Urobilinogen reacts with p-dimethylaminobenzaldehyde of the reagent to form the red colored complex.

Inference: Normal urine gives a faint red color due to the presence of trace amounts of urobilinogen. It is excreted increasingly in urine in hemolytic jaundice where the RBCs are destroyed at a higher rate.

Points to Ponder: Urobilinogen in urine get oxidized to urobilin upon keeping and hence stored urine may not answer the test. Fresh urine is preferred for testing urobilinogen (Fig. 8A-3).

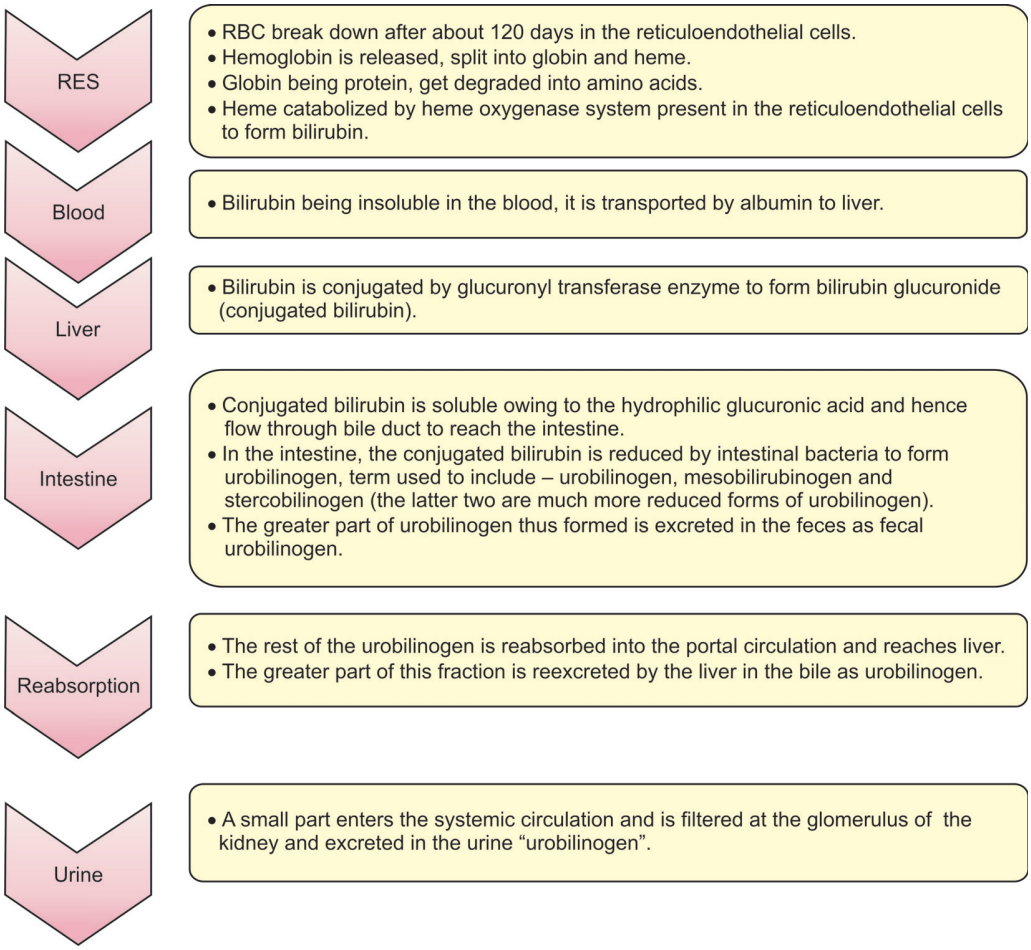

Fig. 8A-3: Urobilinogen formation and excretion in urine

ANALYSIS OF NORMAL CONSTITUENTS OF URINE

Table 8A-1: Physical Properties of Normal Urine

No.	*Experiment*	*Observation*
1	Appearance	Clear
2	Color	Amber yellow
3	Odor	Ammoniacal smell
4	Reaction to litmus	(mostly acidic) Blue litmus turns red
5	Specific gravity	1.015 – 1.025

Table 8A-2: Test for Inorganic Constituents in Urine

No.	*Experiment*	*Observation*	*Inference*
1	Test for chloride	White precipitate	Precipitate due to silver chloride
2	Test for sulfate	White precipitate	Precipitate due to barium sulfate
3	Test for calcium	White precipitate	Precipitate due to calcium oxalate
4	Test for phosphate	Canary yellow precipitate	Precipitate due to ammonium phosphomolybdate
5	Test for ammonia	Phenolphthalein at the tip of the glass rod turned pink	Ammonia vapors emerging from the tube turns the phenolphthalein pink

Table 8A-3: Test for Organic Constituents in Urine

No.	*Experiment*	*Observation*	*Inference*
1	*Test for urea*		
	a) Alkaline hypo-bromite test	a) Brisk effervescence	a) Due to evolution of N_2 gas
	b) Specific urease test	b) Pink color	b) Urea split by urease enzyme to form ammonia making the medium alkaline. In the alkaline medium phenol red used in the test give pink color.
2	*Test for uric acid*		
	a) Benedict's uric acid test	a) Intense blue color	a) Phosphotungstic acid reduced by uric acid to tungsten blue
	b) Schiff's test	b) Black color	b) Silver nitrate reduced by uric acid to metallic silver
3	*Test for creatinine* Jaffe' test	Orange red color	Due to the formation of creatinine picrate
4	*Test for ethereal sulfate*	White precipitate in trace	Precipitate due to barium sulfate
5	*Test for urobilinogen*— Ehrlich's test	Red color	Urobilinogen reacts with p-dimethyl aminobenzaldehyde to give red color

8B. ANALYSIS OF ABNORMAL CONSTITUENTS OF URINE

Clinical laboratory analysis of urine is useful for diagnosis of several clinical conditions e.g. diabetes mellitus, phenyl ketonuria, Maple syrup urine disease, alcaptonuria. Advantage of urine examination is, it involves no pain or any disturbance to the patient. Properly collected, analyzed and interpreted urine laboratory tests are valuable for the practice of Modern Medicine.

1. GLUCOSE

Benedict's Test (see Chapter 1—Reactions of Carbohydrates)

Procedure: To 5 ml of Benedict's reagent taken in a test tube, add 8 drops of urine. Shake well and boil for 1 or 2 minutes or keep it in a water bath for 5 minutes.

Observation: A colloidal precipitate forms and the color of which may be green, yellow, orange or red depending on the in urine. concentration of sugar (see Fig. 1A-3).

Interpretation: In the presence of over 0.2-0.3 percent of glucose, the precipitate form readily. In the absence of glucose the solution may remain clear or will show a turbidity due to precipitated urates.

Color of the precipitate give an idea about the concentration of the sugar solution as shown below.

Blue – absence of reducing sugar
Green – up to 0.5 gm%
Yellow – 0.5 to 1.0 gm%
Orange – >1.0 to 2.0 gm%
Brick Red – ≥ 2 gm%

Normally glucose is absent in urine. Appearance of glucose in urine is referred to as glucosuria. Glucosuria occurs in

- Diabetes mellitus: The high level of glucose in blood crosses the renal threshold for glucose (around 180 mg%) and get excreted in urine.
- Renal glucosuria: Here renal threshold for glucose is lowered. Glucose appears in urine even if it's level remain within normal limits. This may happen in pregnancy and in inherited lowered renal threshold for glucose. It is differentiated from diabetes mellitus by testing blood and urine simultaneously for glucose and if needed glucose tolerance test.

Points to ponder: Benedict's test being a nonspecific test since it involves reduction of cupric ions to cuprous ions by a reducing agent. In the urine several such reducing agents may occur. Such substances are given below.

Carbohydrate substances like fructose, galactose, lactose and pentoses and non-carbohydrate substances like ascorbic acid, homogentisic acid. Presence of glucose can be confirmed by specific test, using glucose oxidase enzyme.

Clinistix: Stiff cellulose strip which turns from red to purple when dipped into urine containing glucose, detect 0.1% glucose or less. It is more sensitive than Benedict's test. Urine containing low amounts of glucose escape detection by the reduction test but detected by clinistix.

Principle of clinistix: Oxidation of glucose by glucose oxidase to produce gluconic acid and hydrogen peroxide. Hydrogen peroxide acted

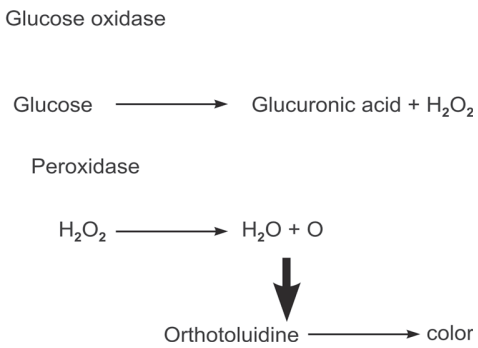

Fig. 8B-1: Principle of clinistix

upon by peroxidase to produce nascent oxygen which in turn acts upon the chromogen (e.g. orthotoluidine) to produce color (Fig. 8B-1).

2. PROTEIN

Heat Coagulation Test (Fig. 8B-2)

Procedure: Fill 3/4th of the test tube with urine Heat the upper 1/3 rd of the urine column by a small flame, so that lower 2/3rd will serve as control. Add a drop of 30 % (v/v) acetic acid to it.

Observation: White turbidity or coagulum.

Interpretation: White turbidity if disappears on addition of acetic acid indicates the presence of phosphates or carbonates. If the white turbidity formed remains or appears or intensifies on adding acetic acid points towards the presence of albumin. Addition of acetic improves the formation of turbidity since the acidification brings the pH of the medium towards 4.7 (IEP of albumin).

Points to ponder: There are chances to miss presence of albumin in the urine if the pH of urine is high and it is not brought down by adding acetic acid. Isoelectric point of human albumin is 4.7.

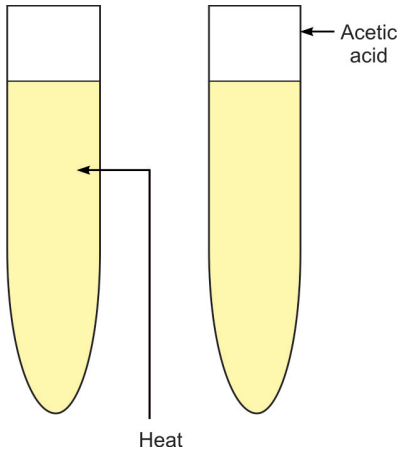

Fig. 8B-2: Heat coagulation test

Normal urine contains less than 250 mg per 24 hours and it escape detection by the usually employed methods. Pathologically different proteins detected in urine-albumin, myoglobin, fibrin and oxyhemoglobin.

- The proteinuria is most commonly seen due to leakage of serum albumin since it is the most abundant and smallest protein in the serum.
- Albumin most often appears in urine due to altered structure of glomerulus in various kidney diseases.
- Albumin may appear in urine by entering below the kidneys (not by glomerular filtration) from blood, exudates or lymph is called **false albuminuria**.
- **Benign proteinuria:** It is transient and not associated with any kidney disaease. Occurs with severe exercise and cold bath.
- **Orthostatic albuminuria:** Albumin appears in urine after prolonged standing

Albustix: Is a stiff cellulose strip impregnated at one end with indicator tetrabromphenol blue buffered at pH around 3 which has a yellow color at pH 3.0. Presence of protein turns it into green - blue. Buffer maintains the pH at 3 and hence pH of urine do not interfere. If protein is absent, the color will be yellow. In the presence of protein the color varies from green to blue. Highly alkaline urine and stale urine (due to the formation of ammonia) may overcome the buffering action of the strip and give a false positive response.

3. KETONE BODIES

Rothera's Test (Fig. 8B-3)

Procedure: Saturate 5 ml of urine with ammonium sulfate crystals and add 2 drops of freshly prepared 2% sodium nitroprusside solution or a little of sodium nitroprusside powder. Shake well. Add 1 ml of liquor ammonia through the sides of the test tube.

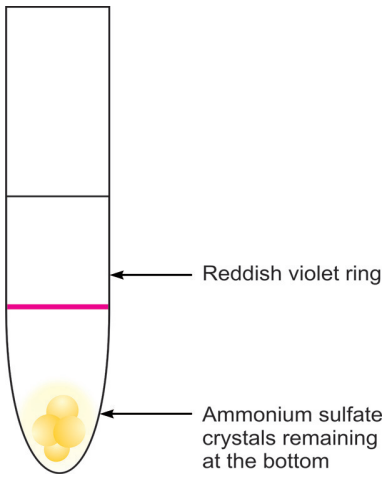

Fig. 8B-3: Rothera's test

Observation: Reddish violet ring at the junction of two liquids.

Principle: Acetone and acetoacetic acid react with sodium nitroprusside (nitroferricyanide) in the presence of alkali to produce a purple color.

Inference: Ketone (acetone) bodies include acetone, acetoacetic acid and β-hydroxybutyric acid. To detect the latter a modified test has to be done. (oxidize the β-hydroxybutyric acid with hydrogen peroxide to form acetoacetic acid. Add a few drops of acetic acid to 2 ml of 1:1 diuted urine with distilled water. Boil for few minutes to discard the acetone and the acetic acid present in the urine. Then add 1 ml of hydrogen peroxide warm gently and carry out Rothera's test. It will give a positive response if β-hydroxybutyric acid is present in the urine.) Normal urine contains approximately 20 mg per 24 hours only. Ketone bodies are produced excessively in the body in starvation and in uncontrolled diabetes mellitus and starvation.

4. BLOOD

Benzidine Test

Procedure: Take 2-3 ml of urine in a test tube. Boil for 5 minutes and cool it. Mix equal volumes of benzidine solution (2-3 ml) and hydrogen peroxide in a test tube and add the boiled cooled specimen of urine into the reagent mixture.

Observation: A transient blue color appears.

Inference and Principle: Peroxidase activity of heme oxidises hydrogen peroxide to release the nascent oxygen which acts upon benzidine to form blue colored compound.

Interpretation: Presence of blood in urine indicates either hematuria (intact RBCs in urine seen in kidney diseases) or hemoglobinuria (Hb in urine).

Points to Ponder

- Benzidine is a carcinogen. So care should be taken while handling the reagent.
- H_2O_2 deteriorates rapidly, so freshly prepared H_2O_2 should be used.
- Boiled cooled urine must be used for the test otherwise peroxidase enzymes of leucocytes present in the urine will interfere with the test.

Strip Test for Detecting Blood or Heme: It is based on the peroxidase activity of heme which splits H_2O_2 to form nascent oxygen which in turn oxidize the chromogen (usual chromogen used is tetramethyl benzidine or orthotolidine) to form the color - on dipping in urine gives a yellow color in the absence of heme (either in RBC or in free Hb) and blue green in the presence of heme. Ascorbic acid and nitrites interfere with it. Formalin if used as a urinary preservative will also give a false negative test.

5. BILE SALT

Hay's Test

Procedure: Take 5 ml of urine in a test tube and sprinkle sulfur powder on the surface of urine.

Observation: Sulfur powder sink to the bottom.

Inference: Bile salts are present in urine otherwise the sulfur powder would have remained on the surface of urine column.

Principle: Bile salts reduce the surface tension. Hence the sulfur powder sinks to the bottom.

Interpretation: Salts of taurocholic acid and glycocholic acid present in the bile regurgitate into blood whenever there is obstruction to bile flow (obstructive jaundice) and will appear in urine. This test is useful to differentiate obstructive jaundice from hemolytic jaundice.

Obstuctive jaundice seen with biliary atresia, obstruction of bile duct due to stones or tumors and obstructive phase of hepatic jaundice.

6. BILE PIGMENT

Modified Fouchet's Test (Fig. 8B-4)

Procedure: To 10 ml urine add 1 ml $MgSO_4$ and boil. While boiling add 10% $BaCl_2$ drop by drop till maximum precipitate is got. Filter and discard the filtrate. Take the filter paper from funnel and dry it by moping it over another paper. After drying add 2 drops of Fouchet's reagent to the precipitate.

Observation: A blue/green color in the presence of bile pigments.

Principle: The precipitate obtained is that of $BaSO_4$ to which bille pigment if any would have adsorbed to it. When Fouchet's reagent to the precipitate (Ferric chloride in Trichloroacetic acid) is added, $FeCl_3$ oxidizes bilirubin to biliverdin and Fe^{3+} (ferric ions) is converted to Fe^{2+} (ferrous ions). This gives the color.

Interpretation: Positive Fouchet's test indicates the presence of conjugated bilirubin in the urine (Only the conjugated bilirubuin can appear in urine in a person with normal kidney). Conjugated bilirubin appears in urine in cases of **obstructive jaundice** and **obstructive phase of hepatocellular jaundice.** So this test is useful to differentiate obstructive jaundice from hemolytic jaundice where it will be negative or weakly positive.

Fig. 8B-4: Modified Fouchet's test

7. UROBILINOGEN

Ehrlich's Test

Procedure: Add 1 ml of Ehrlich's reagent (2% Para dimethylaminobenzaldehyde in 20% HCl) to 10 ml of freshly voided urine. Shake well and keep it in the rack for 5 minutes for the color development.

Observation: Normal urine give only a faint **red** color.

Principle: Urobilinogen forms a colored adduct with Para dimethyl aminobenzaldehyde

Interpretation: Intensity of red color is related to the concentration of urobilinogen in the following manner (see Fig. 8B-5).

No red color: Urobilinogen absent.

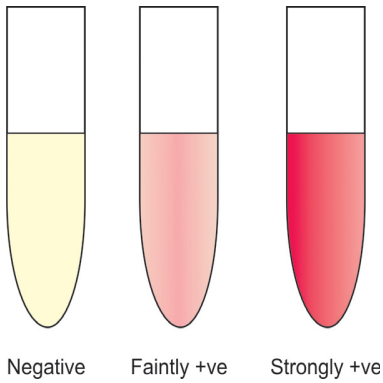

Fig. 8B-5: Ehrlich's test

Faint pink color: Urobilinogen present in normal amounts.

Distinctly red color: Urobilinogen present in increased amounts.

Points to Ponder: Bilirubin if present in the same sample may also react in the same way as that of urobilinogen. In order to avoid this remove bile pigments by adding 2 ml 10% Calcium chloride solution to the urine. Filter and carry out the test with the filtrate.

8C. QUESTIONS

1. Give brief answers:
   a. Significance of odor of urine
   b. Polyuria
   c. Oliguria
   d. Importance of observing the color of urine
   e. Normal pH range of urine
   f. Influence of diet on the pH of urine
   g. Physiological range of specific gravity
   h. Physiological causes of change in volume of urine
   i. Pathological causes oliguria and polyuria
2. Give the excretion rate of the following in urine in a normal person.
   a. Calcium
   b. Phosphates
   c. Sulfates
   d. Chloride
   e. Uric acid
3. Give the principle of the following tests:
   a. Test for chloride
   b. Test for sulfates
   c. Test for calcium
   d. Test for phosphates
4. Give brief answers:
   a. Source of sulfates in urine
   b. Importance of Rothera's test in clinical medicine
   c. Biochemical principle of treatment of urea cycle disorder
   d. Tests useful in the differential diagnosis of jaundice

8D. REAGENT PREPARATION

1. **Concentrated Nitric Acid:** Supply from the bottle (16 N).
2. **3% Silver Nitrate Solution:** Weigh 3 g silver nitrate and add to a small volume of distilled water taken in a 100 ml volumetric flask, shake well and make upto 100 ml.
3. **10% Barium Chloride Solution:** Weigh 10 g Barium chloride and add to a small volume of distilled water taken in a 100 ml volumetric flask, shake well and make upto 100 ml.
4. **2% Potassium Oxalate:** Weigh 2 g Barium chloride and add to a small volume of distilled water taken in a 100 ml volumetric flask, shake well and make upto 100 ml.
5. **Ammonium Molybdate Solution:** Dissolve 100g of molybdic acid in 144 ml of ammonium hydroxide (sp. gravity 0.9) and 271 ml water. Pour the solution thus obtained slowly with constant stirring to 489 ml nitric acid (sp. gravity 1.42) and 1148 ml water. Keep the mixture in warm place for several days. Check the adequacy of keeping in the following manner. Take about 5 ml of this solution in a tube and heat upto 40°C and if no yellow precipitate of ammonium phosphomolybdate is forming, it can be considered as fit for use.
6. **Urease Solution:** Grind 10 gm horse gram or jackfruit seeds (rich sources of urease enzyme)

with 100 ml 30% ethanol using a mortar and pestle.

7. **Fouchet's Reagent:** Dissolve 25 g trichloroacetic acid in about 50 ml of water and add 10 ml of 10% ferric chloride and make upto 100 ml with water.
8. **Ehrlich's Reagent:** Dissolve 2 g p-dimethylaminobenzaldehyde in 100 ml of 20% hydrochloric acid.
9. **Phenolphthalein (pH range–8.3-10, color range – colorless to red):** Dissolve 1 g in 50% alcohol.