URINALYSIS

ACADEMIC HALF DAY

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OBJECTIVES

1. Describe the appropriate urine specimen collection and storage in ambulatory patients and hospitalized patients with Foley catheters in order to accurately interpret the urinalysis.
2. List the possible causes of urine that is cloudy, orange, brown, or red.
3. Predict how the urinalysis will appear in disorders of volume and tonicity.
4. Describe the conditions associated with the following urine casts: Hyaline, Erythrocyte, Leukocyte, Epithelial, Granular, Fatty.

Specimen Collection

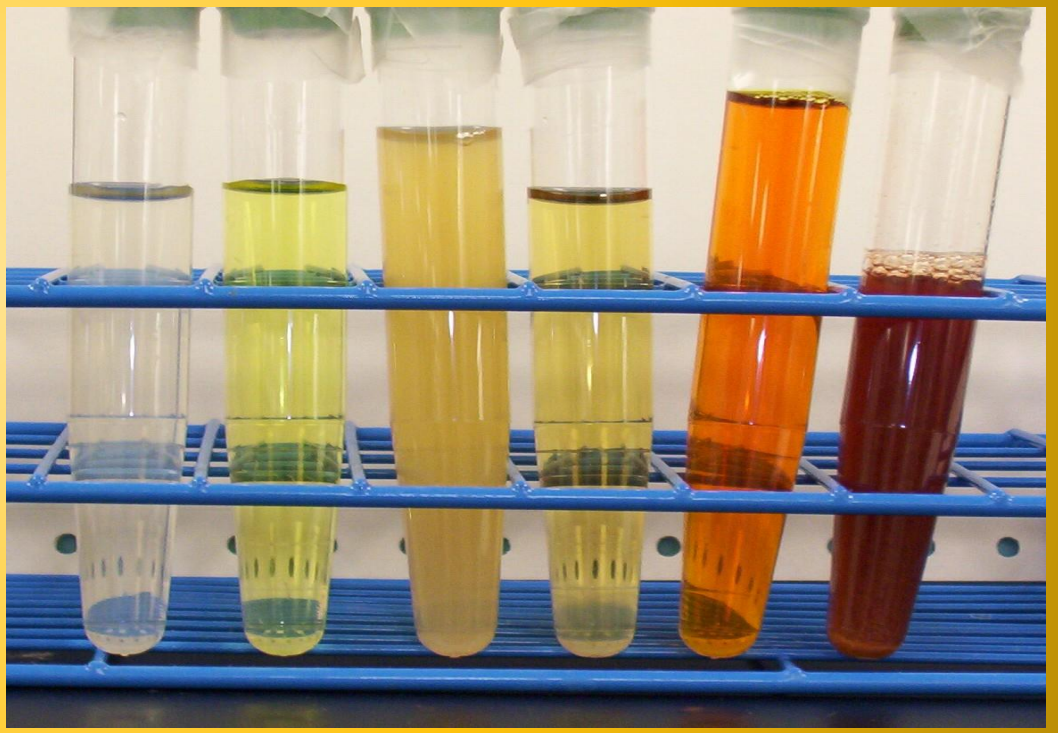
- Avoid exercise 72 hours prior – trauma increases proteinuria and hematuria
- Avoid contamination
  - Males – retract foreskin
  - Females – separate labia and cleanse periurethral area with sterile wipe
  - Midstream “clean catch” collection or bladder catheterization

Specimen Processing

- Prompt processing – should be examined within 1 hour of voiding to minimize breakdown of formed elements
- Macroscopic exam – color and clarity
- Chemical exam - dipstick
- Microscopic exam
  - Centrifuge at 3000rpm for 3-5 minutes
  - Pour off supernatant
  - Place sediment on slide for microscopic exam

Macroscopic Exam

- Color
  - Lighter: dilute
  - Darker: concentrated
  - White: pyuria or phosphate crystals
  - Green: methylene blue, amitriptyline
  - Black: Malignancy, alkaptonuria
  - Red: hemoglobin, myoglobin



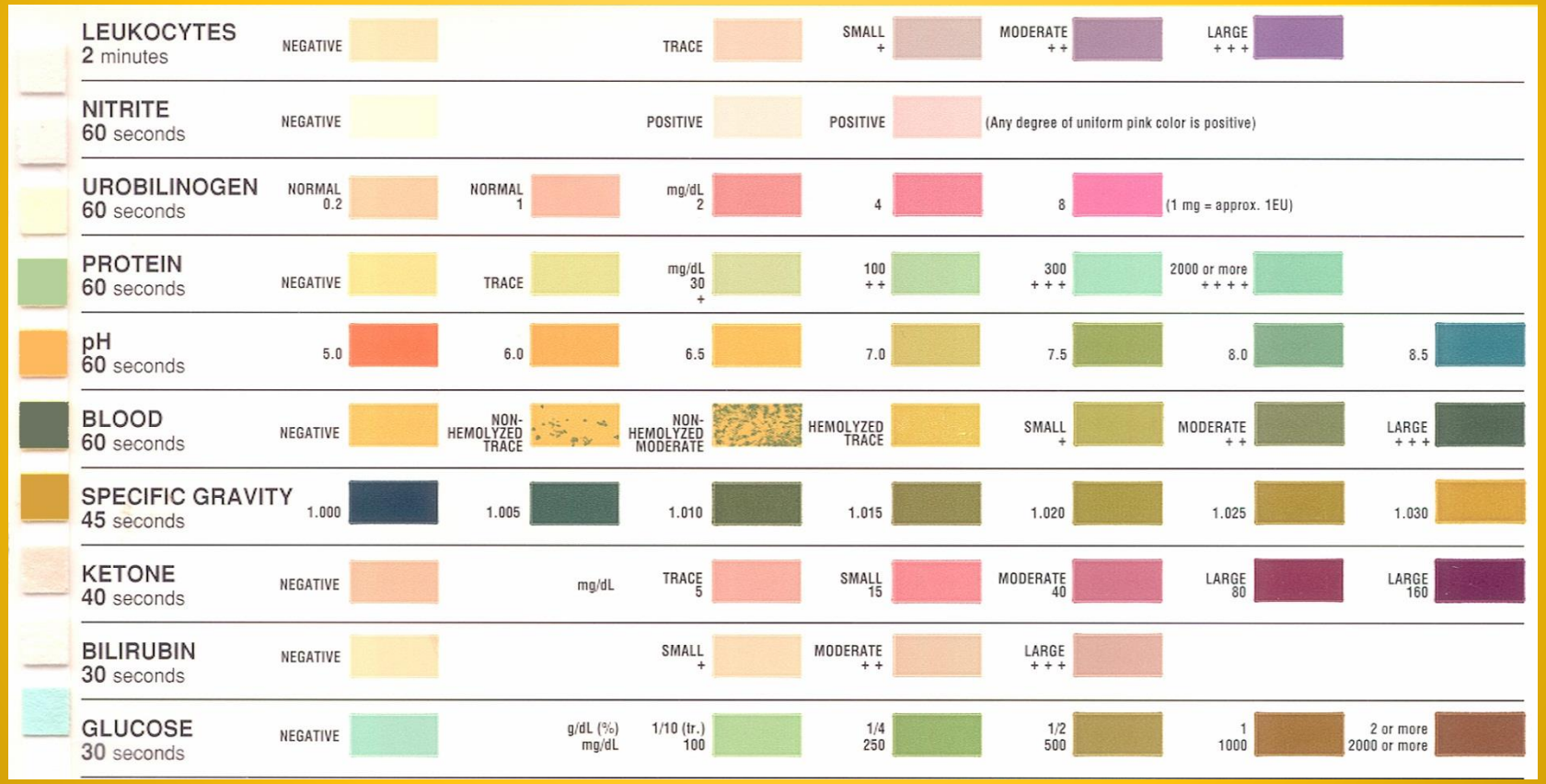
Macroscopic Exam

- Clarity
- Normally clear
- Cloudy
  - amorphous phosphates: white precipitate in alkaline urine
  - amorphous urates: pink precipitate dissolves when heated
  - leukocytes, bacteria, cellular debris
- Foamy
  - proteinuria

Chemical Exam

- When the test strip is dipped in urine the reagents are activated and a chemical reaction occurs.
- The chemical reaction results in a specific color change.
- After a specific amount of time has elapsed, this color change is compared against a reference color chart provided by the manufacturer of the strips.

Chemical Reaction Chart



Urine Dipstick

	Reference Range	Comments
Specific gravity	1.005-1.030	Low with dilute, high with concentrated or with hypertonic product excretion
pH	5.0-6.5	High with acid ingestion, alkaline tide, inability to excrete acid load (renal tubular acidosis), urease-splitting organisms
Blood	None	False positives: myoglobin, hemolysis
Protein	None	Dipstick detects albumin, not other proteins False positives: concentrated urine False negative: non-albumin protein
Glucose	None	Positive when plasma glucose >180mg/dL, pregnancy, Fanconi syndrome
Ketones	None	Detects acetoacetic acid Doesn't detect acetone or beta-hydroxybutyrate
Nitrites	None	Nitrites converted dietary nitrate by bacteria
Leukocyte esterase	None	Positive if > 3 leukocytes/hpf

Urine Microscopy

	Reference Range	Comments
Erythrocytes	0-3/hpf	Need to evaluate erythrocyte morphology
Leukocytes	0-3/hpf	
Casts	None or hyaline	Hyaline – suggest poor renal perfusion Non-hyaline casts – suggest intrinsic injury
Crystals	None	Occur when urine is supersaturated with a specific substance

CASE 1

You are asked to see a 25 year old male with head trauma after a motor vehicle accident for evaluation of hypernatremia. He is making 5L of urine per day. Serum sodium 159. You suspect he has central diabetes insipidus. What do you expect his urine specific gravity to be?

A. 1.005
B. 1.010
C. 1.015
D. 1.020

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In diabetes insipidus, the hypothalamus does not produce ADH, so there is no insertion of aquaporin channels into the apical membrane of the principle cells in the collecting duct. Water is not reabsorbed and the urine osmolality and specific gravity remain low.

Specific Gravity

- Ratio of the weight of urine to an equal quantity of water
- Normal 1.010
- Can use to estimate urine osmolality
  - 1.010 correlates with osmolality of 300mOsm/kg H_2O

Osmolality

- In order to maintain plasma osmolality (# of particles/unit mass) near 285 mOsm/kg the kidney varies urine osmolality from 50 to 1200 mOsm/kg
- Urine osmolality is useful when correlating it with the clinical state of the patient
- Measured by an osmometer
- High glucose concentration increases osmolality
- Certain toxins can increase the osmolality of urine

CASE 2

A 54 year old woman with Sjogren Syndrome comes to the office for evaluation of nephrolithiasis. Her potassium is 3.2, bicarbonate 12. What is a possible diagnosis? What do you think her urine pH will be?

A. 4.5
B. 6.0
C. 7.5

CASE 2

In a Type 1 Distal Renal Tubular Acidosis, there is a failure of the α-intercalated cell to secrete H+ ions and reclaim K+ ions.

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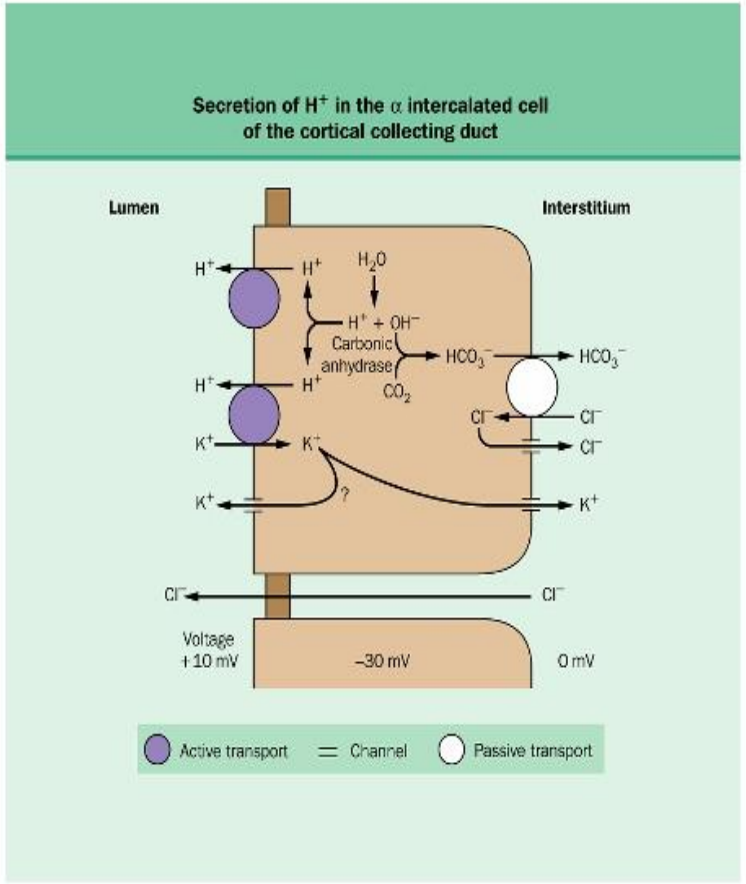

pH

- Acidic pH (5.0-6.0)
  - Diet with animal meat generates high acid load
  - Volume depletion
- Alkaline pH (>7.0)
  - Strict vegetarians can have alkaline urine
  - Type 1 (distal) Renal Tubular Acidosis
  - Infections caused by urease-splitting organisms (Proteus and Pseudomonas species)
  - Acute Tubular Necrosis

CASE 3

A 70 year old male with a diagnosis of Multiple Myeloma comes to see to you for evaluation of proteinuria. His serum glucose is normal and his urine glucose is elevated. He has proteinuria. His serum bicarbonate is 14 , potassium is 3.2 , uric acid is 2.5 , and phosphorus is 1.8 . Dysfunction of which part of the nephron causes these lab results?

A. Glomerulus
B. Proximal tubule
C. Loop of Henle
D. Distal tubule
E. Collecting tubule

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Multiple Myeloma causes proximal tubular dysfunction. This patient has Fanconi Syndrome which is caused by proximal tubular dysfunction. Bicarbonate, glucose, phosphorus, potassium, and amino acids are all absorbed in the proximal tubule, so this patient is not reabsorbing these and they remain in his urine. The acidosis caused by this is called a Type 2 Proximal Renal Tubular Acidosis

Protein

- Dipstick only detects albumin:
  - Trace (5-30mg/dL)
  - 1+ (30mg/dL)
  - 2+ (100mg/dL)
  - 3+ (300mg/dL)
  - 4+ (>1000mg/dL)
- False positives
  - Alkaline urine
- False negatives
  - Light chains
  - Immunoglobulins
  - Sulfosalicylic acid (SSA) test can be used to detect presence of albumin + other proteins

Proteinuria

- The urine dipstick is not very accurate in assessing the severity of proteinuria since the protein concentration is a function of urine volume as well as the quantity of protein present.
- The urine dipstick is also a relatively insensitive marker for initial increases in protein excretion

Proteinuria

- Types of Proteinuria:
  - Glomerular proteinuria: predominantly albumin
  - Tubular proteinuria: Interference with proximal tubular reabsorption, principally due to tubulointerstitial diseases
  - Overflow proteinuria: Increased excretion of low molecular weight proteins can occur with marked overproduction of a particular protein (almost always immunoglobulin light chains in multiple myeloma) – proximal tubule can’t absorb it all
  - Post-renal proteinuria: Inflammation in the urinary tract, which can occur with urinary tract infection, can give rise to increases in urinary protein excretion. The excreted proteins are generally non-albumin (often IgA or IgG), and only small amounts are excreted

Proteinuria

- Urinary protein excretion normally should be less than 150 mg per day (albumin + low molecular weight proteins)
- Normal rate of albumin excretion <20 mg per day
- Persistent albumin excretion between 30 and 300 mg per day is called high albuminuria (microalbuminuria)

Proteinuria

- In diabetics, this is usually indicative of incipient diabetic nephropathy.

- In non-diabetics, the presence of high albuminuria is associated with an increased risk for cardiovascular disease.

Proteinuria - quantification

- 24 hour urine test for protein excretion
  - Gold standard
  - The amount of protein secreted varies by circadian rhythm
  - Problems: it is time consuming, subject to error due to overcollection or undercollection, impractical for many patients
- Protein-creatinine ratio on a random urine sample
  - Correlates well with the 24 hour urine collection
  - Is easy to obtain
  - Limitations: if creatinine in urine is not what it is expected to be (assumes 1g/day of creatinine excreted)

Glucose

- When blood glucose is >180mg/dL, absorptive capacity of the proximal tubule is exceded
- Fanconi Syndrome – proximal tubular dysfunction
  - Glucosuria in the absence of hyperglycemia
  - Also with loss of phosphate, uric acid, amino acids and bicarbonate
- Pregnancy

Ketones

- Dipstick detects acetoacetate but not beta-hydroxybutyrate
- Positive with:
  - Starvation
  - Alcoholic ketoacidosis (but sometimes negative when only ketone is beta-hydroxybutyrate)
  - Diabetic ketoacidosis (dipstick underestimates total ketone excretion, because the main ketone is beta-hydroxybutyrate)
  - Salicylate toxicity
  - Isopropyl alcohol poisoning
- False positive
  - Drugs with sulfydryl groups: captopril

CASE 4

A 25 year old female presents to the hospital with confusion and bruising on her arms and legs. Two days prior she had a diarrheal illness. Labs reveal hemoglobin of 7.5 , platelets 16 . Peripheral smear shows schistocytes. Creatine kinase is normal. Creatinine is 3.7. Urinalysis shows no protein, 3+ blood, + urobilinogen, and microscopy shows no erythrocytes. What is her diagnosis?

A. Rhabdomyolysis with myoglobinuria
B. Thrombotic thrombocytopenic purpura with hemoglobinuria
C. Membranoproliferative glomerulonephritis
D. Obstructive jaundice

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A. Rhabdomyolysis with myoglobinuria (No – CK is normal)

B. Thrombotic thrombocytopenic purpura with hemoglobinuria

C. Membranoproliferative glomerulonephritis (No – should have dysmorphic RBCs on micro)

D. Obstructive jaundice (No – should not have urobilinogen in the urine)

This patient has a hemolytic anemia and TTP likely caused by an E.Coli infection. The anemia with presence of schistocytes on peripheral smear suggests hemolysis. The hemolysis increases unconjugated bilirubin which is insoluble so cannot be excreted by the kidneys, hence no bilirubin in the urine. It is conjugated in the liver and secreted in the gut where it is converted to urobilinogen, reabsorbed in the blood and excreted in the kidney. The presence of blood on urinalysis with no RBCs on microscopy indicates that there is hemoglobin in the urine without cells. Heme pigment is toxic to the tubular cells and causes Acute Tubular Necrosis.

Hematuria

- Presence of blood or intact cells in the urine
- A very alkaline urine or a urine with very low specific gravity can cause RBC to lyse
- RBC can enter the urine anywhere from the glomerulus to the urethra
- Reagent strips can detect 1-2 RBC/hpf
- Greater than 2 RBC/hpf considered abnormal

Blood

- Dipstick measures peroxidase activity
  - **Free hemoglobin – hemolysis**
  - Intact erythrocytes
- 1-3 erythrocytes/hpf needed for positive result
- False positives
  - **Myoglobin**
  - Bacteria that express pseudoperoxidase activity: Enterobacter, Staphyloccoci, Strebtococci species
  - Hypochlorite
  - Rifampin
  - Chloroquin
  - Iodine
  - Alkaline urine
  - Low specific gravity
  - Semen
  - Oxidizing agents to clean the perineum
- False negatives
  - Ascorbic acid
- Blood on urine dipstick with no RBCs on microscopy raises suspicion for:
  - **Rhabdomyolysis (myoglobin) positive for blood with no erythrocytes**
  - **Hemolysis**

Hemoglobinuria

- Hemoglobinuria –presence of free hemoglobin in the urine as a result of intravascular hemolysis

May lead to kidney damage – Acute Tubular Necrosis from heme pigment

CASE 6

A 30 year old man presents to the hospital with severe total body aches after a day of playing basketball outside in Phoenix. His creatinine is 5.6 , potassium is 5.9 . Hemoglobin is normal. Creatine kinase is 12,000 . Urinalysis shows 3+ blood and no erythrocytes. What is the most likely diagnosis?

A. Rhabdomyolysis with myoglobinuria
B. Consumption of too many beets
C. Acute renal colic from nephrolithiasis
D. Renal cell carcinoma
E. Lupus nephritis

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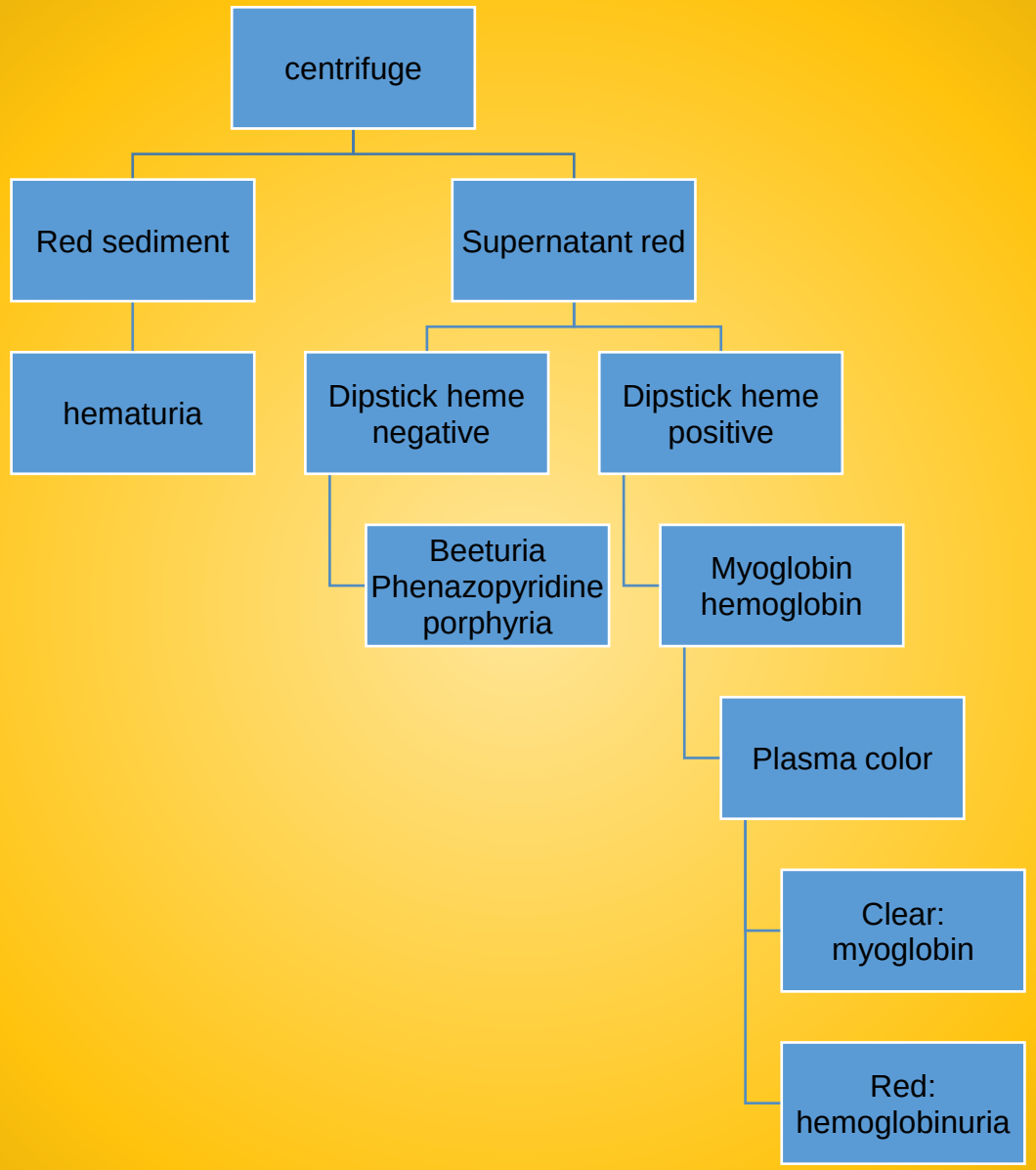
A. Rhabdomyolysis with myoglobinuria
B. Consumption of too many beets (No – urinalysis and micro would both be negative for blood)
C. Acute renal colic from nephrolithiasis (No – Micro would have intact RBCs)
D. Renal cell carcinoma (No – Micro would have intact RBCs)
E. Lupus nephritis (No – Micro would have dysmorphic RBCs or RBC casts)

This patient has rhabdomyolysis caused by breakdown of muscle. Myoglobin gets filtered by the kidneys and causes toxicity to the renal tubules – Acute Tubular Necrosis. The high CK is diagnostic of rhabdomyolysis. The potassium is high because of the ARF and also there is cellular lysis from muscle injury, and the intracellular potassium is coming out of the cells. The presence of blood on urinalysis is because the reagent for heme crossreacts with myoglobin. Whenever you see blood on urinalysis with no RBCs on micro, think rhabdomyolysis or hemolytic anemia.

Myoglobinuria

- Myoglobinuria-small molecular weight heme protein of striated muscle found in urine
- Reacts to same reagent for hemoglobin
- Toxic to renal tubules, may cause acute renal failure
- Cleared from plasma in the first pass, therefore serum is clear of myoglobin

Red Urine


centrifuge
Red sediment
Supernatant red
hematuria
Dipstick heme negative
Dipstick heme positive
Beeturia
Phenazopyridine
porphyria
Myoglobin
hemoglobin
Plasma color
Clear:
myoglobin
Red:
hemoglobinuria

Leukocyte Esterase and Nitrites

- Leukocyte Esterase
  - Enzyme present on leukocytes
  - Positive when 3 leukocytes/hpf present
  - False positive - high glucose or protein concentrations, in the presence of tetracycline, tobramycin, or some cephalosporins
- Nitrites
  - Result from the conversion of nitrates to nitrites by gram-negative bacteria
    - Detects UTI's caused by gram-negative orgnanisms, including: Escherichia coli, Klebsiella pneumoniae, Proteus
    - Does not detect UTI's caused by gram-positive organisms such as Enterococcus that do not produce nitrite
- **Presence of BOTH leukocyte esterase and nitrites on urine dipstick highly suggestive of UTI**
- Absence of BOTH has high negative predictive value for UTI

Bilirubin

- Bilirubin is formed from the breakdown of hemoglobin in the spleen; bound to albumin transported to liver via blood (unconjugated, insoluble can’t be filtered by kidney)
- Bilirubin then is conjugated in liver and excreted via bile duct in duodendum
- Very small amount of conjugated bilirubin may be reabsorbed and filtered by the kidney whenever plasma levels are high.

Bilirubin

- Conjugated bilirubin not usually present in the urine. Thus, its presence suggests:
  - **Severe liver disease**
  - **Obstructive jaundice**

- False positive: chlorpromazine
- False negative: ascorbic acid

Urobilinogen

- Produced in the gut from metabolism of bilirubin into urobilinogen
- Most is excreted in the gut but a small amount is reabsorbed into the blood and excreted in the urine
- **Positive** in the urine and gut: **hemolytic anemia**
- **Negative** in the urine and gut: **biliary obstruction** (because bilirubin does not reach the bowel to be metabolized into urobilinogen)

Urine Bilirubin and Urobilinogen

Condition	Urine Urobilinogen	Urine Bilirubin	Fecal Urobilinogen
normal	Normal 1-4 mg/ 24 hour	-	+
hepatitis	- Normal +	+	+/-
obstruction	-	+	-
hemolysis	++	-	++

Urine bilirubin in liver failure

- If the liver is failing, why would there be conjugated bilirubin at all? Why wouldn't there be a lot of unconjugated bilirubin in the blood that can't be excreted by the kidneys?

- It turns out that the hepatocytes need to both conjugate and excrete bilirubin. Conjugation happens slowly but is easy for the liver to do because the unconjugated bilirubin enters the hepatocyte via passive transport. Excretion requires active transport. When the cells get damaged, they are unable to excrete the conjugated bilirubin. The concentration builds up and the conjugated bilirubin leaks back across the basolateral membrane of the hepatocyte.
- Thus, urine bilirubin is a sensitive way to detect milder liver damage, because the levels or urine bilirubin will be high while serum bilirubin levels are still normal.

What will the urine bilirubin and urobilinogen and fecal urobilinogen look like in each of these patients?

- Has a gallstone obstructing the common bile duct
- Has liver cirrhosis
- Has hemolytic anemia after a diarrheal illness

	BILIRUBIN	UROBILINOGEN	
	URINE	URINE	STOOL
A	-	+	+
B	+	-	-
C	+	+/-	+/-

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Because no bilirubin gets into the duodenum due to the blockage, no urobilinogen is formed. The conjugated bilirubin backs up into the blood. Conjugated bilirubin is soluble and can be filtered by the kidney, so some of it comes out in the urine.

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Injury to the liver parenchyma leads to buildup of conjugated bilirubin. Some of it is excreted in the urine. Some of it goes into the duodenum via the common bile duct, is converted to urobilinogen and a small amount reabsorbed and excreted in the kidney.

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A	-	+	+
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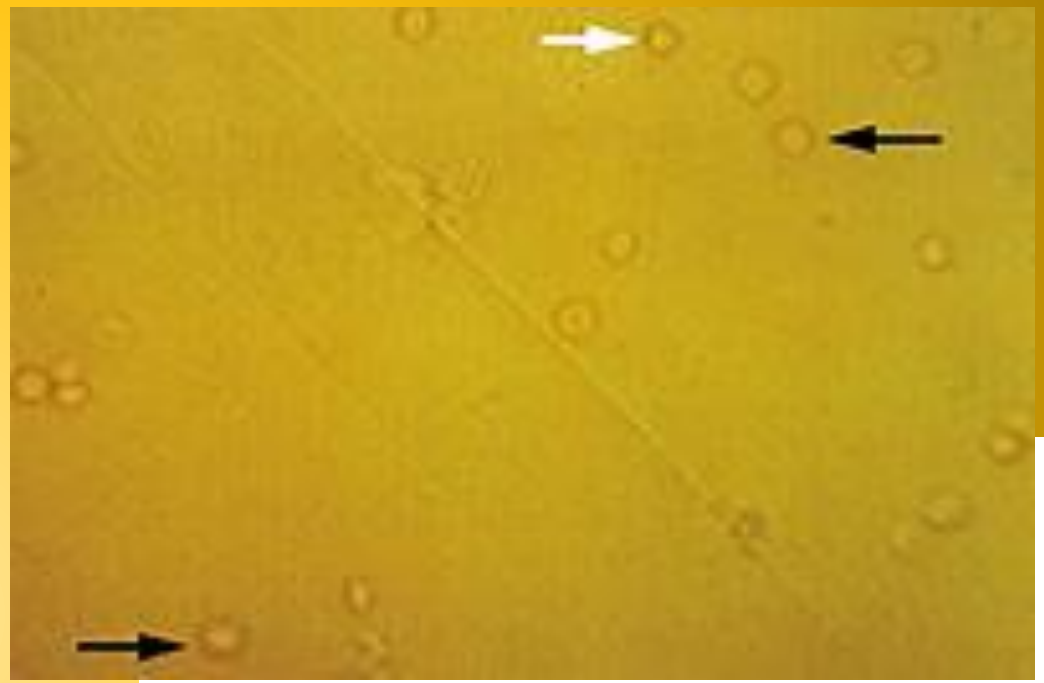
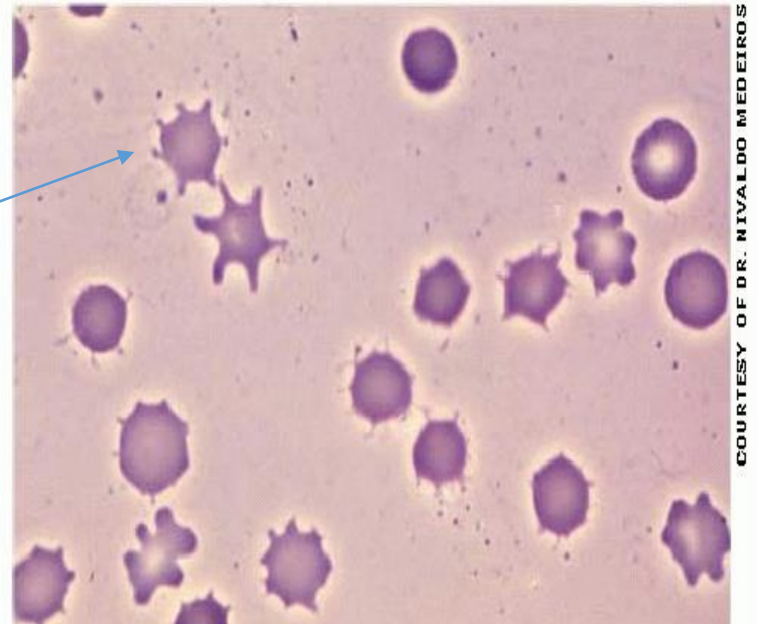
The bilirubin formed from hemolysis is unconjugated. Unconjugated bilirubin is insoluble and can't be filtered by kidney. Therefore, no bilirubin gets into the urine from this patient. This bilirubin then is conjugated in liver and excreted via bile duct in to the duodendum. The bilirubin is metabolized into urobilinogen and a small amount is reabsorbed into the blood and excreted in the urine.

Urine Microscopy

Erythrocytes

- Causes:
  - Glomerular injury
  - Genitourinary tract bleeding
- Isomorphic– urologic process
  - Stone
  - Tumor
  - Infection
- Dysmorphic – glomerular process
  - Acanthocytes – have vesicle-shaped protrusions – highly specific for glomerulonephritis

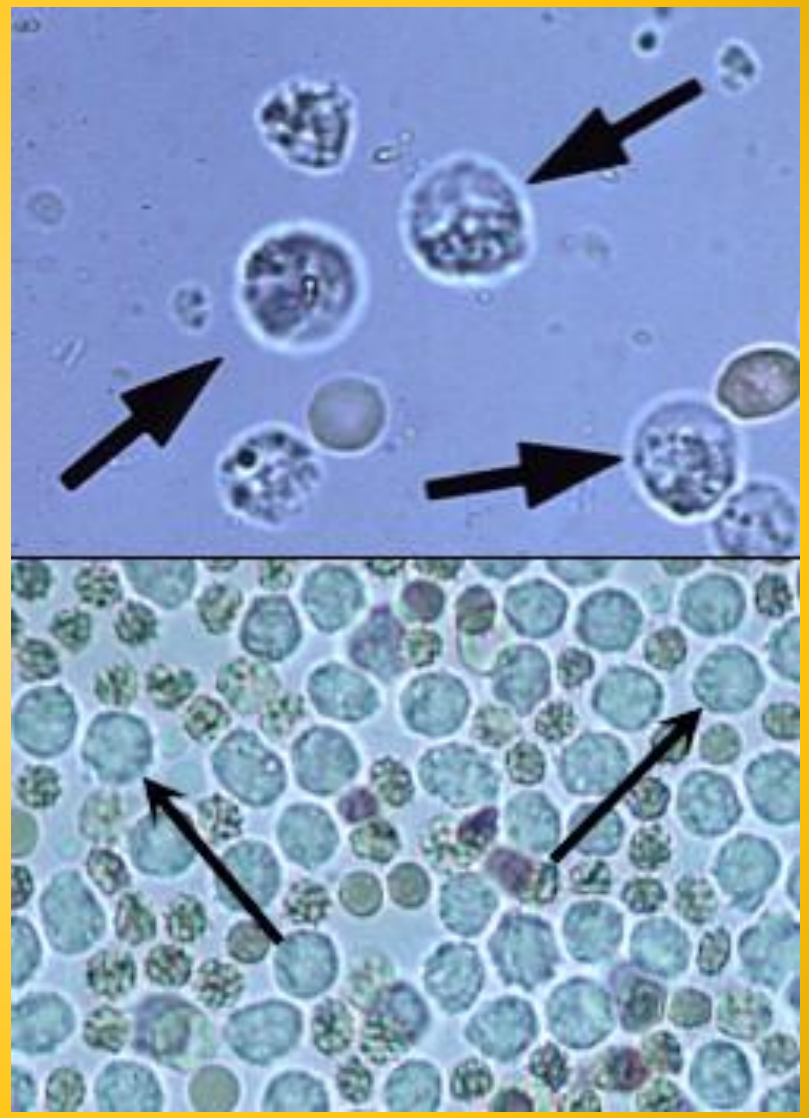




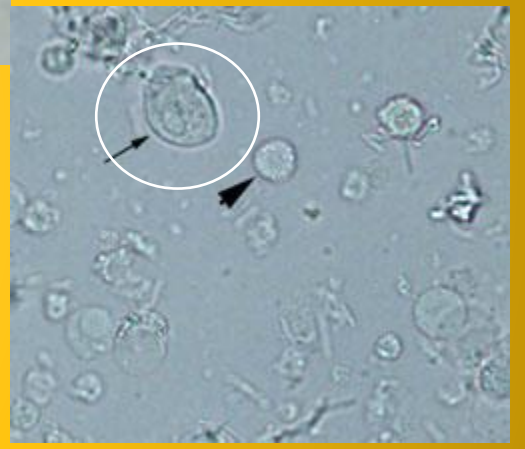
Leukocytes

- Pyuria: >4 leukocytes/hpf
- Sterile pyuria: leukocytes in the urine with negative urine bacterial culture
  - Acute Interstitial Nephritis
    - Often with low-grade proteinuria
    - Causes
      - Drugs: antibiotics, NSAIDs, proton pump inhibitors
      - Kidney transplant rejection (lymphocytes)
      - Kidney stones
  - Mycobacterium tuberculosis (infectious cause of sterile pyuria)

Leukocytes



Epithelial Cells

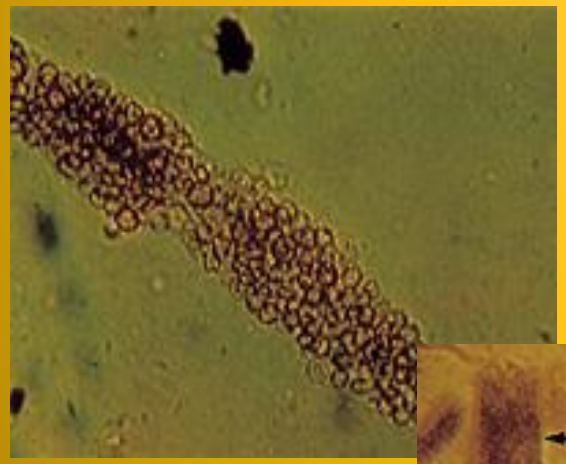


- Renal tubular epithelial cells
  - Large central nuclei
  - 1.5-3 x larger than leukocytes
  - Seen along with pigmented casts in Acute Tubular Necrosis
- Transitional epithelial cells
  - Larger than tubular epithelial cells
  - Originate anywhere from renal pelvis to proximal urethra
- Squamous epithelial cells
  - Large and irregular with small central nuclei
  - Originate from distal urethra or external genitalia
  - Suggests urine contamination

CASE 5

A 14 year old boy comes to your office for evaluation of periorbital edema and leg swelling. Creatinine is 0.5. Urinalysis has 4+ protein and no blood. The urine is foamy. On microscopy, which casts are you most likely to see?

A



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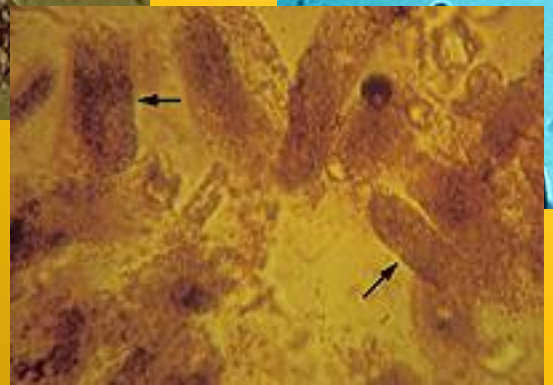

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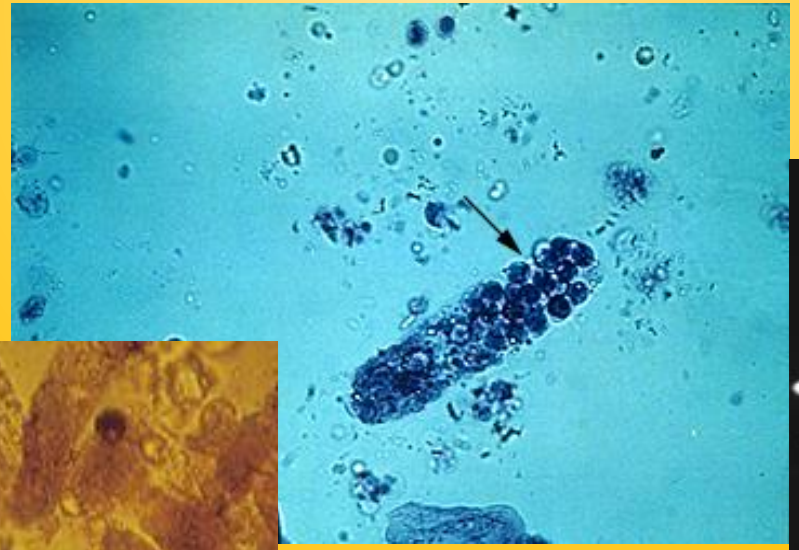

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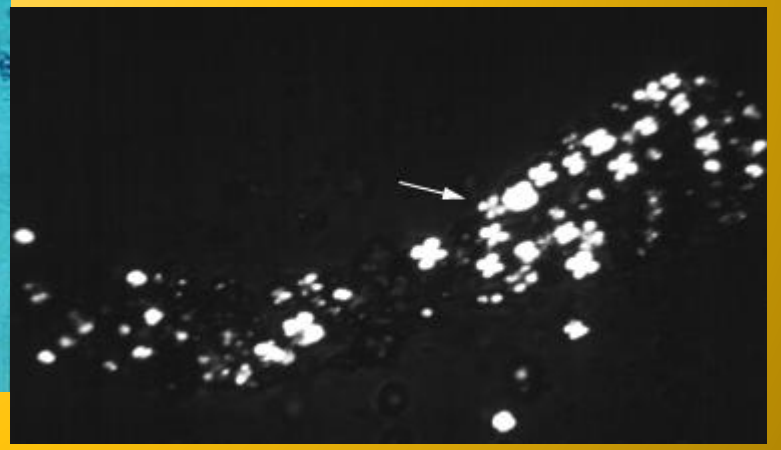

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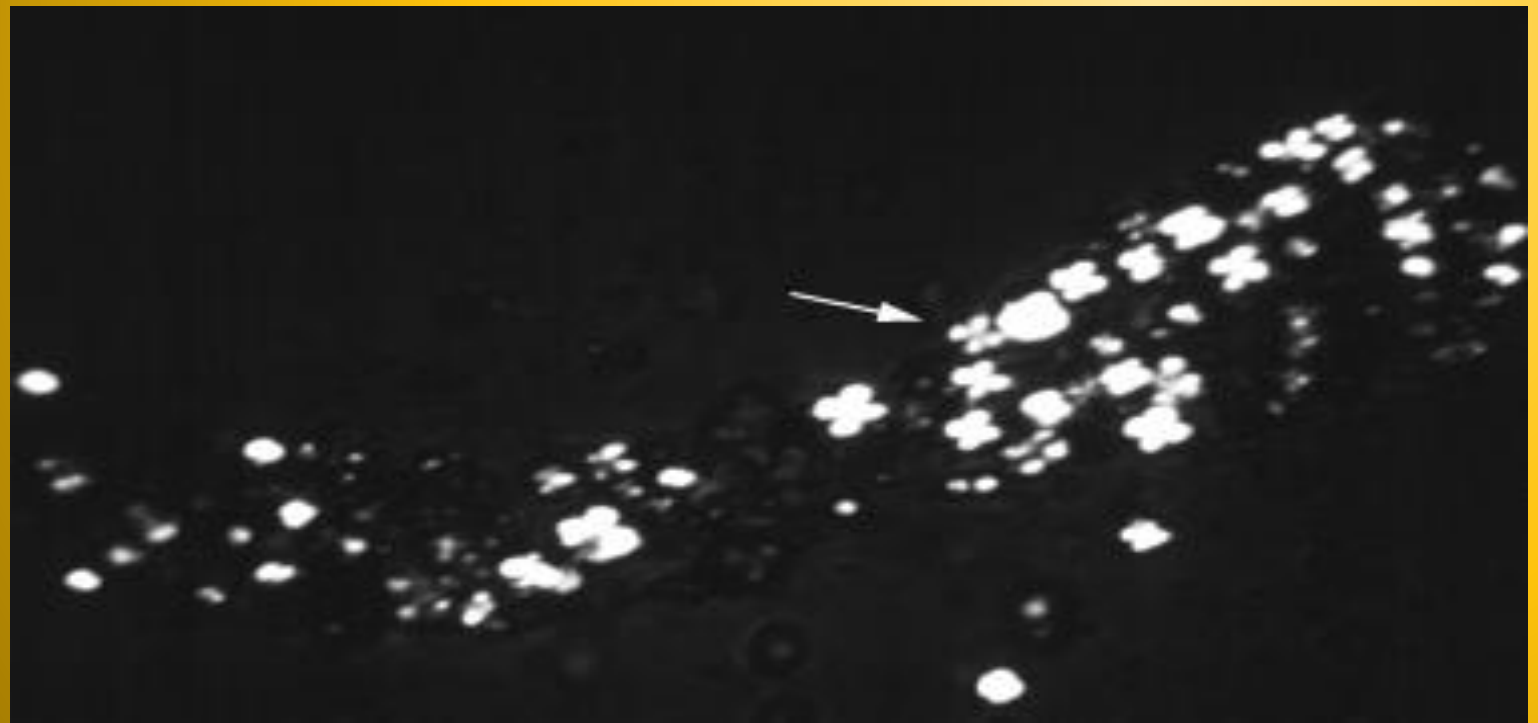


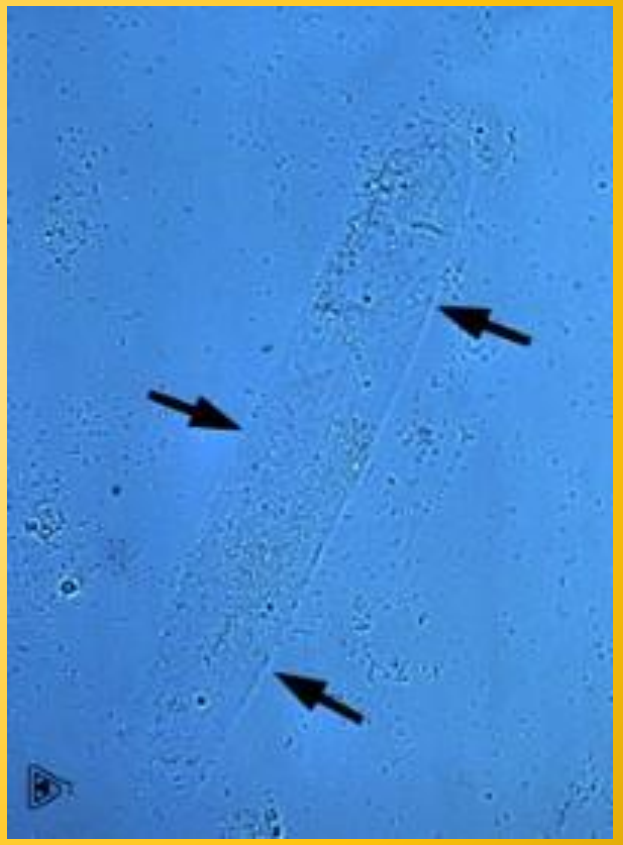
This patient has nephrotic syndrome with high grade proteinuria from Minimal Change Disease. Nephrotic syndrome causes hyperlipidemia and lipiduria. Fatty casts formed by the breakdown of lipid-rich epithelial cells are seen with nephrotic syndrome. Fatty casts have a Maltese Cross appearance under polarized light.

Casts

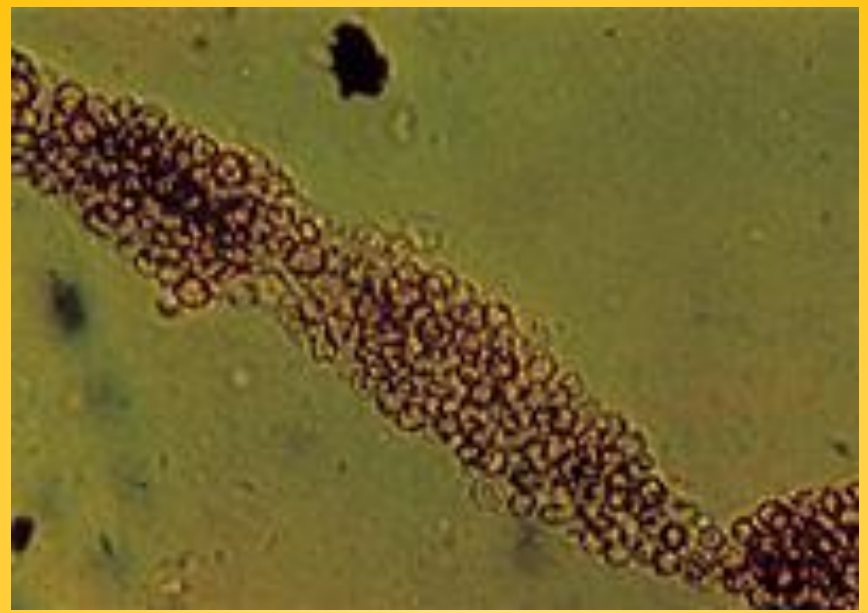
- Casts containTamm-Horsfall mucoprotein, cells or cellular debris, or liprotein droplets
- Cylindrical because formed in the tubular lumen
- Types:
  - Hyaline casts – do not indicate disease (seen with diuretic therapy of concentrated urine)
  - Pigmented granular casts (muddy brown casts) – Acute Tubular Necrosis
  - Erythrocyte casts – Glomerulonephritis
    - Specific but not sensitive for GN
  - Leukocyte casts – Tubulointerstitial inflammation
    - Pyelonephritis
  - Fatty casts – Nephrotic range proteinuria
    - Maltese Cross appearance of cholesterol

Hyaline casts

- Not indicative of disease
- Seen with diuretic therapy or with small volumes of concentrated urine

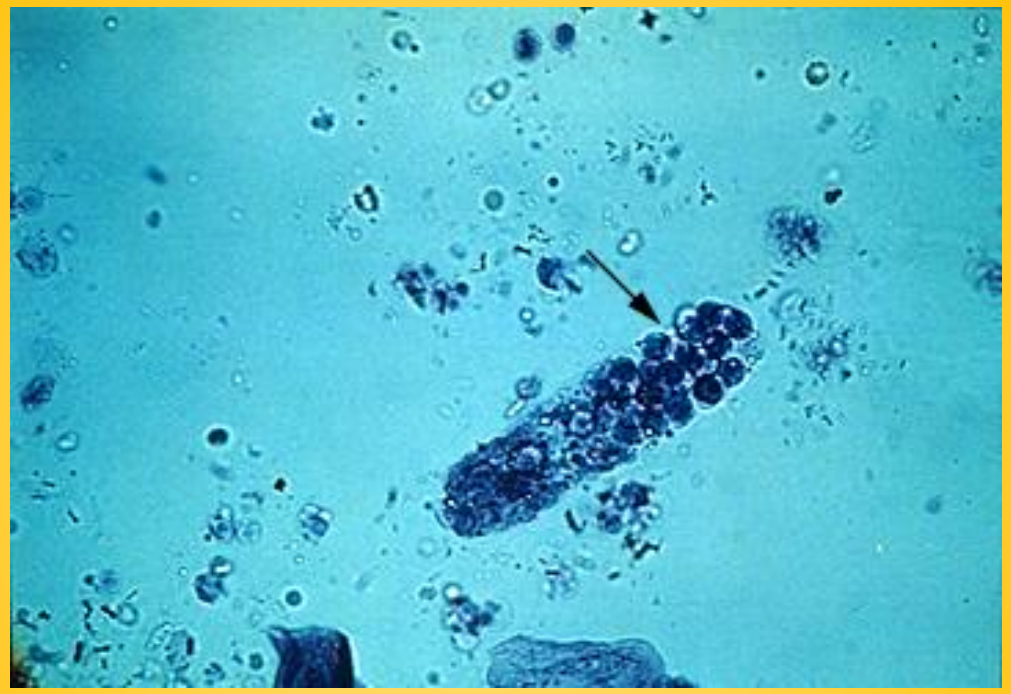


Red Blood Cell Casts



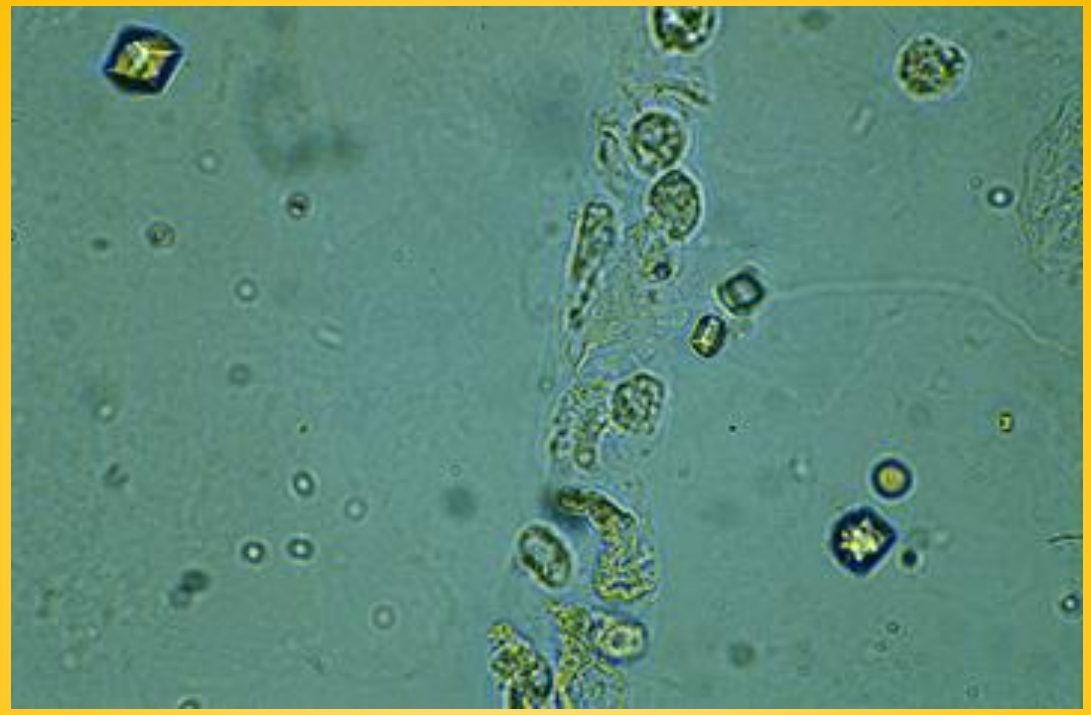
- Diagnostic of glomerulonephritis or vasculitis

White Blood Cell Casts



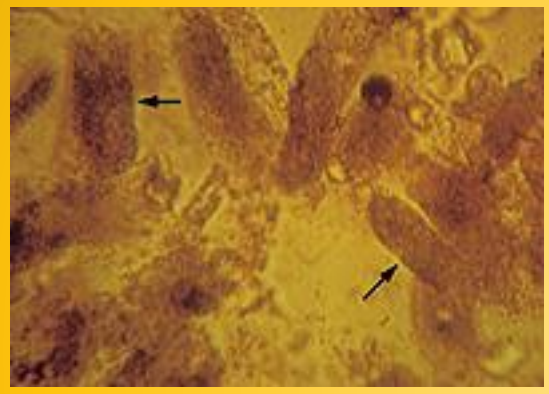
- Seen with tubulointerstitial disease or acute pyelonephritis

Epithelial Cell Casts



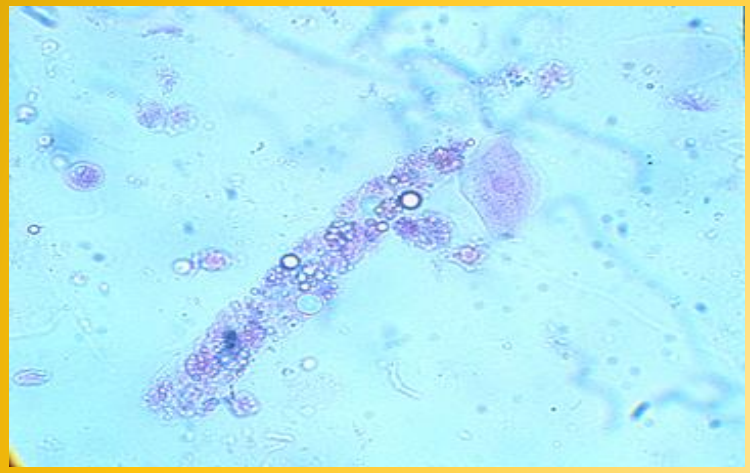
- Seen with acute tubular necrosis and acute glomerulonephritis

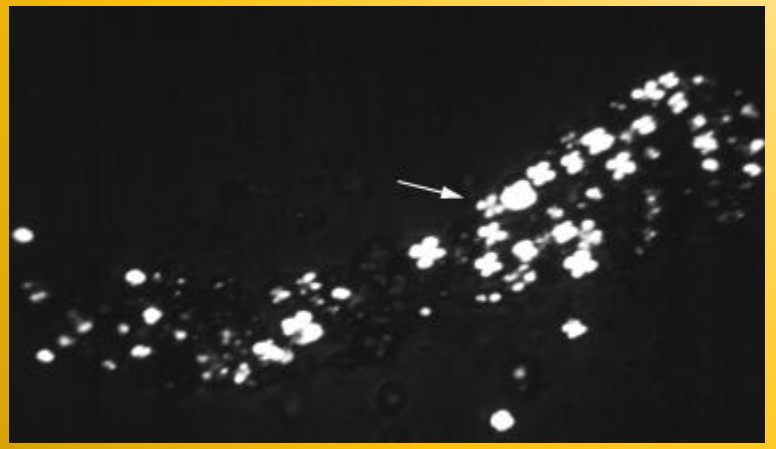
Granular casts



- Represents degenerating cellular casts

Fatty Casts: seen with nephrotic syndrome





- Nephrotic syndrome causes hyperlipidemia and lipiduria
- Fatty casts are formed by the breakdown of lipid-rich epithelial cells

- Fatty casts have a Maltese Cross appearance under polarized light.

Eosinophils

- Wright or Hansel stains
- Causes:
  - UTI
  - Allergy
  - Atheroembolic disease
  - Small vessel vasculitis
  - Rapidly progressive glomerulonephritis
  - Parasitic infections – urinary schistosomiasis
- Poor sensitivity and specificity limit its utility

Review: what will the urine bilirubin and urobilinogen and fecal urobilinogen look like in each of these patients?

- Has a gallstone obstructing the common bile duct
- Has liver cirrhosis
- Has hemolytic anemia after a diarrheal illness

	BILIRUBIN	UROBILINOGEN	
	URINE	URINE	STOOL
A	-	+	+
B	+	-	-
C	+	+/-	+/-

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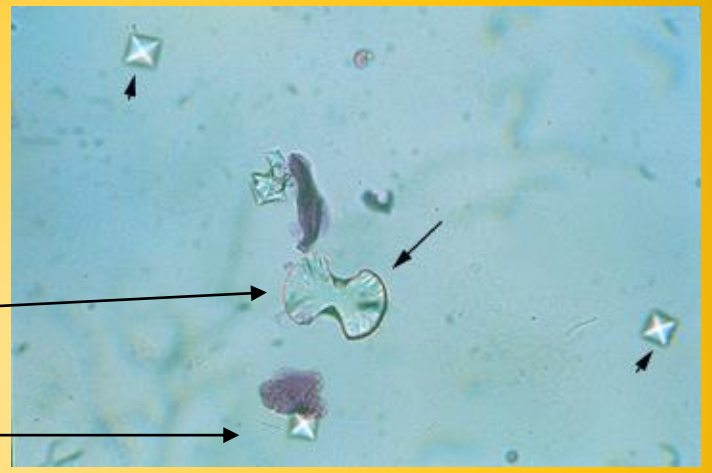
	BILIRUBIN	UROBILINOGEN	
	URINE	URINE	STOOL
A	-	+	+
B	+	-	-
C	+	+/-	+/-

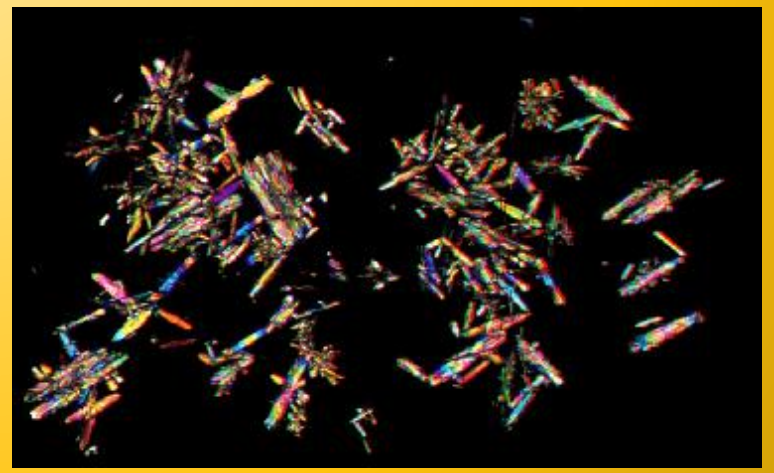
Crystals

Type	Morphology	Comment
Calcium Oxalate	Envelope Dumbell	Not pH dependent Ethylene glycol ingestion
Uric Acid	Amorphous Rosettes Needles	Acid urine, pH <5.8 Polarize light
Calcium Phosphate	Amorphous	Alkaline urine Resemble amorphous uric acid
Triple phosphate (Magnesium ammonium phosphate)	Coffin lid	Alkaline urine Struvite stones
Cystine	Hexagonal plates	Acidic urine Cystinuria
Indinavir	Needle	

Calcium Oxalate Crystals

- More likely to be formed in acidic urine (pH 4.5-5.5)
- Seen with ethylene glycol ingestion
- Monohydrate-dumbell
- Dihydrate-letter envelope
- Polarized monohydrate





Uric Acid Crystals

- Are seen in an acidic urine pH 5.4-5.8
- Polarize light

Calcium Phosphate Crystals

- Form in alkaline urine
- Polarize light

Calcium Phosphate Crystals-Amorphous

- Only form in a relatively alkaline urine (6.2-7.0)
- Do not polarize light
- Resemble amorphous uric acids but don’t polarize light

Triple Phosphate

Magnesium ammonium phosphate

- **Crystals of triple phosphate**
  - colorless, “coffin-lid” prism
  - Only form in alkaline urine (pH >7.0)
  - Component of struvite stones

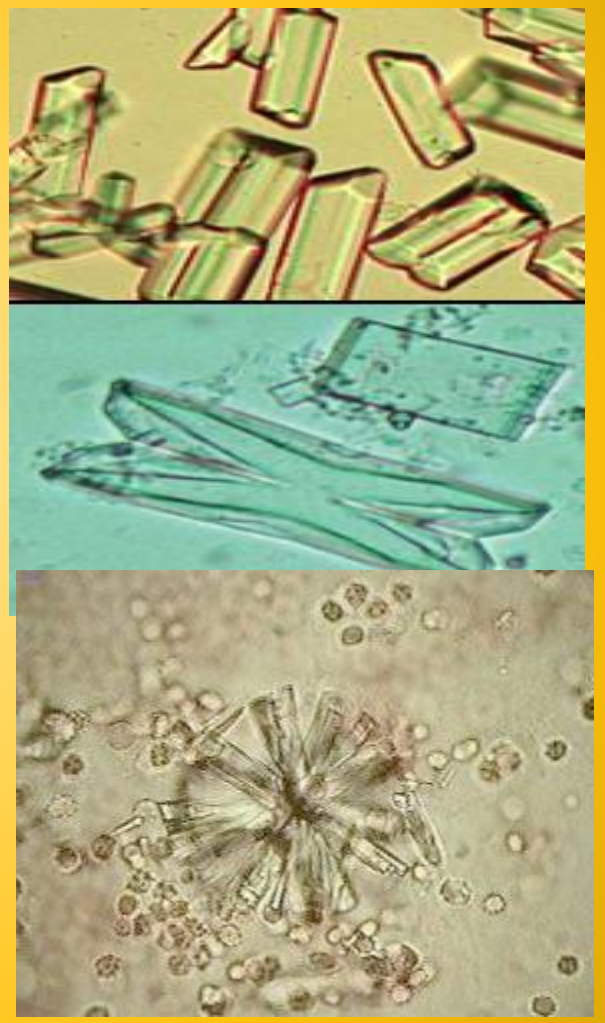
Struvite

- Magnesium ammonium phosphate crystals — (struvite)

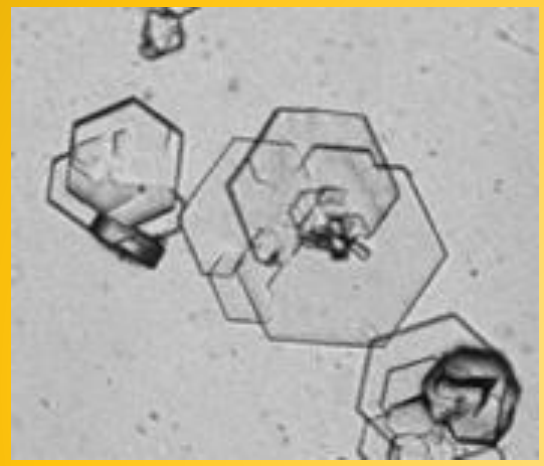
- Normal urine
  - undersaturated with ammonium phosphate
- Stone formation – 2 requirements

  1. struvite stone formation occurs only when ammonia production is increased

  2. the urine pH is elevated to decrease the solubility of phosphate.
  - Both of these requirements may be met when urinary tract infection occurs with a urease-producing organism, such as Proteus or Klebsiella.

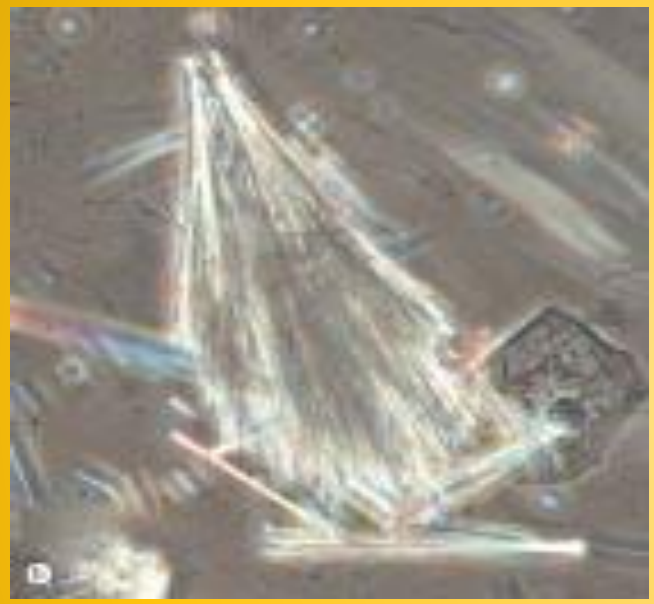


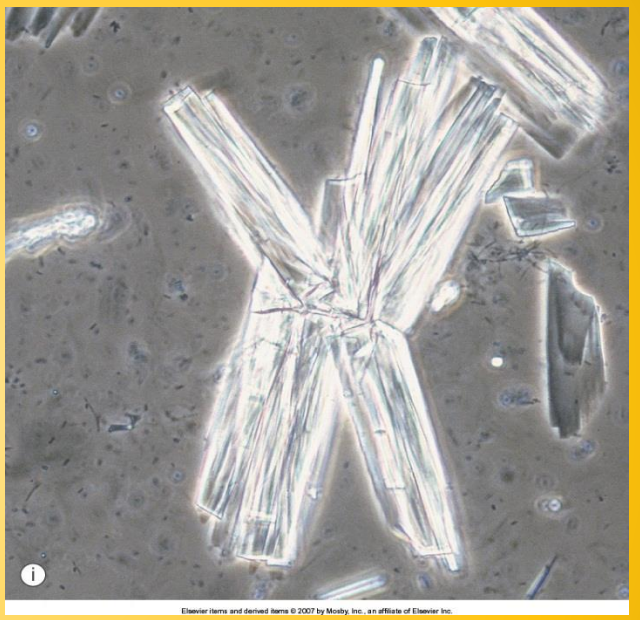
The Cystine crystals



- Are diagnostic of Cystinuria
- Precipitate in acidic urine (pH <6.0)

Crystals Due to Drugs





- Amoxicillin Crystal

Indinavir Crystal