MICROSCOPIC URINE ANALYSIS

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What is Urinalysis?

- Urinalysis – or the analysis of urine – is one of the oldest laboratory procedures in the practice of medicine.
- It is a good test for assessing the overall health of a patient.

  It provides information about:
  - The state of the kidney and urinary tract.
  - Metabolic or systemic (non-kidney) disorders.
- Examples include diabetes mellitus, various forms of kidney failure, and chronic urinary tract infections.

Constituents of Normal Urine

TABLE 25.1 Major dissolved solids in normal urine

Constituent[a]	Grams per 24 hours[b]
Amino acids	0.80
Urea	25.0
Creatinine	1.5
Uric acid	0.7
H^+	pH 4.5–8.0
Na^+	3.0
K^+	1.7
NH_4^+	0.8
Ca^{2+}	0.2
Mg^{2+}	0.15
Cl^-	6.3
SO_4^{2-}	1.4
$H_2PO_4^-$	1.2
HCO_3^-	0–3
Other compounds	2–3

[a]The 24-hour volume and composition of urine vary widely, depending on the fluid intake and diet.
[b]These data are for an average 24-hour specimen with a total volume of 1400 mL.

Abnormal Urine Constituents

TABLE 25.2 Some abnormal urine constituents

Abnormal constituent	Condition	Causes
Glucose (in large amounts)	Glucosuria	Diabetes mellitus, renal diabetes, alimentary glycosuria
Protein	Proteinuria or albuminuria	Kidney damage, nephritis, bladder infection
Ketone bodies	Ketonuria	Diabetes mellitus, starvation, high-fat diets
Pus (leukocytes)		Kidney or bladder infection
Hemoglobin	Hemoglobinuria	Excessive hemolysis of red blood cells
Red blood cells	Hematuria	Hemorrhage in the urinary tract
Bile pigments (in large amounts)	Jaundice	Blockage of bile duct, hepatitis, cirrhosis

*URINE COMPOSITION

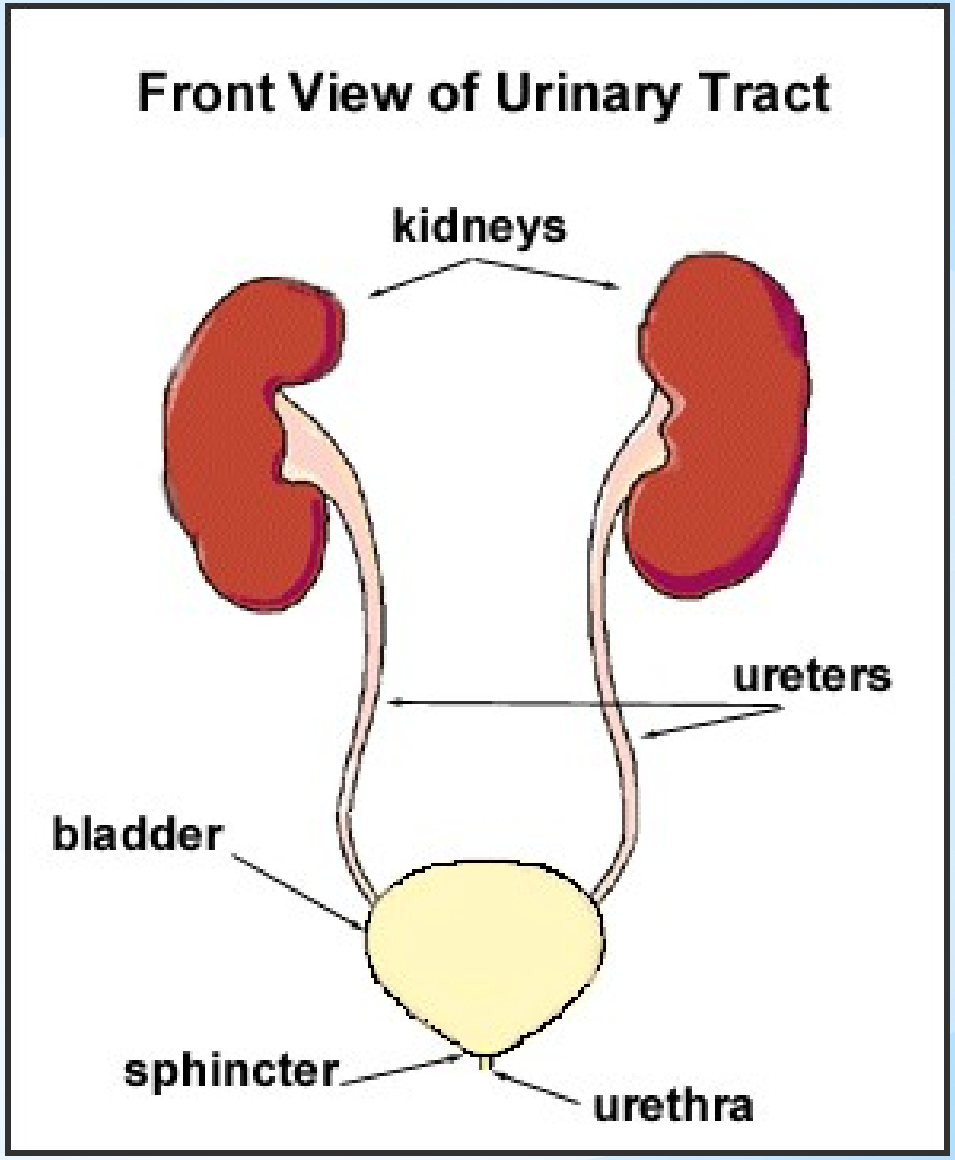
Urine, a very complex fluid, is composed of 95% water and 5% solids .It is the end product of the metabolism carried out by billions of cells and results in an average urinary out put of 1-1.5 L per day.

*Almost all substances found in urine are also find in the blood although in different concentration.

*Urine may also contain formed elements such as cells, casts, crystals, mucus and bacteria.


Urine
0.05% Ammonia
0.18% Sulphate
0.12% Phosphate
0.6% Chloride
0.01% Magnesium
0.015% Calcium
0.6% Potassium
0.1% Sodium
0.1% Creatinine
0.03% Uric acid
2% Urea
95% Water

Anatomy of urinary system



SPECIMEN COLLECTION

-The specimen must be collected in a clean dry, disposable container.

- The container must be properly labeled with the patient name, date, and time of collection. The labels should be applied to the container and not to the lid.

- The specimen must be delivered to the laboratory on time and tested within 1hr, OR it should be Refrigerated

CHANGES OCCUR IN NON PRESERVED SPECIMEN

- Transformation of urea to ammonia which increase pH.
- Decrease glucose due to glycolysis and bacterial utilization.
- Decrease ketones because of volatilization.
- Decrease bilirubin from exposure to light.
- Increase bacterial number.
- Increase turbidity caused by bacteria & amorphous.
- Disintegration of RBCs casts.
- Increase nitrite due to bacterial production of nitrate.
- Changes in color due to oxidation or reduction of metabolic.

URINE ANALYSIS

* Macroscopic
* Chemical
* microscopic

Microscopic Examination

- Most commonly used procedure for the detection of renal and/or urinary tract disease.
- This exam consists of reviewing the solid material suspended in the urine - both chemical and cellular.
- Requires a well-trained laboratory professional who is:
  - skilled in the use of various microscopic techniques such as bright field and phase microscopy
  - able to distinguish normal or contaminating items from abnormal, pathologic elements
  - knowledgeable of the clinical significance of each finding and its relationship to the chemical and physical analysis

Microscopic Procedure

PREPARATION OF SEDIMENT

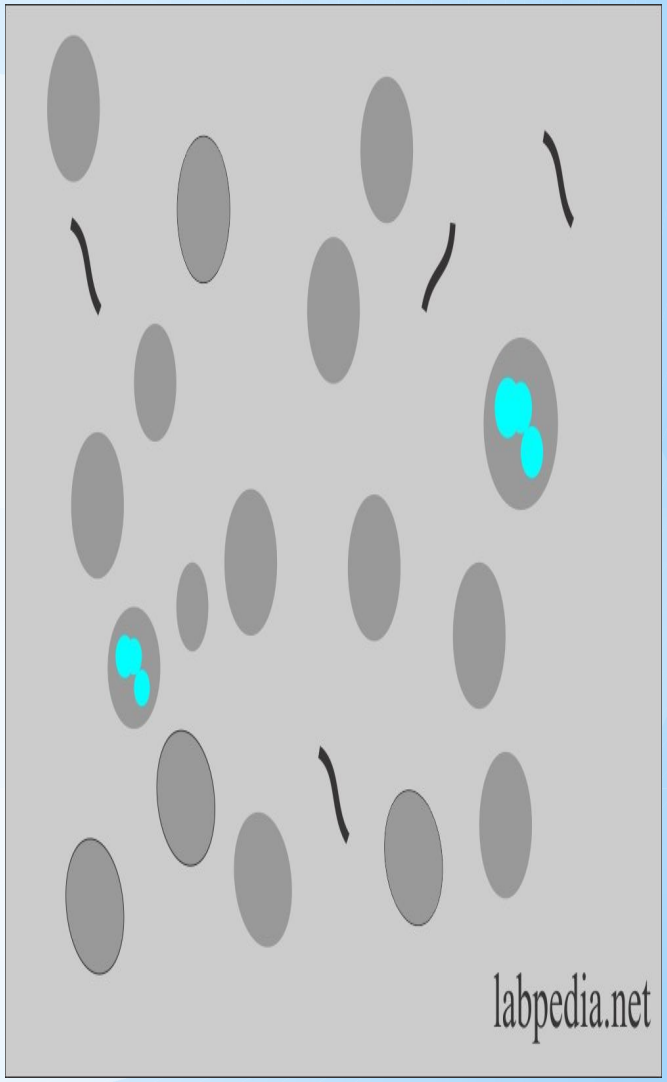
- Take 5-10 ml of urine in a centrifuge tube.
- ii. Centrifuge for 5 minutes at 3000 rpm.
- iii. Discard the supernatant.
- iv. Resuspend the deposit when about one ml of urine left and shake it well.
- v. Place a drop of this on a clean glass slide.
- vi. Place a cover slip over it and examine it under the microscope . This is done by keeping the condense low .

Microscopic Contents

* A variety of normal elements may be seen in urine sediment such as:
  * Red blood cells- Variable and maybe 0 to 2 /HPF
  * White blood cells-Variable in males and females. Maybe 0 to 5 /HPF
  * Various epithelial cells-Variable in males and females but more in females 1 to 5 /HPF
  * Various crystals- Not Found
  * Bacteria- Negative
  * Hyaline cast- 0 to 2 /HPF

Red Blood Cells

1. The presence of RBC in the urine is called **Hematuria.**
2. RBCs are seen as biconcave, non-nucleated discs measuring 7 µm in diameter.
3. The presence of RBC is an indicator of renal disease.
4. These are reported in a routine as the average number seen in 10 HPF (x400).
5. RBCs swell and lyse rapidly in the diluted urine, releasing their hemoglobin and leaving only the cell membrane; these empty cells are called ghost cells.
6. These can only be seen in reduced light. Otherwise, these are missed.



How to differentiate RBCs:

- Oil droplets and air bubbles are highly refractile.
- These are seen on a different level than the other sediments.
- RBCs are smaller than WBCs.
- Add acetic acid to the sediment, lyse the RBCs, and leave behind yeast, oil droplets, and air bubbles.
- Or do the supravital staining.

Dysmorphic RBCs:

- These RBCs vary in size, have cellular protrusions, or may be fragmented.
- Wright's stain can also help to differentiate these RBCs as hypochromic and prominent cellular blebs and protrusion.
- These RBCs are associated with glomerular bleeding.
- These are also seen after Exessive exercise, indicating

Hematuria

Macroscopic hematuria:

* It shows cloudy urine with a red to brown-color.
* This is seen in the following:
  * Trauma, Acute infection, Inflammation, Coagulation disorders.

Microscopic hematuria is seen in:

* Glomerular diseases.
* Malignancy of the urinary tract.
* Renal calculi.
* The possibility of menstrual contamination should be considered in females.

Hematuria may be caused by the following:

* Renal disease, Infections ,Renal stones, Renal tumors, Bleeding disorder ,Anticoagulant therapy.

White Blood Cells (WBC)

* WBCs are bigger than the RBCs measuring an average of 12 μm in diameter.
* In the urine sediment, recognizing the WBCs is easy compared to RBCs.
* Neutrophils contain multilobate nuclei and granules in the cytoplasm.
* WBCs can be identified in the high power and reported as the average number in 10 HPF.
* Neutrophils lyse rapidly in the dilute alkaline urine and begin to lose nuclear details.
* An increased number of WBCs in the urine is called **Pyuria.**

Pyuria may be caused by :

Bacterial infection of the urinary tract

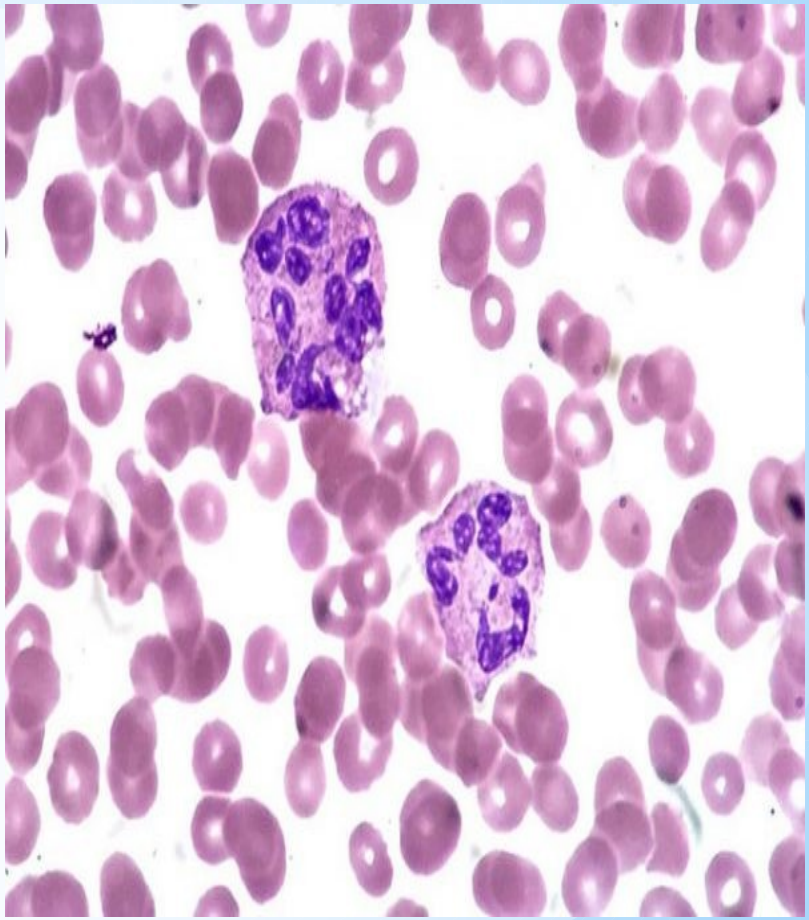
Acute pyelonephritis shows casts along with WBCs, proteins, and bacteria.

Lower urinary tract infection also shows WBCs but a small amount of protein.

Neutrophils

Increased neutrophils are seen in:

*All renal inflammatory diseases.
*Glomerulonephritis.
*Cystitis and urethritis.
*Chronic pyelonephritis.
*Prostatitis.
*Pyogenic infection.
*Acute appendicitis.
*Acute pancreatitis.
*Tuberculosis.
*Urinary bladder tumors.

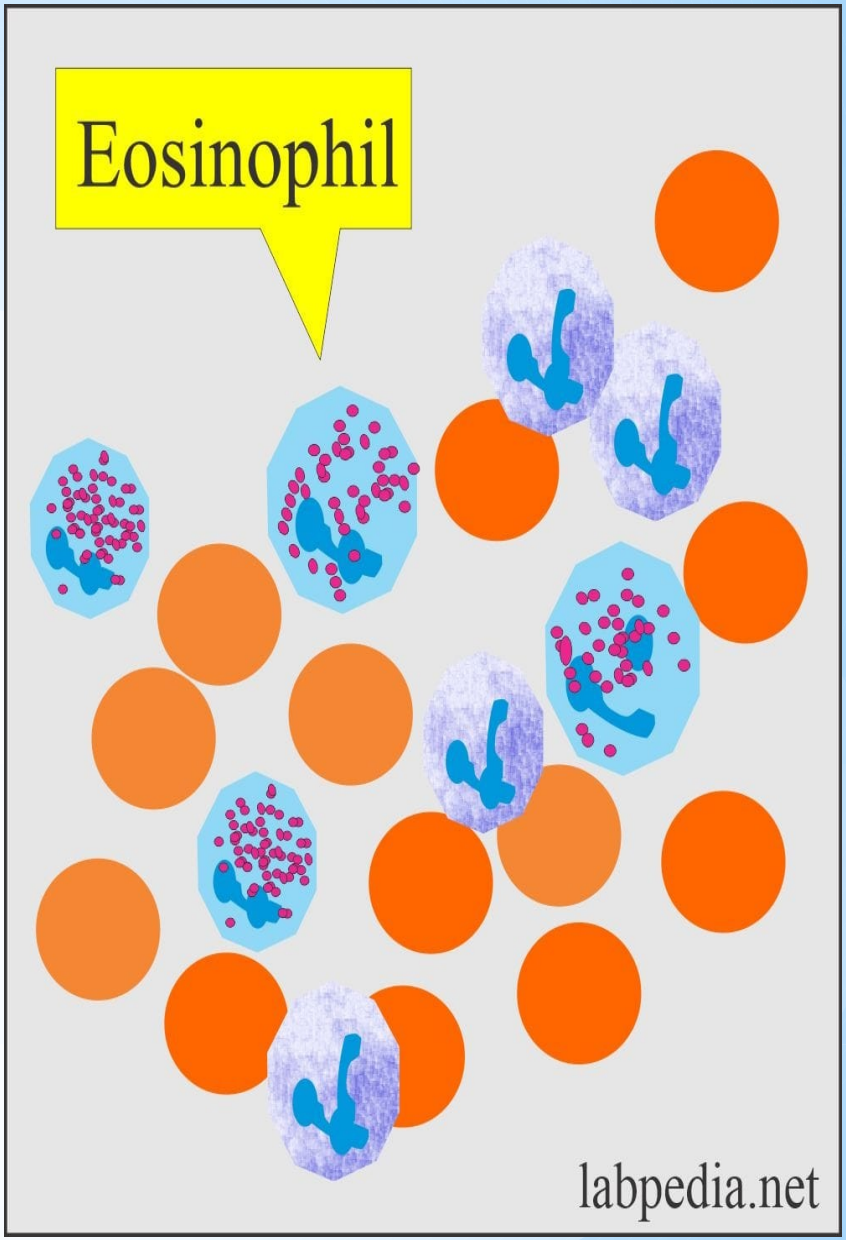


Eosinophils

* Eosinophils usually are not seen in the urine sediment. It may be seen in a few, along with neutrophils.
* On wet preparation, eosinophils are difficult to recognize except for the presence of 2 or 3 lobes.
* The supravital stain detects these.
* Use Wright's stain or a special stain for eosinophils like Methylene-blue and eosin-Y in methanol.

Eosinophils may be seen in:

- Drug-induced interstitial nephritis (hypersensitivity), e.g., penicillin and its derivatives.
- Acute genitourinary diseases like tubulo-interstitial disease.
- These are also seen in renal transplantation rejection.

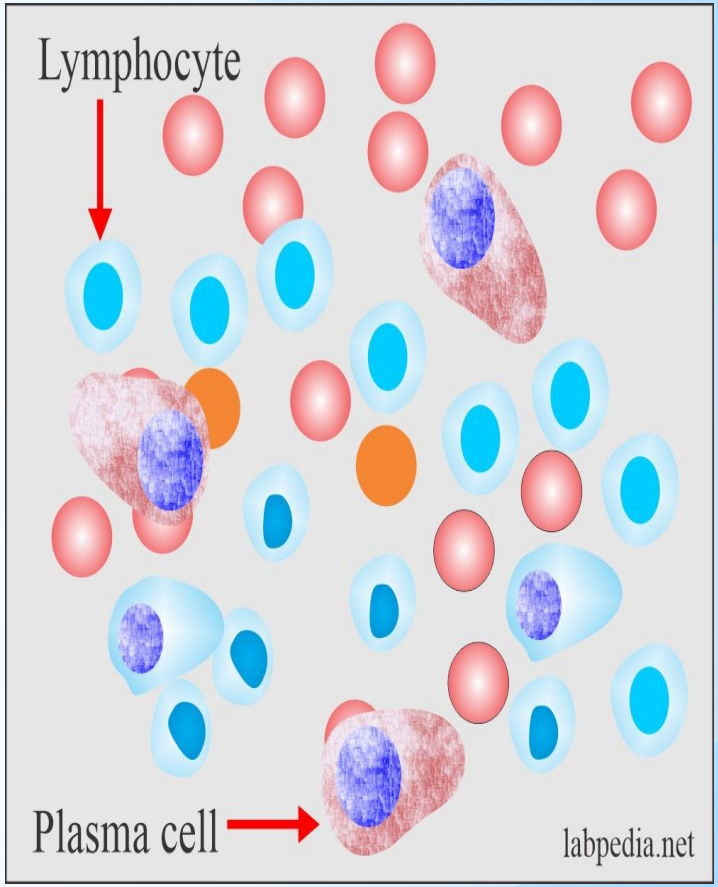


Lymphocytes

* Normal urine may contain a few small lymphocytes which are not recognized.
* Wright's stain can recognize them.

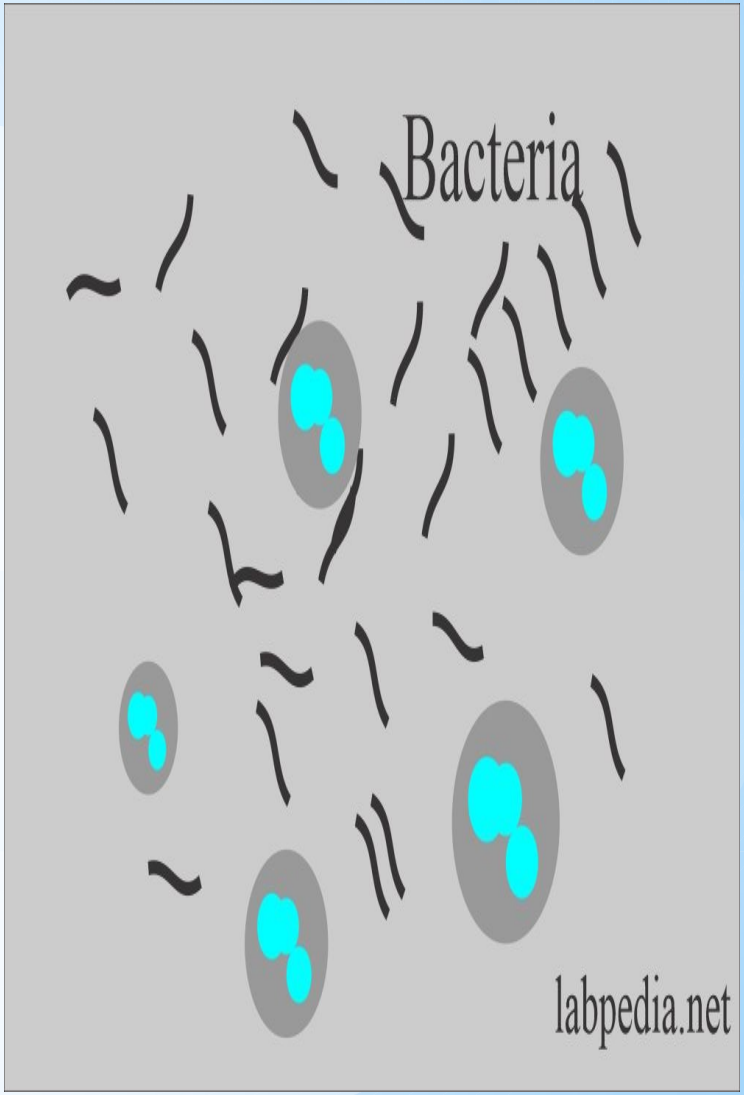
An increased number of lymphocytes is seen in the following:

* The first few weeks of renal transplantation are a sign of rejection.
* Many lymphocytes and plasma cells show renal transplantation rejection and acute renal allograft rejection.



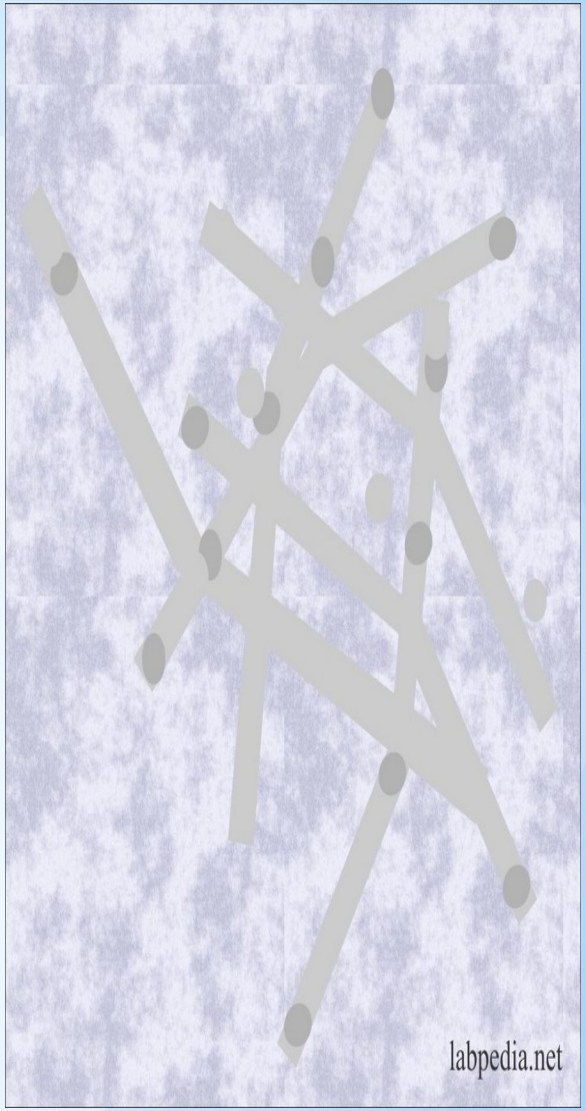
Bacteria in Urine

* Normally, few bacteria are present unless you collect the urine in strict sterile conditions.
* There are bacteria due to contamination from the vaginal secretions, urethra, genitalia, or the container.
* These bacteria multiply if the urine is left at room temperature and give a nitrite test positive and may result in pH 8, which indicates an unacceptable specimen.
* If urine is kept at room temperature in the lab, bacteria can multiply.



Yeast in Urine

* The most common yeast is candida in the urine.
* This may be vaginal contamination due to vaginal infection.
* **This yeast, primarily candida, is seen in:**
  * The patients with diabetes. Because sugar is used as a source of energy for yeast growth.
  * These are also seen in immunocompromised patients.
  * Female with vaginal moniliasis.
  * The infection is suspected when the presence of WBCs accompanies candida.
* **Structure of yeast:**
  * These may be seen as small, refractile oval structures that may be laking buds.
  * These are strongly gram-positive.



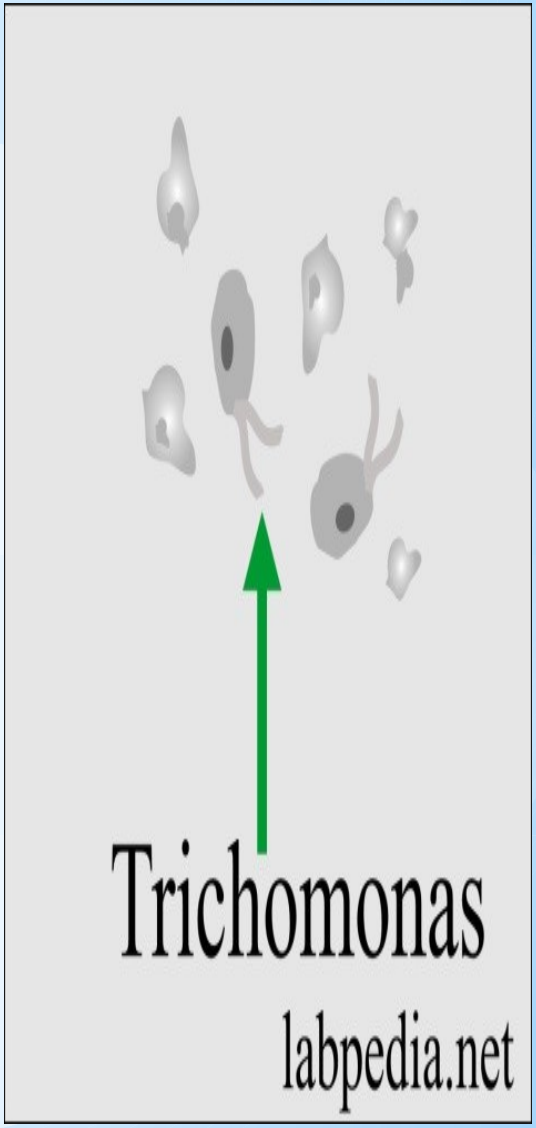
Trichomonas (parasites)

* This protozoan is the common cause of vaginal infection called Trichomonas vaginitis caused by Trichomonas vaginalis.
* Trichomonas is a sexually transmitted disease and causes inflammation in the female.
* While the infection of the male urethra and prostate is asymptomatic.

Structure of Trichomonas:

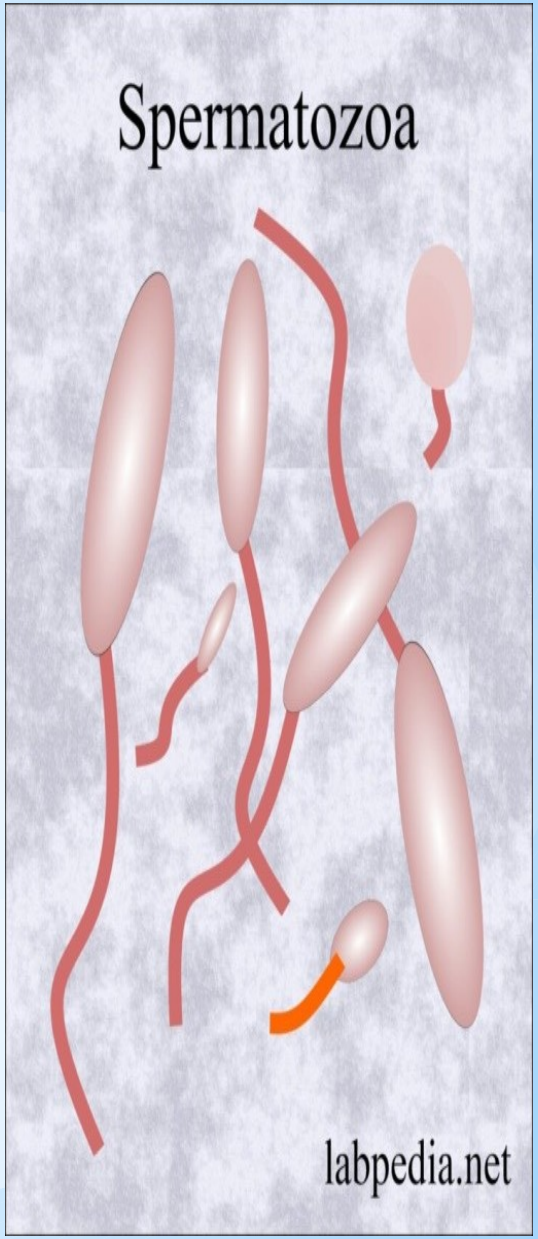
  * Trichomonas is a pear-shaped flagellate with an undulating membrane.
  * It is easily identified in a wet preparation and the urine sediment by its rapid movement under the microscope.
  * These protozoa reside in the prostate and vagina.

* Wet preparation from the vagina or urethra by direct swab preparation can diagnose it.



Spermatozoa

* The spermatozoa are oval, slightly tapered heads and long flagellate-like tails.
* Urine is toxic to sperms, so they are non-motile in the urine.
* **Urine showing spermatozoa-**
* There should be a lab protocol when reporting spermatozoa in the urine, particularly female urine. There may be legal consequences.
* These may be seen in males and females following intercourse (coitus).
* Spermatozoa in the urine is significant in infertility cases and sexual abuse.
* In males may be seen after a nocturnal emission or ejaculation.
* Also seen in diseases of the genital tract.
* Their presence in the urine is of significance in the case of retrograde ejaculation, where the sperms go into the urinary bladder instead of the urethra.



Epithelial cells

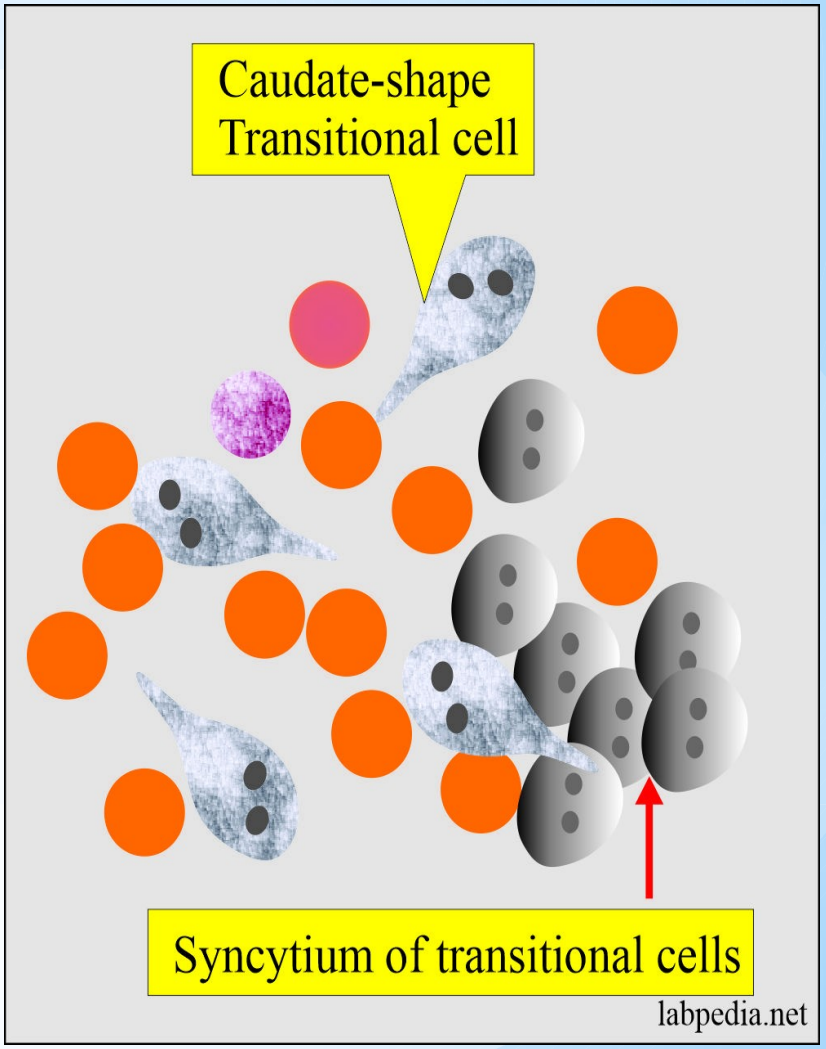
* Mainly these cells are shed from the urethra and urinary bladder.
* Few renal epithelial cells are seen normally in the sediment.
* Epithelial cells are the following types :
  * Squamous epithelial cells
  * Transitional epithelial cells
  * Renal epithelial cells.

Squamous epithelial cells:-

* These represent normal sloughing and have no pathologic significance.
* Midstream clean catch will have less number of these cells.
* These cells have little significance but are helpful for focusing on the microscope.

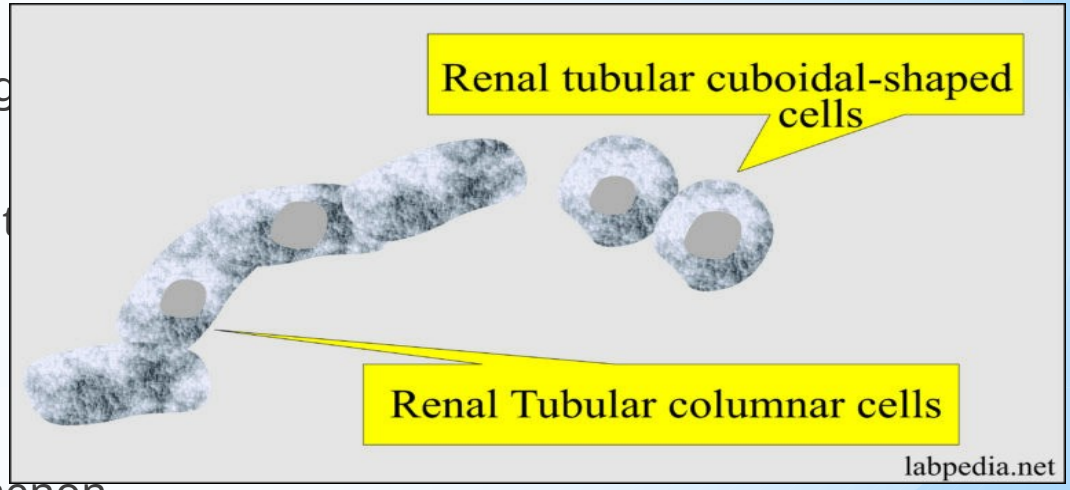
Transitional Epithelial cells

* These are stratified epithelial lining of the urinary tract system, starting from the kidney's pelvis to the urinary bladder base in the female and proximal part of the male urethra.
* **Morphology:**
  * These are smaller than the squamous epithelial cells.
  * These have various shapes, like spherical, polyhedral, and caudate forms.
  * All forms have a clear central nucleus.
  * These cells take origin from the lining of the renal pelvis, calyces, ureter, urinary bladder, and upper part of the urethra.



Renal Epithelial Cell

* **The function of renal tubular cells:**
  * The main function of these cells is reabsorption.
  * In hemoglobinuria, these cells contain yellow-brown hemosiderin pigments.
  * In the case of bilirubin, the color of these cells is deep yellow.
* **Significance:**
  * When present in groups, it indicates severe renal damage.
  * Acute tubular necrosis.
  * It is seen in heavy metal poisoning
  * Drug-induced toxicity.
  * Hemoglobin and myoglobin toxicit
  * Viral infections like HBV.
  * Viral infections.
  * Allergic reactions.
  * Acute allogenic Rejection phenomenon.



Casts Cells

Formation of the cast:

* These casts are precipitated proteins in the tubules. It forms in the lumen of distal convoluted tubules and collecting ducts.
* Their shape is representative of the tubular lumen with parallel sides and mostly rounded ends.
* The casts dissolve easily in the dilute alkaline urine.

Composition of the cast:

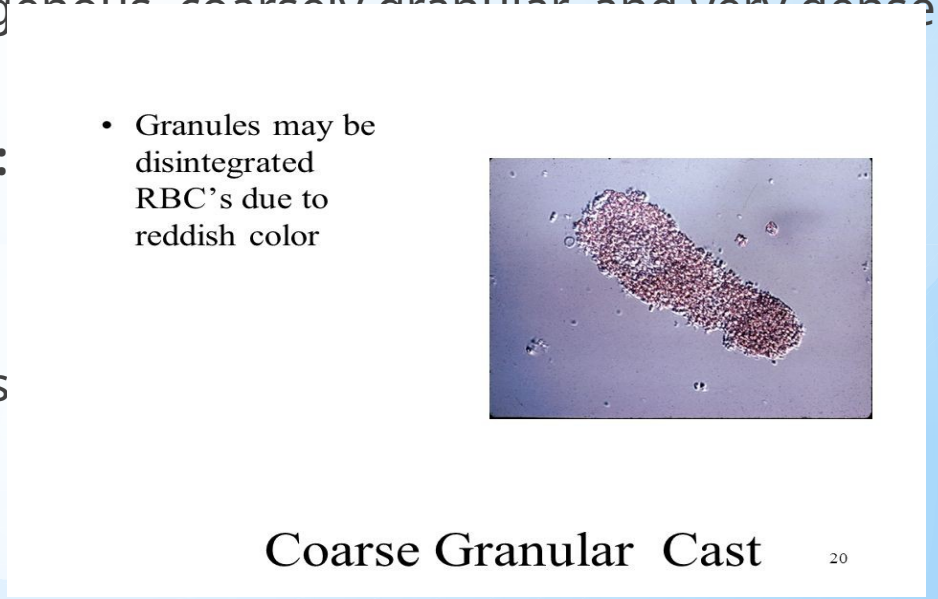
* The major component is the Tamm-Horsfall protein. This protein is produced from the renal tubular epithelium and upper collecting ducts.
* Other proteins are albumin and Immunoglobulins.
  * The width of the cast depends on where the cast is formed.
* These casts lead to blockage of the urinary outflow in the tubules.

CASTs in Urine

* Urine sediment after the centrifugation shows the crystals and casts.
* Casts are formed in the collecting tubules.
* Various types of urine casts have different significance.
* Depending upon the various reason, there are the following types of casts:

* Granular cast:

* Granular casts appear homogenous, coarsely granular and very dense.
* These casts are colorless.
* **Granular casts are found in:**
* Acute tubular necrosis.
* Pyelonephritis.
* Advanced glomerulonephritis



* **Hyline cast:**
  * These are clear and colorless and form when the tubules contain precipitated proteins.
  * Hyaline casts may be seen in physiologic states like Exessive exercise and mild renal disease.

Hyaline casts are found in

* Malignant hypertension.
* Chronic renal diseases.
* Diabetic nephropathy.
* Congestive heart failure.
* Pyelonephritis.
* Glomerulonephritis.

*There are WBCs in a protein matrix.

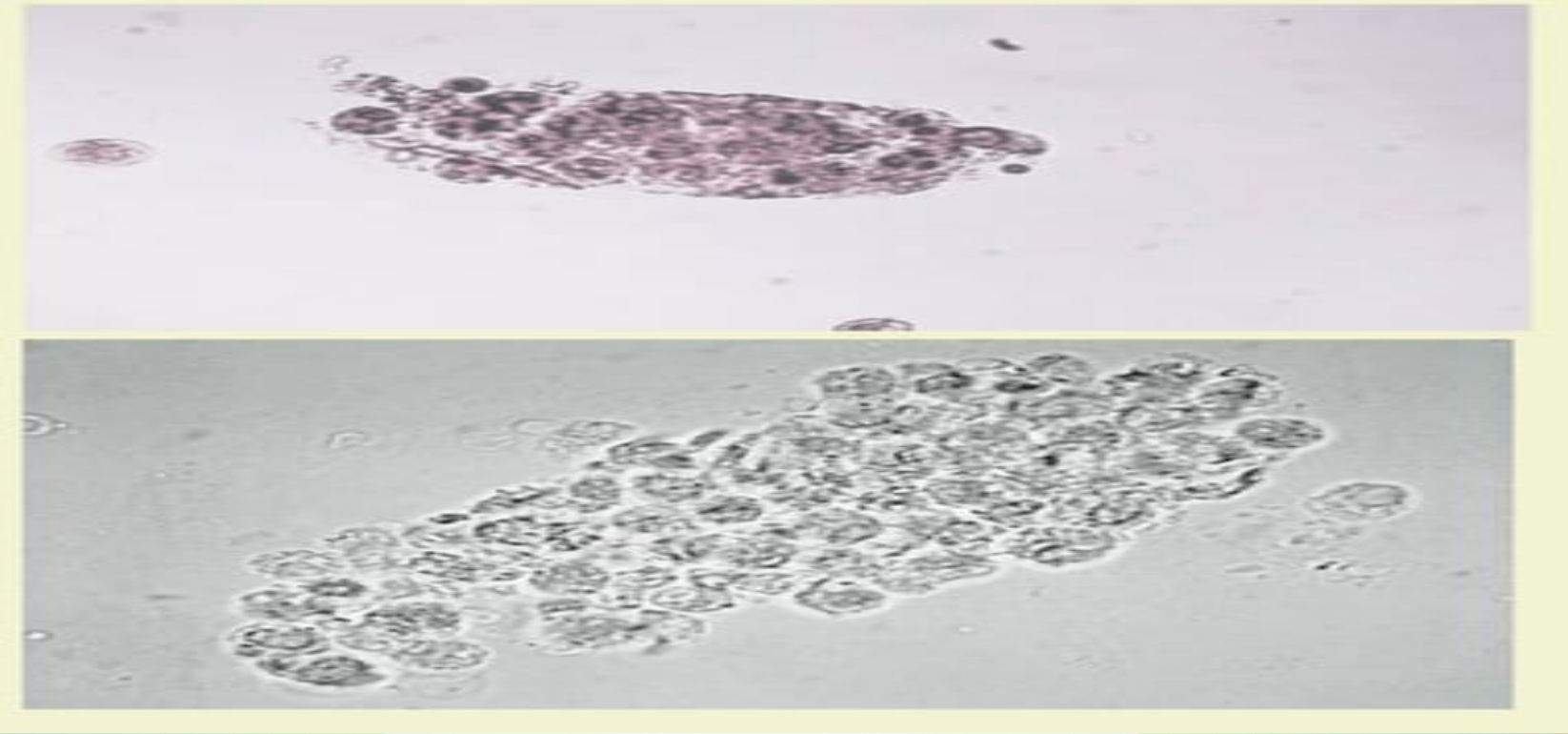
*These take origin from kidney tubules.

WBC casts are found in:

*Lupus nephritis.

*Acute glomerulonephritis.

*Pyelonephritis



* **Epithelial cast:**
  * Renal epithelial cell casts formed from the shedding of the renal tubular cells.
  * These shed cells undergo degenerative changes.

Epithelial cell casts are found in:-

Acute tubular necrosis.

Pyelonephritis.

Acute glomerulonephritis.

Viral infection like Cyto megalo Virus (CMV.)

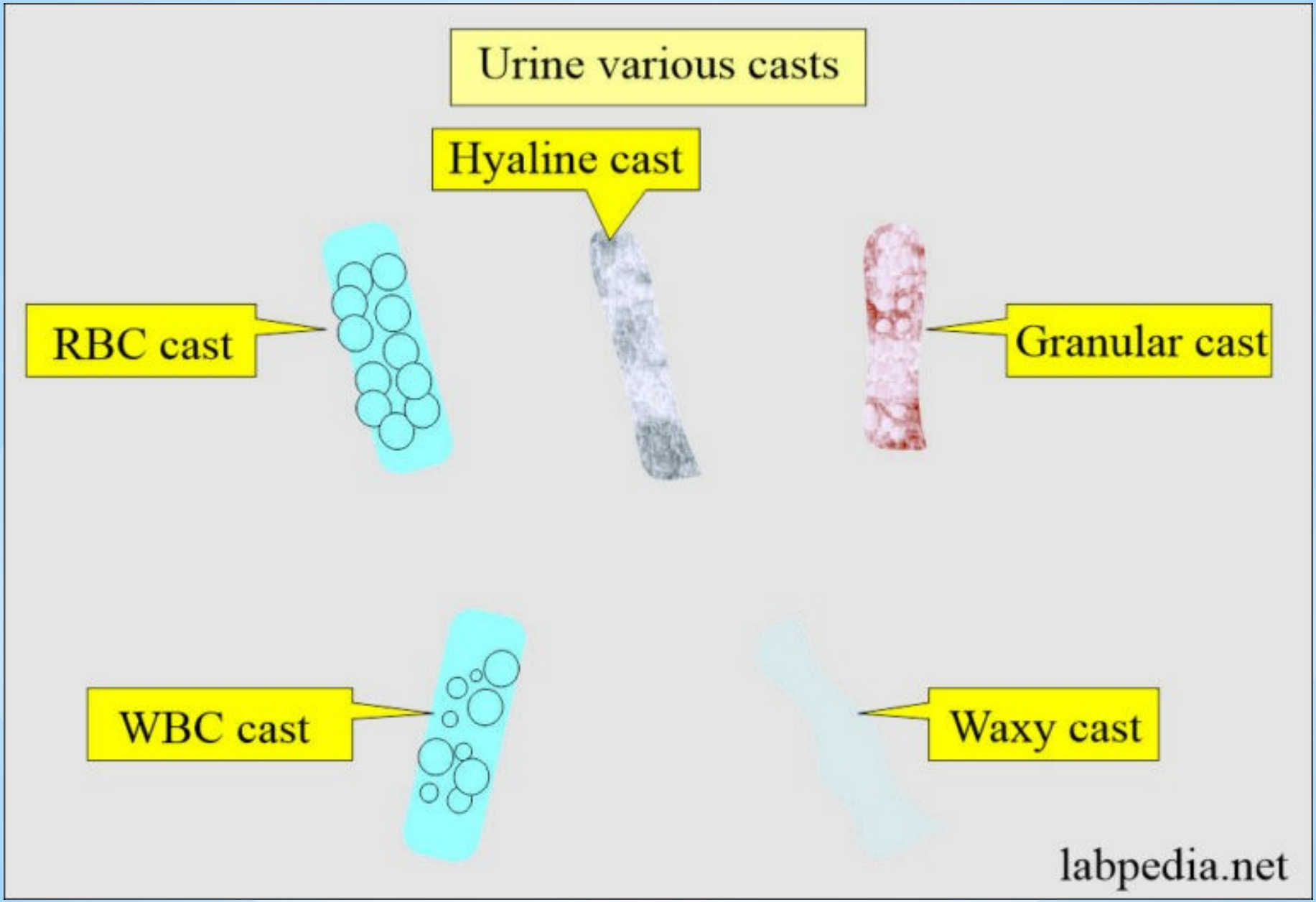
Drugs intoxication like salicylates overdose.

*Waxy cast:

- *Waxy casts formed from the degeneration of the granular cast.

Waxy casts are found in:-

- *Nephrotic syndrome.
- *Severe renal failure.
- *Malignant hypertension.
- *Diabetic nephropathy.


Urine various casts
Hyaline cast
RBC cast
Granular cast
WBC cast
Waxy cast
labpedia.net

Urinary Crystals

- Most are not clinically significant but are reported
- True geometrically formed structures or as amorphous material
- Must differentiate from the few abnormal crystals indicating liver disease, inborn errors of metabolism, and damage to tubules
- Iatrogenic: caused by medications or treatments
- Report: rare few, moderate, many

Crystal Formation

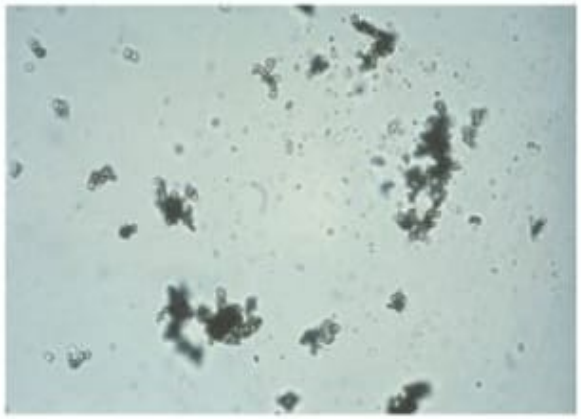
- Precipitation of urine solutes: salts, organic compounds, and medications
- Formation based on temperature, solute concentration, and pH
- Many crystals in refrigerated specimens
- High specific gravity needed in fresh specimens

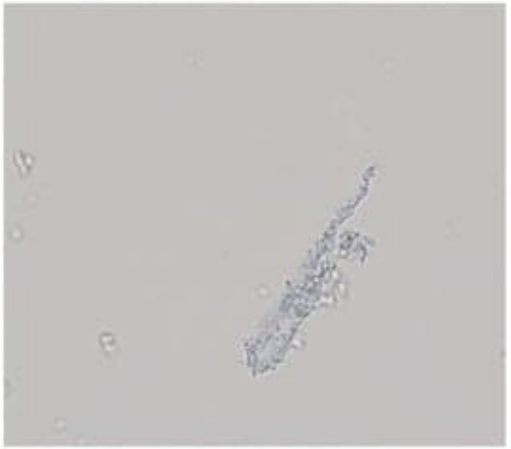
General Identification Techniques

- Most have characteristic shapes and colors
- Most valuable ID is urine pH
- Classification: normal acid, normal alkaline
- All abnormal crystals are found in acid urine
- Polarized microscopy characteristics are valuable in ID

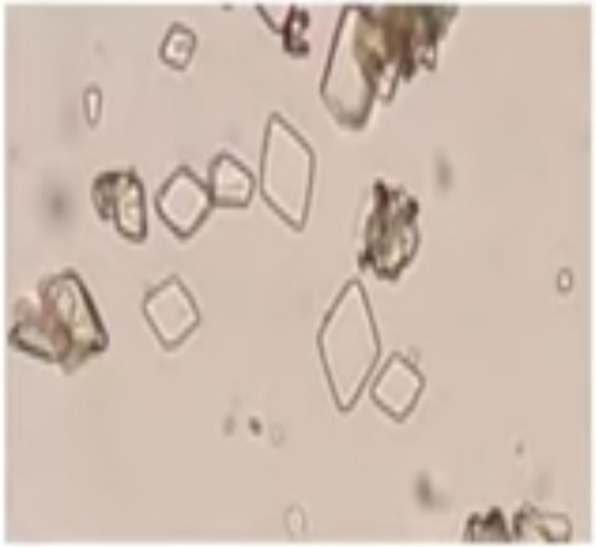
Normal Crystals in Acid Urine

- Amorphous urates
  - Yellow-brown granules microscopically
  - Urine sediment has pink color due to the pigment uroerythrin attaching on surface of granules
  - Often in clumps; may resemble casts
  - pH usually greater than 5.5





URIC ACID



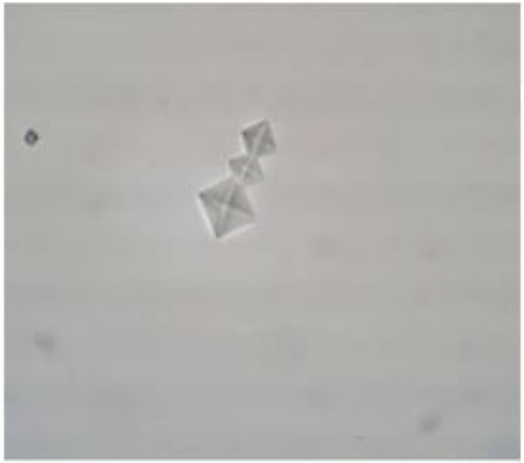
Barrel, diamond, and rosette shape

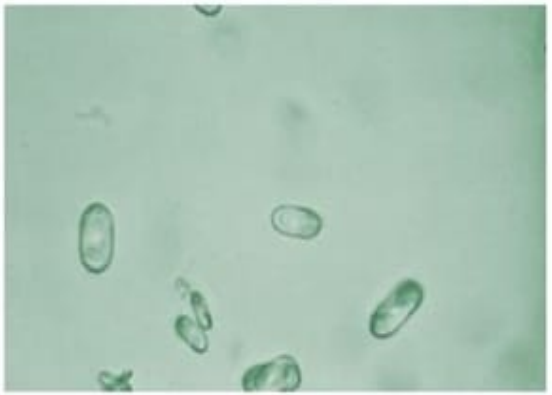
Orange-brown or yellow in color.

Protein-rich diet, which increases uric acid in the urine.

Calcium Oxalate Crystals

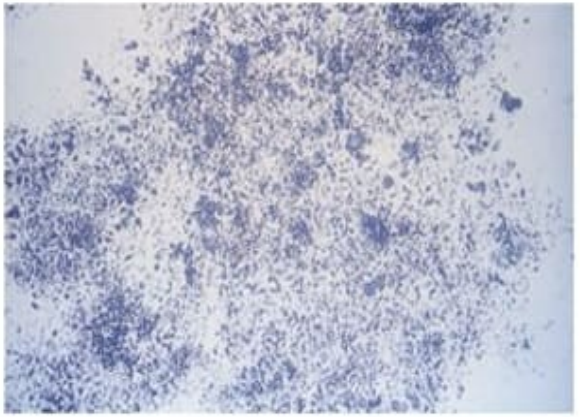
- Acid and neutral pH
- Dihydrate is envelope or two pyramid–shaped
  - Most common
- Monohydrate is oval or dumbbell shaped
  - Antifreeze poisoning
- Calcium oxalate is a major component of renal calculi





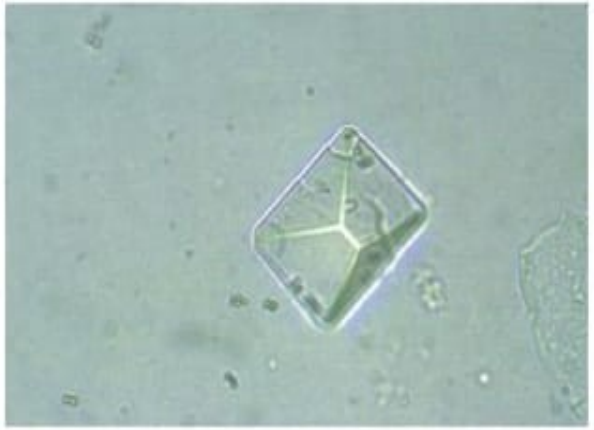
Amorphous Phosphates

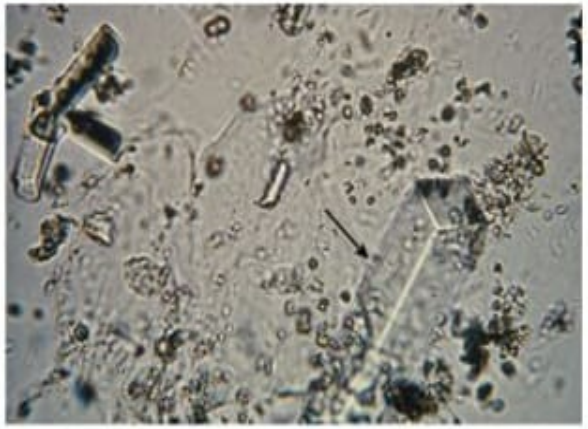
- May appear similar to amorphous urates
- Differentiate
  - Alkaline pH and heavy white precipitate after refrigeration



Normal Crystals in Alkaline Urine

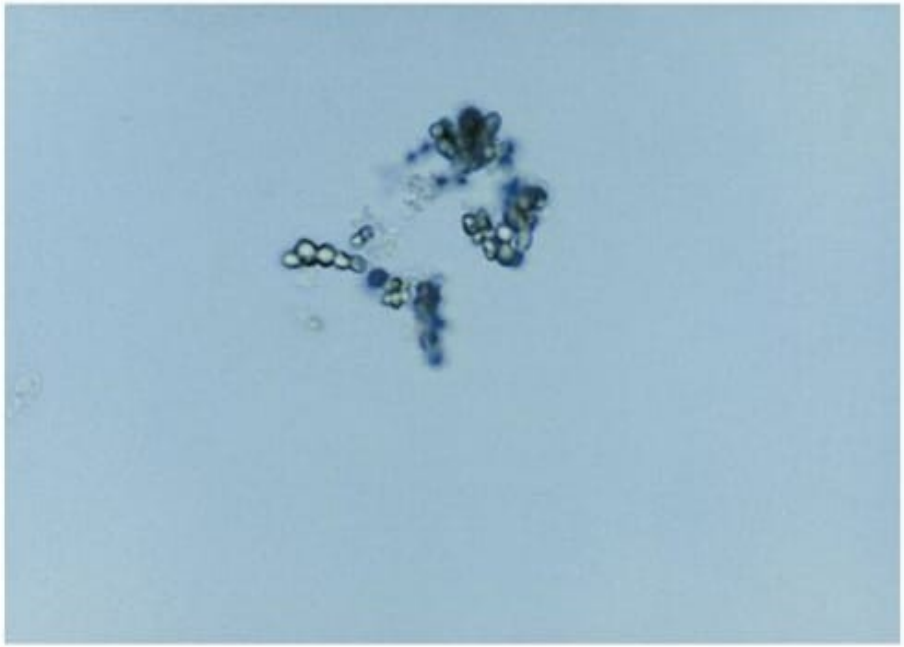
- Triple phosphate
- Colorless, prism, or coffin-lid shaped
- Highly alkaline urine and urinary tract infections (UTIs)
- Polarize
- No clinical significance

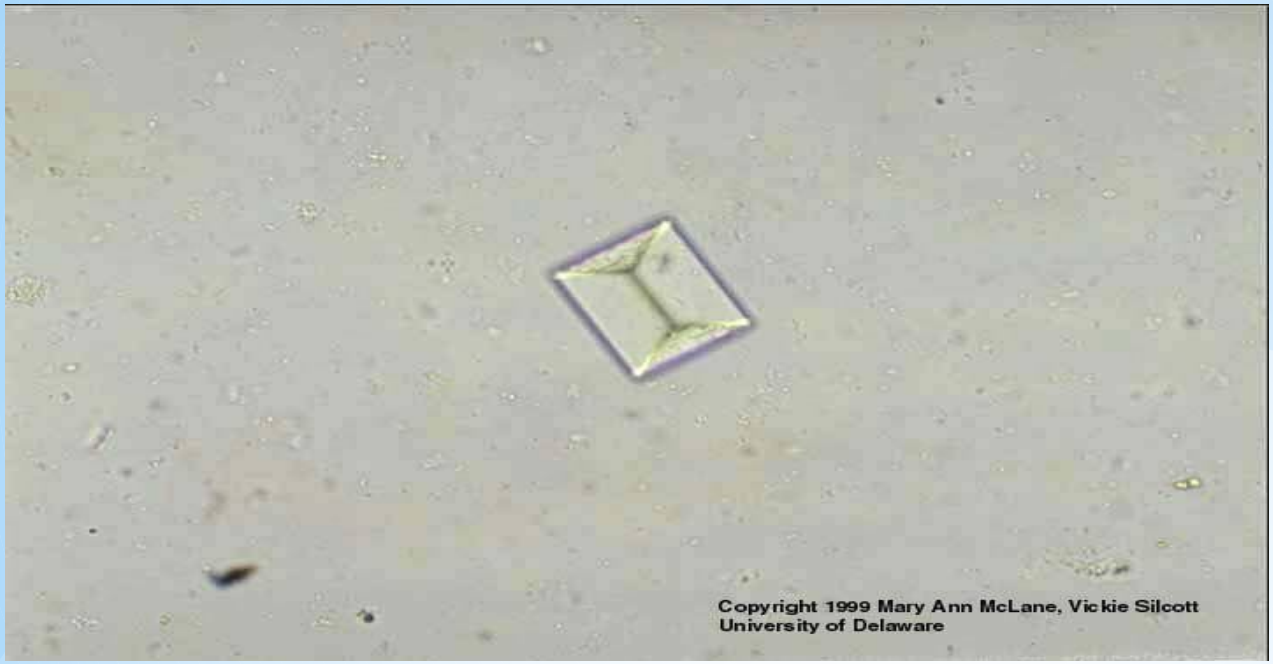




Calcium Phosphate and Carbonate

- Phosphate
  - Flat rectangles and thin prisms in rosettes
  - No clinical significance
- Carbonate
  - Small, dumbbell, and spherical shapes
  - Gas produced with addition of acetic acid
  - No clinical significance

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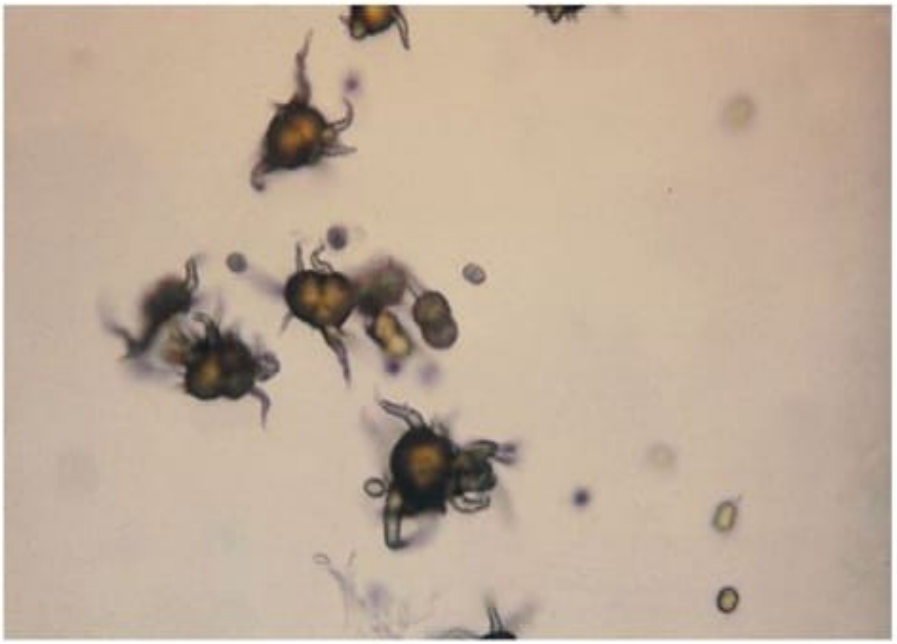
Calcium
carbonate crystal

Ammonium Biurate Crystals

- Yellow-brown, spicule-covered spheres; “thorny apples”
- Only urates in alkaline urine
- Old specimens and with urea-splitting bacteria



AMMONIUM BIURATE CRYSTALS

COLORED

Yellow brown/
Brown

Spherical bodies with protrusions!

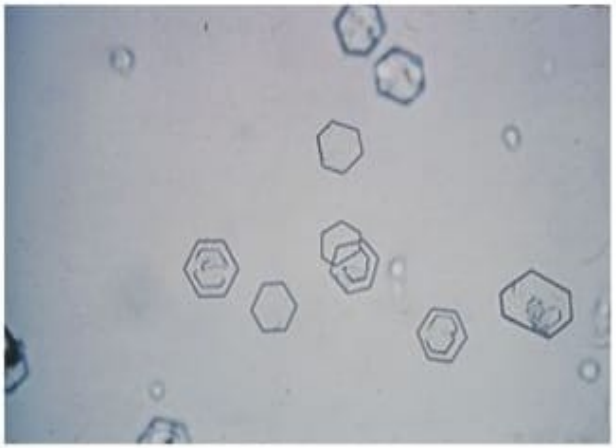
THORN APPLE

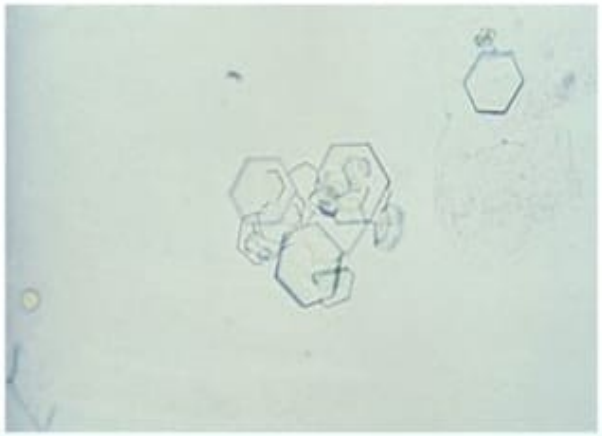
Alkaline urine

- Normal urine
- Old/ poorly preserved urine
- Can occur together with triple phosphates and calcium phosphates in the case of bacterial urinary tract infection with urease-positive bacteria

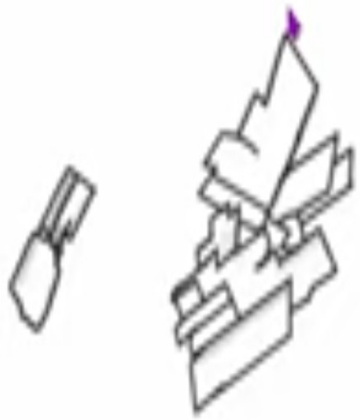
Abnormal Crystals

- Cystine crystals
  - Hexagonal, thin and thick plates
  - Similar to uric acid
  - UA polarizes but only thick cystine crystals polarize
  - Seen in cystinuria: inability to reabsorb cystine
  - Confirm: cyanide nitroprusside





CHOLESTEROL



Colorless

Rectangular plates with a **notch** in one or more corners

Acidic urine

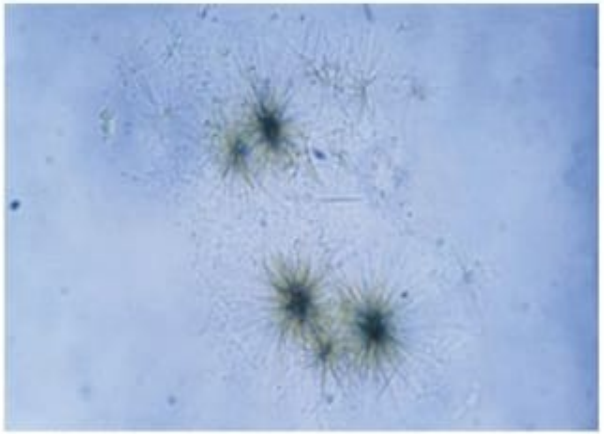
Nephrotic syndrome.

These appear after the urine sample has been refrigerated !!

Not to be confused with glass fragments!

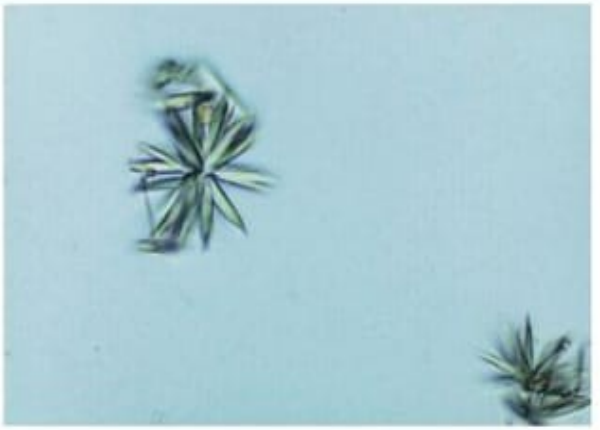
Liver Disease Crystals

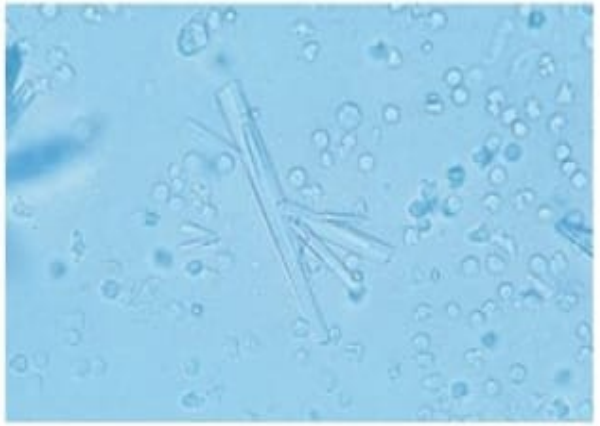
- Tyrosine crystals
  - Fine yellow needles in clumps or rosettes
  - Seen with leucine crystals
  - Inherited amino acid disorders
- Leucine crystals
  - Yellow-brown spheres with concentric circles and radial striations



Sulfonamide Crystals

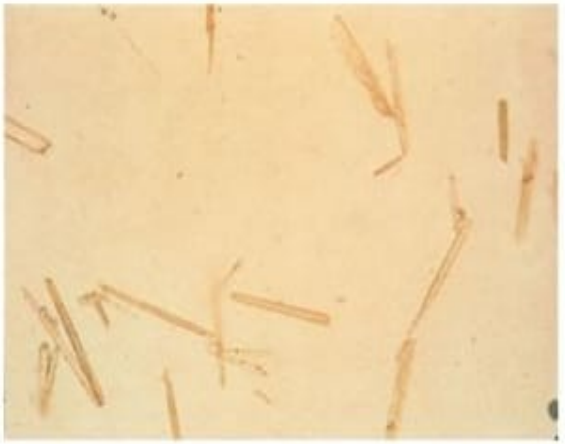
- Possibility of tubular damage if crystals are forming in the nephron
- Shapes most frequently encountered include needles, rhombics, whetstones, sheaves of wheat, and rosettes with colors ranging from colorless to yellow-brown

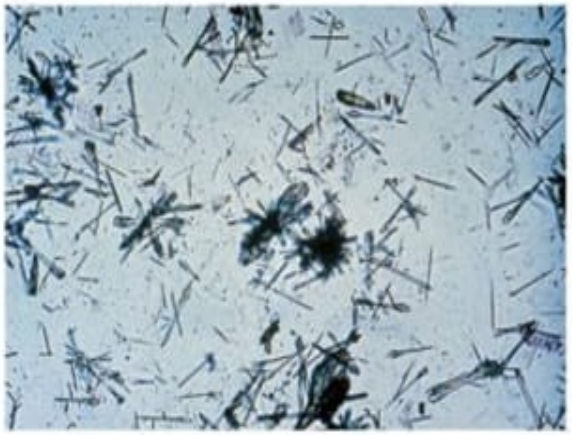




Ampicillin Crystals

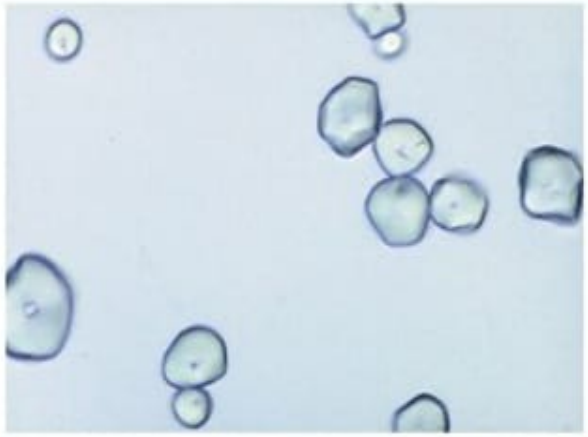
- Ampicillin crystals appear as colorless needles that tend to form bundles following refrigeration

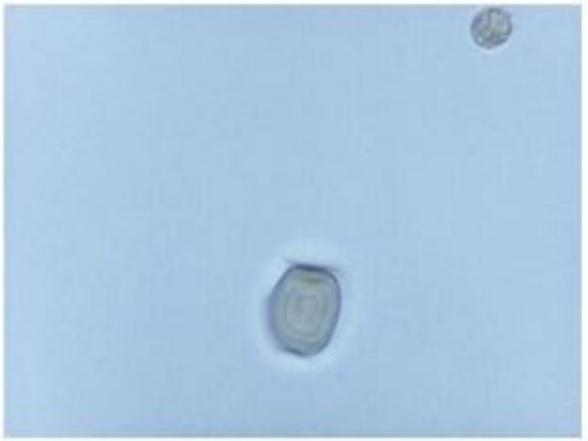




Urinary Sediment Artifacts

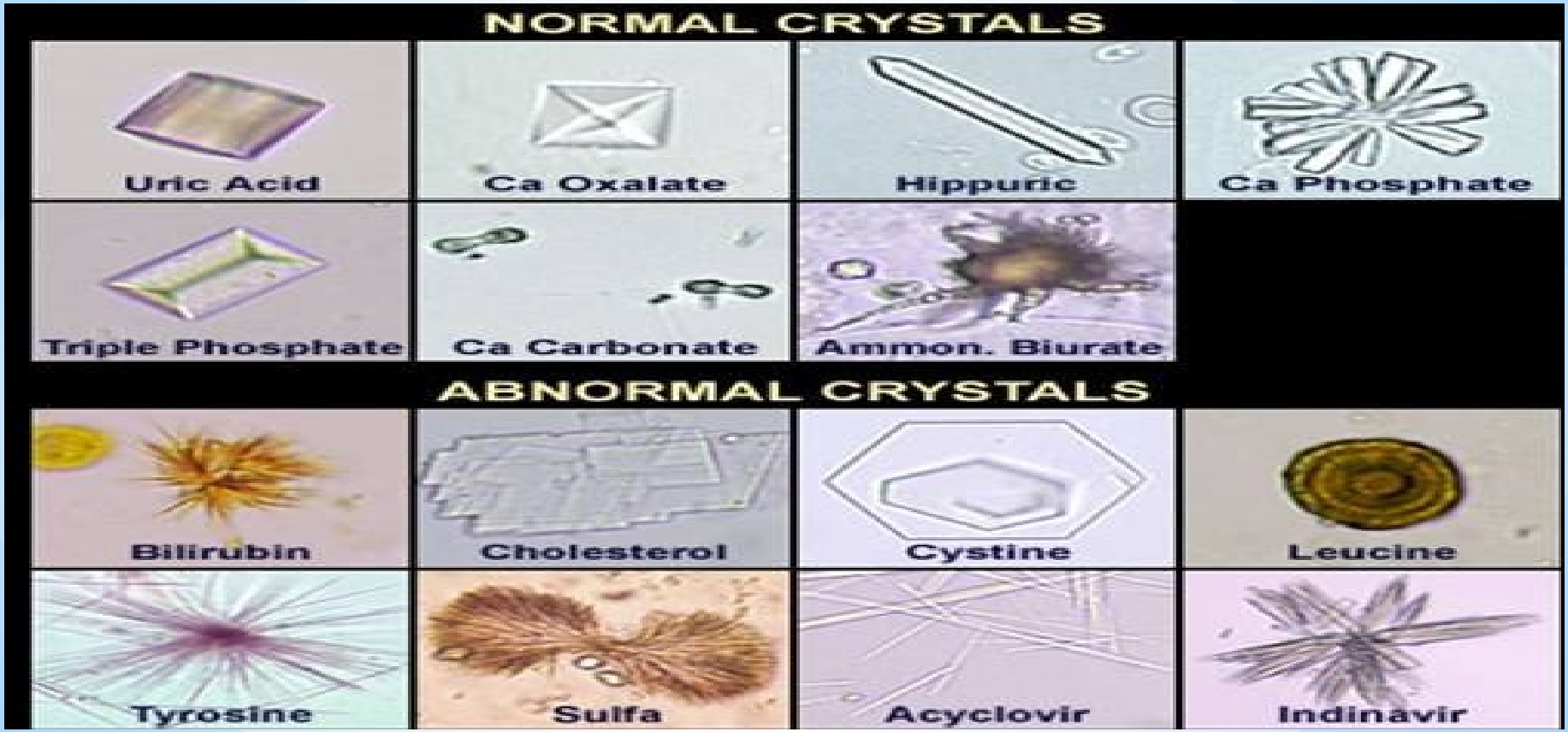
- Material fibers, meat and vegetable fibers, and hair
- Starch, oil droplets, air bubbles, pollen grains, vegetable fiber, hair, diaper fiber





Microscopic Crystals

A variety of normal and abnormal crystals may be present in the urine sediment.



Normal crystals

Crystals	Ph of urine	Identification	Remarks
Uric acid	Acidic	•Barrels, rosettes, rhomboids, needles or hexagonal plates •Amber in color •Soluble in alkali	Urate nephrolithiasis or acute urate nephropathy, gout and leukemia
Calcium oxalate	Acidic or alkaline	•Can be bihydrate (envelope shape) or monohydrate (ovoids, dumbells or rods)	Dietary oxalate ingestion (spinach, tomato, cabbage and asparagus) and nephrolithiasis
Calcium phosphate	Alkaline	•Large, flat shaped plates or wedge shaped prisms •Prisms often appear in rosettes	In rare cases, could be caused by hypoparathyroidism
Triple phosphate	Alkaline	•Coffin lid appearance •Made of magnesium ammonium and phosphate	In patients with struvite stones and in urinary tract infection with *Proteus*
Calcium carbonate	Alkaline	•Spheroids with radial striations •Colorless to yellow-brown •Readily observed at low magnification	Normal
Ammonium biurate	Alkaline	•Thorn apple appearance •Yellow-brown with irregular protrusions or spiky thorns	Old or longstanding urine sample

Patients 1. A 45-year-old man comes to his physician after spending a second sleepless night with excruciating lower abdominal pain. The pain seemed to come in waves and was unrelieved by aspirin, tylenol, or lying or standing in any position. He had not experienced any similar pain before. On physical examination there are no abnormal findings.

Characteristic	Result
WBC/hpf	2-5/hpf
RBC/hpf	>100/hpf
Casts	None
Other	Occasional squamous epithelial cells

Characteristic	Result
Color	Dark Yellow
Appearance	Cloudy
Leukocyte Esterase	Neg
Nitrite	Neg
pH	6.0
Protein	Neg
Blood	3+
Specific Gravity	1.015
Ketones	Neg
Glucose	Neg
Bilirubin	Neg

- **Questions: What abnormal findings are present?**

There is cloudiness with microscopic hematuria.

- **What is the differential diagnosis?**

Hematuria can be due to numerous causes: inflammation, trauma, glomerulonephritis, calculi, instrumentation, neoplasms, etc.

- **What diagnosis do you suspect?**

The severe pain suggests a renal calculus.

- **What other studies could be done?**

Radiographic studies. A plain film will demonstrate about 80% of stones (those with calcium). An IVP (Intra Venous Pylogram) may show a filling defect. The urine could be strained to catch a passed stone so that it could be sent for analysis. Uric acid stones suggest gout, magnesium ammonium phosphate stones suggest urinary tract infection.

Summary

* Urinalysis in an important clinical diagnostic test.
* Urinalysis can reveal diseases that have gone unnoticed because they do not produce striking signs or symptoms.
* Urinalysis provides information about the kidney, urinary tract, and systemic (non-kidney) disorders.
* The results of the macroscopic, chemical and microscopic analysis must be interpreted together to arrive at a proper diagnosis.
* Although urinalysis is easily performed with reagent & Microscopic test, the results are dependent on:
* Thus, technologists and technicians performing these tests must be properly trained, especially in correctly recognizing microscopic elements.


THANK
YOU