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URINALYSIS

Learning objectives

Upon completion of this chapter, the students will be able to:

- List the major organic and inorganic chemical constituents of urine.
- Describe the physical appearance of urine and discuss their clinical significance in freshly voided urine.
- Describe the proper technique for performing chemical testing.
- Describe the relationship of urinary chemical test to the diagnosis of urogenital pathology.
- List and identify the normal and abnormal crystals found in the urine along with their clinical importance.

INTRODUCTION

- **Urine** is a liquid by-product of different metabolism undertaken in the body of organisms
- The cellular metabolism generates many by-products such as urea, uric acid, creatinine and the like….which are rich in nitrogen and must be cleared from the bloodstream
- These by-products are expelled from the body by micturation, which is the primary method for excreting water-soluble chemicals from the body.

Urine Formation

- Urine is formed by the combination processes of filtration, tubular reabsorption and secretion.
- Glomerular filtration: a process of ultrafiltration regulated mainly by arterial hydrostatic pressure.
- Substances with molecular weights >68,000 g/mol (e.g. cells, lipoproteins, and most proteins) cannot pass through the glomerulus.

Urine Formation

- Tubular Reabsorption: the return back of essential substance to the systemic circulation.
  - Useful compounds (e.g. glucose, water, electrolytes, amino acids, and vitamins) are efficiently reabsorbed from the glomerular filtrate by the renal tubules.
- Tubular Secretion: the addition of substances to the renal tubules.
  - Important to maintained body homeostasis by the secretion of water, electrolytes and other substances to the renal tubules.

Urine Quantity

- Regarding to the quantity, the average urine production may vary according to the species of animals
- Studies indicated that the volume of urine may range from 16-50 ml/kg/day in large animals and 10-40ml/kg/day in small animals.
- Urinations per day depending on state of hydration, activity level, environmental factors, weight and the health status
- Producing too much or too little urine need medical attention.

Why Urinalysis?

- Whenever an evaluation of renal function is needed, the first step to be considered is a routine analysis.
- The urinalysis affords this routine test and valuable aids to diagnose renal pathology.
- It is also one of the most helpful indicators of health and disease status related to various endocrine or metabolic abnormalities linked to kidneys function.
- It is also an important method for monitoring the course of a disease as well as the efficacy of treatment.

The Composition of Urine

Normal Urine Constituents

- Water (about 95%)
- Urea
- Creatinine
- Uric acid
- Electrolytes

Abnormal Urine Constituents

- Glucose
- Protein
- Bile pigments
- Blood cells
- Cast and
- Microbes

Factors Affecting The composition of Urine

- Diet and nutritional status
- Condition of body metabolism
- Status of kidney function
- Level of contamination with pathogenic microorganisms (bacteria) or even non-pathogenic microflora

Collection of Urine Specimen

In order to make urinalysis reliable, the urine must be collected properly.

- Improper collection may invalidate the results of the laboratory, no matter how carefully and skillfully the tests are performed.
- Animal urine may be collected in four ways:
  - Metabolism cage: best method for collecting serial urine samples for diagnosing polyuria.
  - Free-flow sample during urination, preferably mid-stream.
  - Catheterization: this procedure is prone to contamination of blood and tissue.
  - Cystocentesis: also prone to contamination of blood and tissue.

Types of Urine Specimen

- First Morning Specimen
- Random Specimen
- Postprandial
- Mid- stream Specimen

- **First Morning Specimen**- a specimen obtained during the first urination of the day.
  - Most concentrated
  - Bladder incubated
- Best for nitrite and protein tests and microscopic examination

Types of…..

- **Random Specimen** - a specimen taken at any time during examination.
  - Most convenient
  - Most common
  - Good for chemical screen and microscopic examination
- **Postprandial :** a specimen obtained 2 hours after meal.
  - Good for glucose test.
- **Mid- stream Specimen** - a specimen obtained from the middle part of the first urine.
  - It is commonly used for routine urinalysis.
  - It is also important for bacteriological urine culture.

Preservation of Urine Specimen

Urine should be examined immediately soon after collection because some urinary components are unstable.

- If urine specimen can not be examined immediately, it must be refrigerated or preserved by using different chemical preservatives.
- Long standing of urine at room temperature can cause :
  - Growth of bacteria
  - Break down of urea to ammonia leading to an increase pH of the urine and cause of calcium and phosphate precipitation
  - Oxidation of urobilingen to urobilin.
  - Destruction of glucose by bacteria.
  - Lyses of RBCs, WBCs and casts.

Preservation of…..

Method of Preservation of Urine Specimen

- **Physical Method**
  - Refrigeration
- **Chemical Method :** *Use of chemical preservatives such as*
  - Thymol
  - Toluene
  - Formaldehyde
  - Hydrochloric acid (HCl)
  - Chloroform
  - Boric acid
  - Sodium carbonate

Preservation of…..

Methods	Advantages	Disadvantages
Refregeration (2-6 ^{O}C)	No chemical Interferences	Use for a short period of time (3-6 hours).
Freezing	For specimen transport	May destroy formed elements
Toluene (Till it forms thin layer over the Urine)	Preserves acetone, Reducing Substances like protein	Flammable
Thymole (small crystal 5 mm diametre/100ml urine)	Preserves most constituents	Can cause false positives for proteins

Preservation of…..

Methods	Advantages	Disadvantages
Chloroform (1 tablet/60 ml urine)	Preserves urine aldosterone level	Settles to the bottom
Formaldehyde (1 drop/30 ml urine)	Preserves formed elements	Interferes with glucose evaluation
HCL (1 drop/15 ml urine)	Stabilizes steroids, catecholamine's	Formed elements are destroyed
Boric acid (75mg/10ml of urine	Preserves chemicals and formed element	Precipitate uric acid
Sodium Carbonate (few amount)	Preserves poryphrines and urobilinogen	Interferes with other urine constituents

Categories of Urine Tests

According to their degree of accuracy, urine tests are grouped into three broad categories:

- Screening tests
- Qualitative tests
- Quantitative test

Categories of Urine Tests

- Screening tests tell us the presence or absence of substances, and the results are reported as positive or negative.
- Qualitative tests are test which give rough estimate to the amount of substance present. They are also called semi-quantitative tests.
- The results are graded as negative, trace, +1, +2, +3 or +4.
- Quantitative tests are tests that determine accurately the amount of substances to be tested.

Type of Examination in Routine Urinalysis

Physical Examinations
- Volume
- Color
- Odor
- Foam
- pH
- Specific gravity

Chemical Examinations
- Glucose
- Protein
- Ketones
- Bilirubin
- Urobilinogen
- Blood
- Nitrite
- Leukocyte Esterase

Microscopic Examinations
- RBCs
- WBCs
- Epithelial cells
- Casts
- Bacteria
- Yeasts
- Parasites
- Crystals
- Artifacts

PHYSICAL EXAMINATION

Quantity of Urine (Volume)

- Normally - varies with food and water intake, climate and exercise
- Abnormal (Increased amount **(polyuria)**)
  - ✓ Chronic interstitial nephritis - the kidney cannot reabsorb urine
  - ✓ Diabetes mellitus – due to strong osmotic activity of glucose in the distal tubules of kidney
  - ✓ Diabetes insipidus – influence of ADH
  - ✓ Diuretics - cause rapid formation of urine
  - ✓ Excessive fluid intake

Physical Examination.....

- **Decreased amount (Oliguria)**
  - Acute interstitial nephritis
  - Reduced fluid intake
  - Dehydration from any cause
  - Gastrointestinal disorders with vomiting and diarrhea
  - Fever
  - Exercise
  - Cardiac decompensation - interferes with renal circulation

Physical Examination.....

Color -Color of urine is normally formed due to the presence of urochromes.

- Interpretation
  - Pale yellow to yellow brown - normal color
  - Colorless to pale yellow (**usually low specific gravity and polyruia except in diabetes mellitus**) due to
    - ✓ Chronic interstitial nephritis
    - ✓ Diabetes mellitus
    - ✓ Diabetes insipidus
    - ✓ Excessive intake of water or fluids
    - ✓ Hypoadrenocorticism
    - ✓ Generalized nephritis and pyelonephritis

Physical Examination.....

- Dark yellow to yellow brown (**concentrated urine with a high specific gravity and oliguria**)
  - Acute nephritis
  - Decreased fluid intake
  - Dehydration
  - Fever
  - Prolonged vomiting or diarrhea
- Yellow brown to greenish yellow
  - Bile pigments and urobilinogen usually produce a greenish foam when shaken

Physical Examination.....

- Red, wine or brown
  - Cloudy - hematuria
  - Translucent - hemoglobinuria
- Brown to brownish black
  - Normal in horse as urine is yellow when voided, but turns a deep brown color upon standing due to oxidation
  - Azoturia - myoglobinuria
  - Methemoglobinuria
  - Melanin in standing urine
- Green/ Blue
  - Methylene blue

Physical Examination.....

Transparency

- Recorded as: Clear, Cloudy and Flocculent
- Interpretation
  - Normal urine is clear on being voided, except in the horse which is normally thick and cloudy due to calcium carbonate crystals and mucus.
- Substance which cause turbidity of the urine are:
  - Bacteria
  - Epithelial cells
  - Erythrocytes
  - Leukocytes
  - Mucus
  - Fat
  - Crystals

Physical Examination.....

Foam

- When normal urine is shaken after collection, a small amount of white foam is produced.
- If foam is abundant and slow to disappear, it is an indication of high concentration of protein **(proteinuria)**
- Bile salts produce a green or yellow foam.
- Hemoglobin is formed red to brown foam.

Physical Examination.....

Odor

- Urine from feline, porcine and canine species normally has a strong odor.
- A strong ammonia odor may indicate the presence of bacteria, as bacteria convert urea to ammonia.
- A sweet fruity odor is produced by ketone bodies from conditions such as:
  - Diabetes mellitus
  - Pregnancy ketosis
  - Acetonemia

Physical Examination.....

Specific Gravity

- The specific gravity of urine is a measurement of the relative amount of solids in the solution (Urine).
- It is an indication of the degree of tubular re-absorption or concentration by the kidney.
- Under normal renal function and metabolism, the specific gravity varies inversely with the volume of urine excreted.
- If large volumes of urine are excreted, the specific gravity is usually low, and vice versa.

Methods of Determination

- Urinometer - requires large volume of urine
- Refractometer - only one drop of urine is needed

Physical Examination.....

Normal Values of Urine specific gravity for d/t species

Species	Range	Average
Horse	1.020-1.050	1.035
Cow	1.025-1.045	1.035
Sheep and Goat	1.015-1.045	1.030
Pig	1.010-1.030	1.015
Dog	1.015-1.045	1.025
Cat	1.020-1.040	1.030
Man	1.010-1.030	1.020

Physical Examination.....

Interpretation

Decreased specific gravity

- Chronic interstitial nephritis: due to inability to reabsorb water
- Uremia - if advanced
- Hypoadrenocorticism
- Renal amyloidosis
- Generalized nephritis and pyelonephritis
- Mobilization of effusions or edema fluids
- Fluid therapy
- Treatment with diuretics

Increased specific gravity

- Acute interstitial nephritis - due to defect in infiltration and inability to concentrate urine
- Cystitis – due to products of inflammatory reaction
- Diabetes mellitus
- Reduced fluid intake
- Dehydration
- Vomiting and diarrhea - if prolonged
- Hypovolemic Shock
- Fever
- High temperature
- Excessive panting or sweating

CHEMICAL EXAMINATION

CHEMICAL EXAMINATION

- Chemical evaluation of urine commonly done through reagent strip and sometimes by variety of specific techniques.
- In reagent strip the required reagents are coated on strip pads.
- Urine is placed on the strip or the strip is immersed in the urine and allowed to react with the reagents.
- The results are compared to the color chart located on the outside of the container.

Chemical Examination

- All test results are usually reported in a semi-quantitative manner (i.e., negative, trace, 1+ to 4+).
- The urine test strips are marketed under different names like Multistix, Combistix, BiliLabstix and Uristix.
- They usually measure pH, protein, glucose, ketones, bilirubin, blood/hemoglobin, and urobilinogen and etc….

Chemical Examn…..

pH

- The pH of urine can range from 4 (strongly acidic) to 7 (neutral) to 9 (strongly basic).
- Standing urine will become basic due to loss of CO_2 and bacterial production of ammonia from urea.
- The kidneys regulate blood pH by excreting bicarbonate, ammonium ion, and phosphates.
- Urine pH reveals more about metabolic status and systemic health than about the kidneys.

Chemical Examn…..

pH

- The normal range of pH varies with species, diet and metabolism.
- Carnivores have a neutral to acid urine, while herbivores have a neutral to alkaline urine.
- Could be measured using lithmus paper
- Normal Values of pH for different species

Species	Range	pH
Horse	alkaline	7-9
Cow	alkaline	7-9
Sheep and Goat	alkaline	7-9
Pig	alkaline to acid	5-9
Dog	acid	6-7
Cat	acid	6-7
Man	alkaline to acid	4.8-7.5

Chemical Examn…..

Interpretation

Acidic

- Normal in carnivores
- Nursing calves and foals
- Starvation
- Fever
- Acidosis
- Prolonged muscular activity
- Administration of acid salts

Alkaline

- Normal in herbivores
- Diet high in roughage or vegetable matter
- Cystitis
- Urine retention
- Rapid absorption of effusions
- Alkalosis
- Alkaline therapy

Chemical Examn.....

Protein

- Test for urinary protein is one of the most important and valuable parts of the routine urinalysis.
- Albumin is one of the commonest proteins, which appears in urine during a pathological condition.
- It often occurs as a symptom of renal disease.
- Globulins are excreted less frequently.
- Bence Jones protein is a specific type of globulin excreted in multiple myeloma.
- Normal urine does not contain any protein

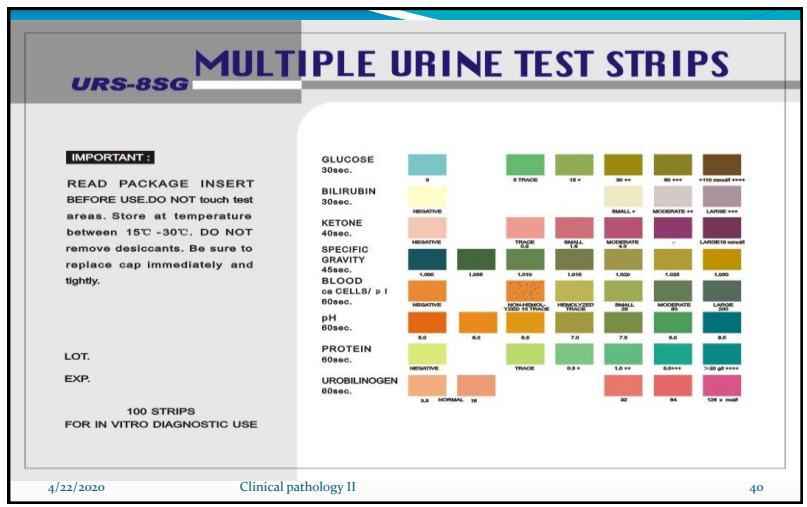
Chemical Examn.....

- **Methods of test for proteinuria**
  - Strip test
  - Robert's test
  - Heller's test
  - Sulfosalicylic acid test
- The general principle of the last three tests is that protein can be precipitated or become turbid by means of a chemical, usually a strong (concentrated) acid.

Chemical Examn.....

Procedure for all reagent strip test

- Dip the reagent strip briefly into the specimen.
- Remove excess urine by tapping the edge of the strip against the rim of the urine Container.
- Compare the color of the test area after 6o seconds with the color chart supplied by the manufacturer.



Chemical Examn.....

Robert's Test

- **Principle**
- The principle of this test is based on the precipitation of protein and formation of white compact ring using conc Nitric acid (HNO_3).

Procedure

- Place 3-5 ml of clear urine in a test tube.
- Add 3 ml Robert's reagent (mixture of $MgSO_4$ and HNO_3)to the side of the tube and allow to lay beneath the urine.
- A white ring at the zone of contact indicates a positive test.
- The ring must be read within 3 minutes after adding the reagent

Chemical Examn.....

Heller's test

- **Principle :** same as Robert`s Test
- **Procedure :**
  - Perform the test as Robert's test using concentrated nitric acid instead of Rober's Reagent.
  - This is very commonly used.
  - At the junction of the two liquids a white ring is formed, if urine contains protein.

Chemical Examn.....

Sulphosalicylic Acid Test

Principle

- This test is based on the precipitation of protein (particularly albumin) by sulphosalicylic acid.

Procedure

- Place 3ml centrifuged urine in a test tube.
- Add 3 ml of 20 % sulphosalicylic acid.
- Mix thoroughly and estimate the amount of turbidity 10 minutes later.
- Appreciate the presence of turbidity in the solution for positive test.

Chemical Examn.....

Interpretation

- Protein is normally absent or present in trace amount due to physiological stresses.
- Physiological or functional proteinuria - is transient due to a temporary increase in glomerular permeability as a result of congestion in the capillaries.
- Examples are:
  - Excessive ingestion of proteins
  - Emotional stress
  - Excessive muscular exertion
  - Convulsions

Chemical Examn.....

Pathologic proteinuria : can be classified as

- **Renal proteinuria**
  - Nephritis
  - Nephrosis
- **Post-renal proteinuria** – due to contamination by exudates or blood after the urine leaves the renal tubules
  - ✓ Pyelitis
  - ✓ Ureteritis
  - ✓ Cystitis
  - ✓ Urethritis
  - ✓ Vaginitis
  - ✓ Prostatitis
  - ✓ Urolithiasis
  - ✓ Trauma with hemorrhage

Chemical Examn.....

Glucose

- It is the principal sugar in blood, serving as a major metabolic fuel for the tissues .
- Normally almost all the glucose, which enter into the glomerular filtrate is reabsorbed into the circulation by the kidney tubules
- The presence of detectable amount of glucose in the urine is known as **glycosuria.**
- **Causes of Glycosuria**
  - Physiological
  - Pathological

Chemical Examn.....

Physiological

- Excessive carbohydrates
- Anything that stimulates sympathetic nervous system (Excitement, stress)
- Pregnancy

Pathological

- DM
- Hyperthyroidism
- Hyperadrenalism
- Hyperpitutarism
- Some diseases of pancreas

Chemical Examn.....

Types of Urinary Sugar (Glucose)Tests

- Test for urine sugar is used to detect diabetes mellitus and to monitor the effectiveness of diabetic control.
- There are various tests for glucose which may be applied to urine.
- The most frequently used are :
  - Non specific reduction test (Benedict test)
  - Enzymatic tests based on the action of glucose oxidase (Strip or colorimetric methods).

Chemical Examn.....

Benedict test

- This test is based on the ability of glucose to act as reducing substances.

Principle

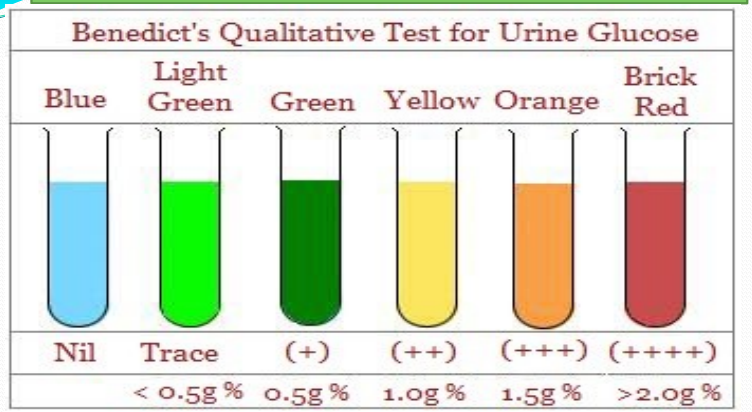
- In boiled benedict solution, glucose can reduce (convert) the blue copper (II) in Benedict solution to copper (I) oxide, which is orange to red in color.
- A positive reaction is graded as a change in color ranging from blue to green, yellow, orange and finally red.

Chemical Examn.....

Procedure:

- Measure 8 to 10 drops or 0.5 ml of well-mixed urine in a test tube.
- Add 5 ml of Benedict's qualitative reagent. Mix well.
- Place in boiling-water bath for exactly 5 minutes (or boil in naked flame for exactly 2 minutes.)
- Remove from the boiling-water bath and immediately cool to room temperature in a cold water bath (about 10 minutes).
- Observe the color change.
- A positive reaction depends on the presence of a fine yellow, orange, or brick red precipitate.
- The test is then graded on the basis of the color of the mixed solution.

Chemical Examn.....



Chemical Examn.....

Determination of Ketone Bodies

- Ketone bodies are a group of three related substances such as, acetone, acetoacetate (acetoacetic acid or diacetic acid), and β -hydroxybutyrate (β -hydroxybutyric acid).
- Ketone bodies are normal products of fat metabolism.
- They are normally not detectable in the blood or urine.
- Under normal metabolism, fat is broken down in the tissues to glycerol and fatty acids.

Chemical Examn.....

- Whenever there is inadequate carbohydrate in the diet or a defect in carbohydrate metabolism or absorption, the body metabolizes increasing amounts of fatty acids which is converted into excessive ketone bodies.
- When the rate of formation of ketone bodies is greater than the rate of their use, their levels begin to rise in the blood (ketonemia) and in the urine (ketonuria).
- The excessive production and accumulation of ketone bodies may lead to ketosis.

Chemical Examn.....

Tests for Ketone Bodies

- Some of the commonly used tests for ketone bodies are the following:-
  - Reagent strip tests (Ex. Ketostix),
  - Lang's test,
  - Rothera's test.

Principle of the Tests

- The general principle for the tests mentioned above is both ketone bodies give a purple color when react with alkaline sodium nitroprusside.

Chemical Examn.....

Procedure for Rothera's Test

- To 5 ml of fresh urine, add ammonium sulphate crystals until saturated (about 1 g).
- Add 2 drops of sodium nitroprusside reagent and mix thoroughly.
- Overlay with ammonium hydroxide solution (28% full strength).
- If ketone bodies are present, a red to purple color will develop. The color may not appear for 10-15 minutes.
- Report the test as positive or negative.

Chemical Examn.....

***Lang's Test* Procedure**

- Pour about 5 ml of fresh urine into a test tube.
- Add 5 drops of glacial acetic acid and a few drops of saturated solution of sodium nitroprusside and mix.
- Slowly overlay with ammonium hydroxide (28%, full strength)
- If ketone bodies are present, a purple or reddish purple colour will appear
- Report the test as positive or negative.

Chemical Examn.....

Causes of ketosis include:

- Late pregnancy and lactation, especially in dairy cows
- Pregnancy toxemia in sheep and goats
- Diabetes mellitus
- Acidosis
- Mild fever (if prolonged)
- Impaired liver function
- Infectious diseases causing a caloric imbalance
- Starvation or fasting
- Vomiting and diarrhea (if prolonged)
- A high fat diet
- Hyperadrenocorticism
- Hyperpituitarism
- Excessive female sex hormones

Chemical Examn.....

Determination of Bilirubin

- Bilirubin is a waste product that must be eliminated from the body.
- It is formed by the breakdown of hemoglobin
- The water insoluble form of bilirubin is often referred to as free bilirubin or unconjugated bilirubin or indirect bilirubin.
- In the liver bilirubin is converted to a water soluble product by conjugation with glucuronic acid to form bilirubin glucuronide.

Chemical Examn.....

- The water-soluble form is called conjugated bilirubin. It is also called direct bilirubin.
- The liver cells that form the conjugated bilirubin excrete it into the bile and then transported into the intestinal tract through the bile duct.
- In the small intestine this conjugated bilirubin is converted by intestinal bacteria to urobilinogen or stercobilinogen.
- In the urine, this water soluble bilirubin can often be excreted by the kidneys.

Chemical Examn.....

Tests for Bilirubin

- Tests for bilirubin are based on the oxidation of bilirubin to biliverdin.
- Specimen**:** Freshly passed urine is required.
- Urine containing bilirubin should be analyzed immediately after collection (with in 2 hrs of voiding).
- The following tests are used to detect bilirubin in the urine.
  - ***Harrison's (Fouchet's) Test***
  - ***Gmelin Test***

Chemical Examn.....

Procedure for *Harrison's (Fouchet's) Test*

- Add 5 ml of a 10% solution of barium chloride to 10 ml of urine.
- Mix, and let stand for a few minutes.
- Filter through with a small filter paper.
- Let the filter paper to dry
- Add one or two drops of Fouchet's reagent.
- **Fouchet's Reagent**
  - Trichloroacetic acid ---------- 25gm
  - Distilled H_2O ----------100ml
  - 10% Ferric chloride (FeCl) ----------10ml
  - Mix well.
- A blue to green color indicates a positive reaction.

Chemical Examn.....

Procedure for Gmelin Test

- Bile pigments are oxidized by acids to coloured derivatives.
- Take 2 ml of concentrated nitric acid in a test tube
- Add 2 ml of urine along the side of the test tube
- At the junction of the two fluids, a play of colour – green, blue, violet etc. will be observed if bile pigment is present

Chemical Examn.....

Causes of bilirubinuria include:

- Biliary obstruction
  - Complete - bilirubinuria without urobilinogen
  - Partial - bilirubinuria with urobilinogen
- Liver disease - bilirubinuria may precede clinical jaundice and is therefore an early indication of liver disease
  - Hepatitis
  - Hepatic necrosis
- Hemolysis
- Acute enteritis
- Intestinal obstruction

Chemical Examn.....

Determination of Urobilinogen

- A very small amount of urobilinogen about 1 percent is excreted in the urine which gives the urine its characteristic color with the other color pigments
- Urobilinogen is normally present in urine. But, it will not be present in the case of obstructive jaundice.
- An increases in urobilinogen is detected on hemolytic jaundice or liver disorder in which liver function is impaired.

Chemical Examn.....

Test for Urobilinogen

- ***Qualitative Ehrlich's Test for Urobilinogen***
- ***Reagent strip methods***

Principle

- The test depends upon the reaction between urobilinogen and paradimethylaminobenzaldhyde to form a cherry (deep) red.

Chemical Examn.....

Procedure:

- Place 10 ml urine in a test tube. Allow warming to room temperature.
- Add 1 ml Ehrlich's reagent and mix.
  - para-dimethylaminobenzaldehyde ---------- 2 gm
  - HCl concentrated ---------- 20 ml
  - Distilled H_2O ---------- 80ml
- Let stand 3 to 5 minutes
- Normal amounts of urobilinogen in the urine sample will change the solution to pink.
- Abnormally high amounts of urobilinogen will change the solution to a Cherry red color.

Chemical Examn.....

- ***Decreased or absence of urine urobilinogen***
  - Obstruction of biliary passages
  - Decreased destruction of erythrocytes
  - Impaired intestinal absorption
  - Nephritis
- ***Increased amount of urine urobilinogen***
  - Hepatitis
  - Cirrhosis of liver
  - Haemolytic jaundice

Chemical Examn.....

Dtermination of Blood in the urine

- The presence of free Hemoglobin in the urine is referred to as hemoglobinuria.
- Hemoglobinuria is usually related to hematuria- a condition when intact red blood cells are present in the urine.
- Hematuria is used to indicate bleeding somewhere in the urinary tract.
- Hematuria can also be distinguished from hemoglobinuria by centrifugation and microscopic examination of the sediment from a fresh urine specimen.

Chemical Examn.....

Tests for hematuria /hemoglobinuria

- The tests commonly used are for the detection of hemoglobin in urine
  - ✓ Guaiac
  - ✓ Benzidine
  - ✓ Reagent Strip Tests

Principle

- Peroxidase of the hemoglobin molecule liberates oxygen when react with hydrogen peroxide
- The librated oxygen reacts with an organic reagent or chromogen (gum guaiac or benzidine) to give a colored compound (blue or green).

Chemical Examn.....

Procedure for Guaiac

- Pour 4 drops of urine and a few drops of conc acetic acid in one test tube.
- Pour the following into a second test tube
  - A few drop of Guaiac ($C_6H_4(OH)OCH_3$)
  - 2 ml ethanol (95%)
  - 2 ml fresh 3% H_2O_2
- Mix the above slowly
- Pour the same amount into the side of the urine tube.

Chemical Examn.....

Procedure for Benzidine

- In a test tube, dissolve a small quantity of benzidine in glacial acetic acid
- Add 2 ml of urine to this
- Add one ml of fresh hydrogen peroxide and mix
- Development of green to blue colour is positive for blood

Chemical Examn.....

Haemoglobinuria

- Bacillary haemoglobinuria (Clostridium haemolyticum)
- Leptospirosis
- Babesiosis
- Photosensitization
- Severe burns
- Incompatible blood transfusion
- Plant poisoning
- Myoglobinuria

Haematuria

- Acute nephritis
- Nephrosis
- Trauma to urethra
- Severe infection – anthrax,
- Chemical poisonings – Copper
- Sweet clover poisoning
- Parasites - Dioctophyma renale and Dirofilaria immitis in canines

Chemical Examn.....

Bile Salts

- Bile salts occur in the urine in certain diseases, especially in jaundice,
- But the small quantities which appear in urine are difficult to detect by chemical tests.
- The presence of bile salt in the urine reduce the surface tension of the fluid.

Principle

- Bile salts in the urine lower the surface tension of urine that allow the sulphur particles to sink to the bottom of the tube.

Chemical Examn.....

Procedure

- Place about 10 ml of urine in a test tube
- Sprinkle a little dry sulphur powder on to the surface of urine.
- Observe sulphur particles: If the sulphur powder sink immediately, the urine is positive for bile salts

Microscopic Examn.....

Microscopic Examn.....

- Microscopic examination of urine is also called as the "liquid biopsy of the urinary tract"
- Urine consists of various microscopic, insoluble and solid elements in suspension.
- Since they settle down on standing and centrifugation they are known as urinary deposits or sediments.
- Examination of urinary deposit is helpful for diagnosis and management of urinary tract diseases

Microscopic Examn.....

- These elements are mostly found in acid and hypertonic urine
- They deteriorate rapidly in alkaline and hypotonic solution
- A mid-stream, freshly voided, first morning specimen is preferred since it is the most concentrated
- The specimen should be examined within 2 hours of voiding because cells and casts degenerate upon standing at room temperature.
- If preservative is required, then crystal of thymol or drops of formalin (40%) is added to urine specimen

Microscopic Examn.....

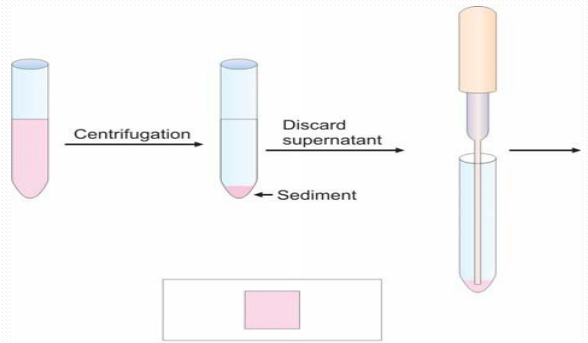
Centrifugation and Examination of the urine specimen

- Mix the specimen well, as casts tend to settle out.
- Pour 10-12ml of the sample into a conical tube or test tube and centrifuge at 1500-2000rpm for 5-10 minutes.
- Remove supernatant, leaving a small amount of urine in the tube (1ml).
- Suspend the sediment and take one or two drops of the unstained sediment to examined along with the stained sediment
- Place one to two drops of "UriStain" (mostly composed of Crystal Violet, Ethyl Alcohol, Safranin and Water & Stabilizers, Ammonium Oxalate) in the remaining sediment and mix.

Microscopic Examn.....

- On a clean slide, place one drop from stained and one drop from unstained sediment and cover each drop with a coverslip by avoiding bubbles.
- Examine with low power and subdued light, and examine the entire area.
- Casts mostly found along the edges of the coverslip and they are counted under low power and differentiated under high power.
- Red blood cells, leukocytes, and epithelial cells and others are counted at least in ten fields to make a report

Microscopic Examn.....



Microscopic Examn.....

Classification of Sediment

- Urine sediments can be categorized into organized and non-organized sediments based on the substances they are composed of.
- Of the organized fraction having clinical importance the followings are included:
  - Epithelial cells
  - Leukocytes
  - Erythrocytes
  - Casts
  - Microbes (Bacteria, yeast, fungi, protozoa, parasite ova)
- From the unorganized elements : crystals, pigments and fat droplets are primarily concern.

Microscopic Examn.....

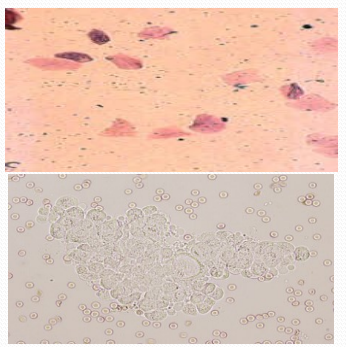
Epithelial Cells

- Few epithelial cells (0-2 / LPF) are normally seen in the urine
- Their number could be increased where there is renal pathology elsewhere in the system.
- Are of three types: Squamous, Transitional and Renal tubular epithelial cells
- Large squamous cells are derived from the lower urethra and vagina.
- Transitional epithelial cells are from the urethra, bladder, ureters or renal pelvis.
- The smaller round to polyhedral cells come from the renal tubules.

Microscopic Examn.....

Squamous Epithelial Cells

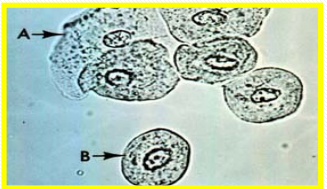
- These are large cells, rectangular in shape, flat with abundant cytoplasm and a small central nucleus
- They are best seen under low power objective (x10).
- Presence of large numbers indicated contamination of urine with vaginal fluid.

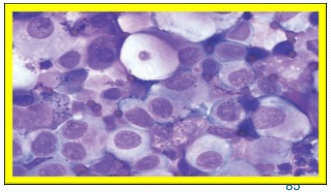


Microscopic Examn.....

Transitional Cells

- These cells are large, and oval, or pear-shaped (caudate cells)
- Generally much larger than WBC with abundant cytoplasm
- Has distinct nucleus located centrally
- Large numbers in urine occur after catheterization and in transitional cell carcinoma.

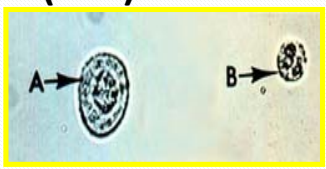


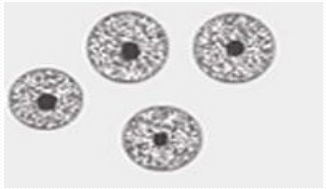


Microscopic Examn.....

Renal Tubular Epithelial (RTE) Cells

- These cells are small (about the same size or slightly larger than white blood cell) polyhedral, or oval with granular cytoplasm
- Has a single, large, refractile, eccentric nucleus is often seen
- Increased numbers are found in conditions causing tubular damage like acute tubular necrosis, pyelonephritis, viral infection, allograft rejection, and salicylate or heavy metal poisoning.





Microscopic Examn.....

Leukocytes

- Under normal conditions, only a few white blood cells are present. Normal range: 0-2 WBC/HPF.
- Appearance: normally, clear granular disc shaped
- Measure 10-15 µm, with visible nuclei
- In hypotonic urine they are swollen and show Brownian movement; are called as glitter cells
- Predominantly, they are polymorphonuclear (neutrophils)
- Clumps of numerous white cells are indicators of infections and injury to urinary tract.

Microscopic Examn.....

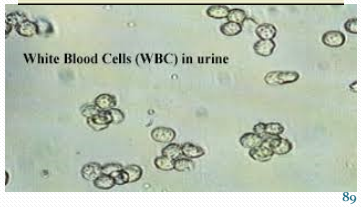
Leukocytes.....

- Increased numbers of white cells occur in fever, pyelonephritis, lower urinary tract infection, tubule-interstitial nephritis, and renal transplant rejection.
- In urinary tract infection, following are usually observed in combination:
  - Clumps of pus cells or pus cells >10/HPF
  - Bacteria
  - Albuminuria
  - Positive nitrite test

Microscopic Examn.....

Leukocytes.....

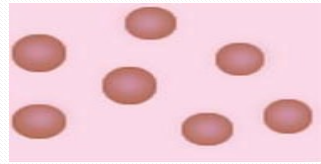
- Cytoplasm contains granules
- Nucleus is segmented (lobed) because of most of them are nuetrophils
- Can be found in single or clumped form



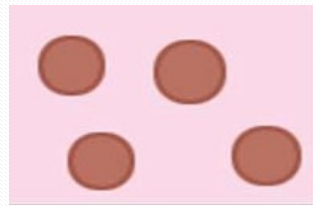
Microscopic Examn.....

Erythrocytes

- In a fresh urine sample, they appear as small, smooth, yellowish, anucleate biconcave disks called as isomorphic red cells)



- However, they may appear as swollen (thin discs of greater diameter, 9-10 µ) in dilute or hypotonic urine

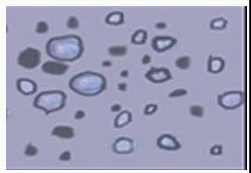


Microscopic Examn.....

Erythrocytes.....

- They may appear crenated (smaller diameter with spikey surface) in hypertonic urine.

- In glomerulonephritis, red cells are typically described as of dysmorphic (i.e. markedly variable in size and shape)
- > 80% of dysmorphic red cells is suggestive of glomerular pathology



Microscopic Examn.....

Casts

- Casts are cylindrical bodies formed either in the distal convoluted tubules or the collecting ducts of the kidney
- Since the walls of the tubule act as a mold for cast formation, the width of the tubule determines the width of the cast
- Thus, narrow casts are formed in the distal tubules while broad casts are formed in the collecting ducts.

Microscopic Examn.....

Casts.....

- The matrix of all casts is Tamm-Horsfall protein, a glycoprotein secreted by the distal loop of Henle and the distal tubule
- Although there are several types of casts, all urine casts are basically hyaline.
- But various types of casts are formed when different elements get deposited on the hyaline material
- They are of two main types:
  - **Non-cellular:** Hyaline, granular, waxy, fatty
  - **Cellular:** Red blood cell, white blood cell and epithelial cell

Microscopic Examn.....

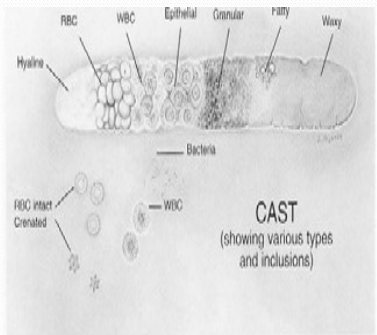
Casts.....

- Its formation is enhanced by:
  - Acidity of urine
  - Increased solute conc
  - Decreased urine flow rate (urine stasis)
  - Presence excess amount of plasma proteins
- A significant number of urinary casts may indicate the presence of renal disease.
- Casts are best seen under low power objective (×10) with condenser lowered down to reduce the illumination.

Microscopic Examn.....

Casts

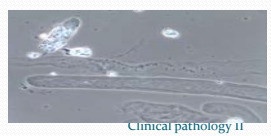
- *Types of cast*
  - Hyaline cast
  - Granular cast
  - Epithelial cast
  - Waxy cast
  - Fatty cast
  - Blood cast
  - Leukocytic cast



Microscopic Examn.....

Hyaline Cast

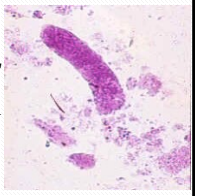
- These are the most common type of casts in urine and are homogenous, colorless, transparent, and refractile
- They are cylindrical with parallel sides and blunt rounded ends and low refractive index.
- It needs phase microscopy to enhance visualization
- Increased amounts seen with dehydration, fever, emotional stress, strenuous exercise

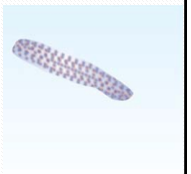


Microscopic Examn.....

Granular Cast

- It is an aged cellular cast
- Characterized by degenerated cellular debris that makes it granular in appearance.
- These are cylindrical structures with coarse or fine granules embedded in Tamm-Horsfall protein matrix
- They are seen in strenuous exercise and in fever, acute glomerulo-nephritis, and pyelonephritis

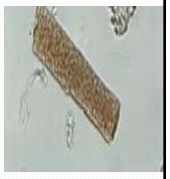


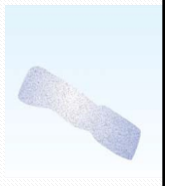


Microscopic Examn.....

Waxy Cast

- They form when hyaline casts remain in renal tubules for long time (prolonged stasis).
- They have homogenous, smooth glassy, cracked or serrated margins and irregular broken-off ends.
- The ends are straight and sharp and light yellow in color.
- They are most commonly seen in end-stage renal failure

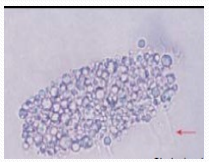


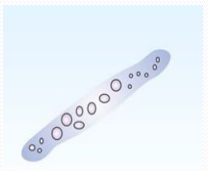


Microscopic Examn.....

Fatty Cast

- These are cylindrical structures filled with highly refractile fat globules (triglycerides and cholesterol esters) in protein matrix.
- Highly refractile due to fat content
- Easily identify using polarized microscopy:
- It is the pathologic finding, often seen in nephrotic Syndrome

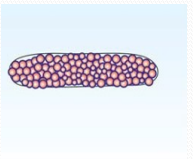




Microscopic Examn.....

RBC Cast

- A hyaline cast incorporated with RBC to the inside matrix
- They may appear yellow to reddish-brown color due to hemoglobin pigmentation.
- These have greater diagnostic importance than any other cast.
- It help to differentiate blooduria due to glomerular disease from other causes.



Microscopic Examn.....

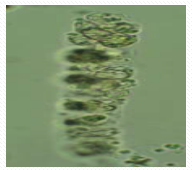
WBC Cast

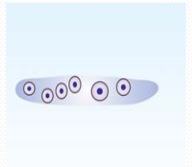
- Cylindrical structures with white blood cells embedded in Tamm-Horsfall protein matrix
- Identify by looking for lobed nucleus
- The presence is an indication of pathologic condition (mostly pyuria)

Microscopic Examn.....

Epithelial Cell Cast

- Mostly it is the incorporation of renal tubular epithelial cells in hyaline matrix that have been sloughed off.
- They are seen in acute tubular necrosis, viral renal disease, heavy metal poisoning, and acute allograft rejection.





Microscopic Examn.....

Crystals and Amorphous

- Crystals are refractile structures with a definite geometric shape due to 3-dimensional arrangement of its atoms and molecules.
- Amorphous material (or deposit) has no definite shape and is commonly seen in the form of granular aggregates or clumps
- Crystals in urine can be divided into two main types:
  - Normal (seen in normal urinary sediment) and
  - Abnormal (seen in diseased states).

Microscopic Examn.....

Crystals and Amorphous

Normal crystals

- Calcium oxalate
- Triple phosphates
- Uric acid
- Amorphous phosphates
- Amorphous urates
- Ammonium urate

Abnormal crystals

- Cysteine
- Cholesterol
- Bilirubin
- Tyrosine
- Sulfonamide and
- Leucine

Microscopic Examn.....

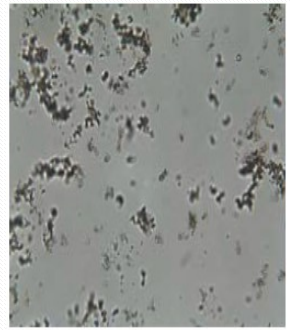
Crystals.....

- The presence of crystals in urine depends on pH, solubility and concentration of crystalloid.
- Amorphous urates and uric acid, calcium oxalate and hippuric acid found in acid urine
- Alkaline urine may contain triple phosphates, amorphous phosphate, calcium carbonate (especially in the horse) and rarely ammonium biurate crystals
- Pathologic crystals found in acid or neutral urine include cystine, tyrosine, leucine and bilirubin

Microscopic Examn.....

Amorphous Urates

- These crystals have no distinct form and appear as sand-like granules
- Macroscopically appear as a pink/ yellow sediment after urine centrifugation
- Found in acid pH urine



Microscopic Examn.....

Uric Acid Crystals

- Found in acid pH urine
- Appear in several forms (diamond, round, plates)
- But diamond shape is the most common form
- Having multicolored when polarized
- Increased numbers are found in gout and leukemia

Microscopic Examn.....

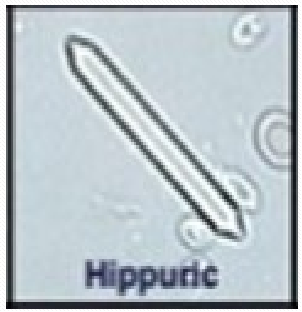
Calcium Oxalate Crystals

- Appear in acid pH urine
- It is the most frequently observed crystal in urine
- Has octahedral shape, often referred as an “envelope” shape
- Multicolored when polarized
- Their increased number in fresh urine (oxaluria) may also suggest oxalate stones /ethylene glycol poisoning.

Microscopic Examn.....

Hippuric

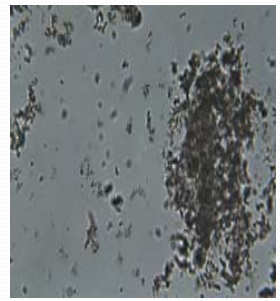
- It is found in acid, neutral or slightly alkaline urine
- Having colorless appearance
- Most commonly appear in the form needle
- It presence indicated a toluene intoxication



Microscopic Examn.....

Amorphous Phosphates

- Like Amorphous urate has no distinct form and appear as sand-like granules microscopically
- Macroscopically appear as a white sediment just after centrifugation
- It is a crystal found in alkaline pH urine



Microscopic Examn.....

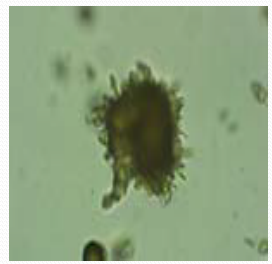
Triple Phosphate Crystals

- The most frequently observed crystal in alkaline urine
- Characterized by Colorless with 4-6 sided prisms
- Referred as 'coffin lid crystals'

Microscopic Examn.....

Ammonium Biurate Crystals

- Alkaline pH urine
- Yellow spheres with spicules on surface
- Referred as 'thorny apple crystals'
- Significant when found in fresh urine



Microscopic Examn.....

Abnormal crystal

Crystal	Urine rxn	Color	Forms	Dissolved by	Appearance
Bilirubin	Acid	Yellow or dark red	Have various aggregation of tiny amorphous needle shape or thorny shape.	-	
Leucine	Acid	Yellow	Spheres with radial and concentric striations	NaOH	

Microscopic Examn.....

Abnormal crystal

Crystal	Urine rxn	Color	Forms	Dissolved by	Appearance
Tyrosine	Acid	Color-less	Fine needles usually arranged in sheaves (bound) with a constriction at middle	NH4OH HCl	
Cystine	Acid	Color-less	Haxagonal plates	HCl