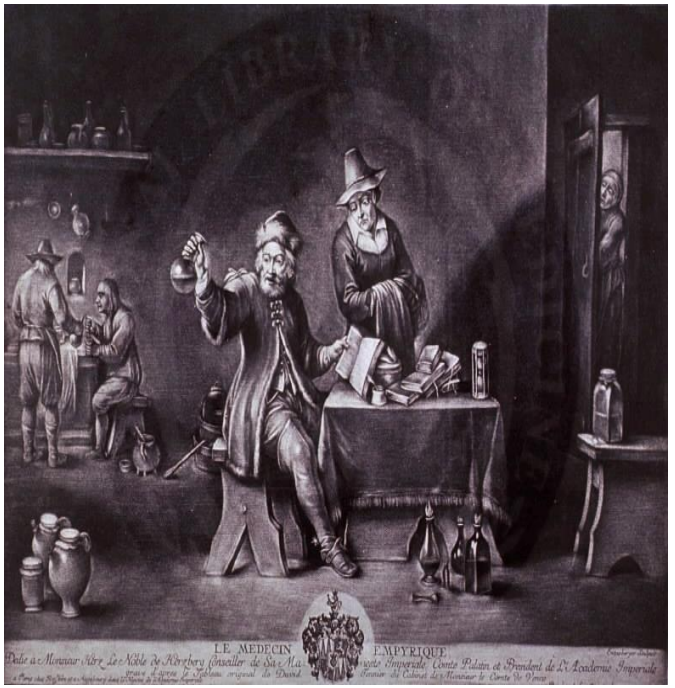
URINE ANALYSIS

What is urine analysis?

- Urine analysis, also called Urinalysis – one of the oldest laboratory procedures in the practice of medicine.
- Also knows as Urine-R&M (routine & microscopy)
- Is an array of tests performed on urine, and one of the most common methods of medical diagnosis.



Courtesy of the National Library of Medicine

Why urinalysis?

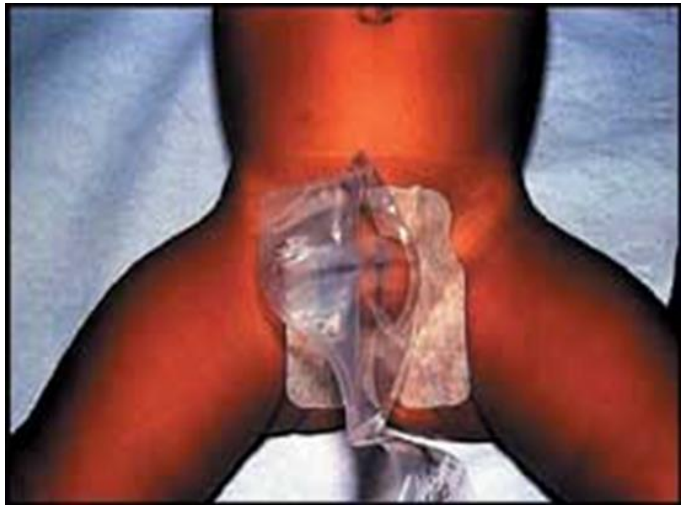
- General evaluation of health
- Diagnosis of disease or disorders of the kidneys or urinary tract
- Diagnosis of other systemic disease that affect kidney function
- Monitoring of patients with diabetes
- Screening for drug abuse (eg. Sulfonamide or aminoglycosides)

Collection of urine specimens

- Improper collection---- may invalidate the results
- Containers for collection of urine should be wide mouthed, clean and dry.
- Analysed within 2 hours of collection else requires refrigeration.

Types of urine sample

Sample type	Sampling	Purpose
Random specimen	No specific time most common, taken anytime of day	Routine screening, chemical & FEME
Morning sample	First urine in the morning, most concentrated	Pregnancy test, microscopic test
Clean catch midstream	Discard first few ml, collect the rest	Culture
24 hours	All the urine passed during the day and night and next day Ist sample is collected.	used for quantitative and qualitative analysis of substances
Postprandial	2 hours after meal	Determine glucose in diabetic monitoring
Supra-pubic aspired	Needle aspiration	Obtaining sterile urine



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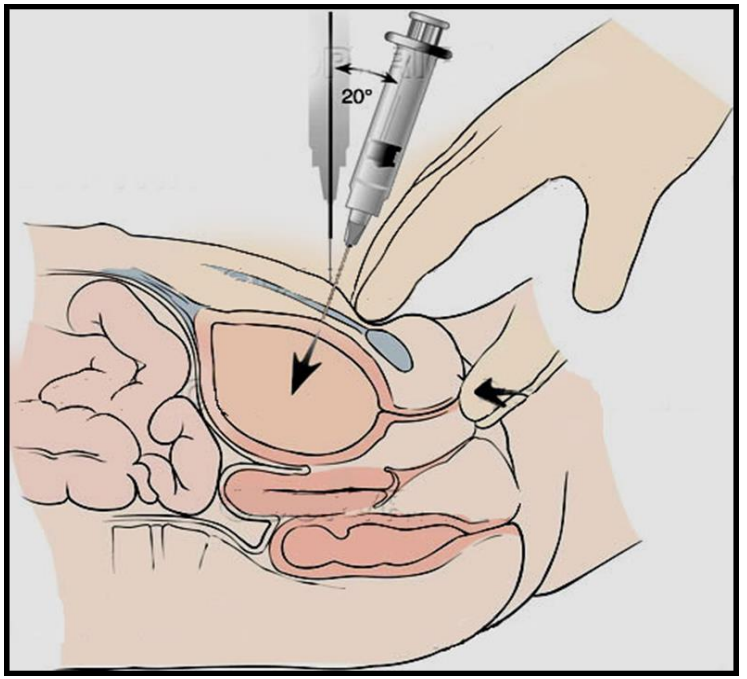

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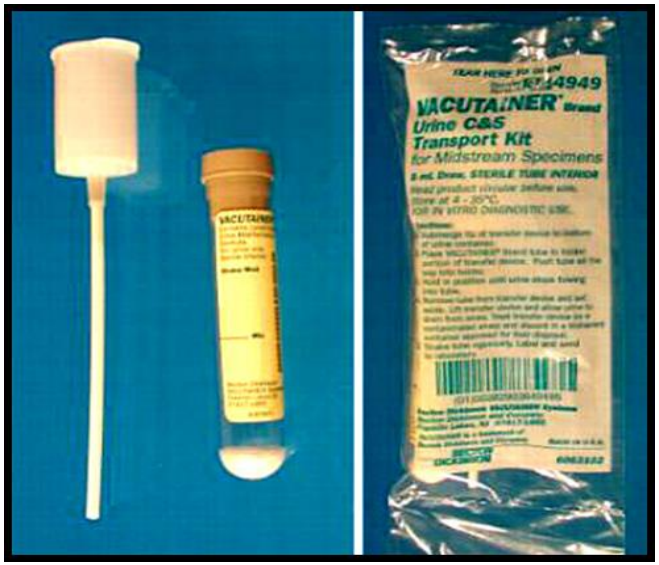
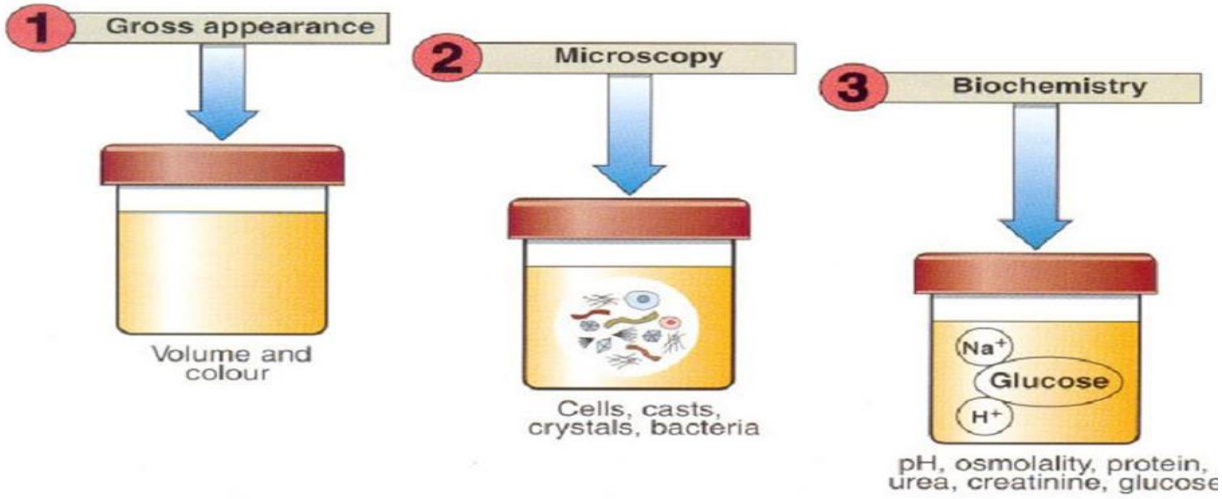


c

a: clean catch urine collection method
in children
b: Suprapubic aspiration of urine.
c: Urine storage and transportation kit

Urinalysis ;What to look for?

- Urinalysis consists of the following measurements:
  - Macroscopic or physical examination
  - Chemical examination
  - Microscopic examination of the sediment
  - Urine culture



Physical examination of urine

Examination of physical characteristics:

- Volume
- Color
- Odor
- pH and
- Specific gravity
  - The refractometer or a reagent strip is used to measure specific gravity

Physical examination continued…

Volume

- Normal- 1-2.5 L/day
- Oliguria- Urine Output < 400ml/day
  Seen in
  - Dehydration
  - Shock
  - Acute glomerulonephritis
  - Renal Failure
- Polyuria- Urine Output > 2.5 L/day
  Seen in
  - Increased water ingestion
  - Diabetes mellitus and insipidus.
- Anuria- Urine output < 100ml/day
  Seen in renal shut down

Color

- Normal- pale yellow in color due to pigments urochrome, urobilin and uroerythrin.
- Cloudiness may be caused by excessive cellular material or protein, crystallization or precipitation of non pathological salts upon standing at room temperature or in the refrigerator.
- Colour of urine depending upon it's constituents.
-

Physical examination continued

Color

- Abnormal colors:
  - Colorless – diabetes, diuretics.
  - Deep Yellow – concentrated urine,
    excess bile pigments, jaundice

Blue Green	Pink-Orange-Red	Red-brown-black
Methylene Blue	Haemoglobin	Haemoglobin
Pseudomonas	Myoglobin	Myoglobin
Riboflavin	Phenolpthalein	Red blood cells
	Porphyrins	Homogentisic Acid
	Rifampicin	L -DOPA
		Melanin
		Methyldopa

	Pathological	Non pathological
White	Chyle Pus	Phosphates
Yellow to Orange	Bilirubin Urobilin	Concentrated urine Carrots Senna Riboflavin Acriflavine sulfasalazine
Pink to Red	Haemoglobin Myoglobin Porphyrins Red blood cells	Beets(anthocynin) Aminopyrine Methyldopa Food color Bromosulfonphthalein Pyridium Senna

	Pathological	Nonpathological
Red to Brown to Purple	Porphobilinogen Uroporphyrin	
Brown to Black	Homogenistic acid Melanin Myoglobin Methaemoglobin Phenol Porphyrins	Chloroquine Iron compounds Levodopa Metronidazole Quinine
Blue to Green	Biliverdin Pseudomonas infection	Acriflavine Azure A Methylene blue Vit B Phenyl salicylate Amitryptiline

ODOUR

- Fruity/sweet odour- presence of ketones.
- Pungent smell- presence of bacteria/ specimen contaminated with bacteria.
- Sweaty feet- Isolvaleric acidemia
- Misty/mousy odour- Phenylketonuria.
- Maple syrup- Congenital metabolic disorder.
- Fishy odour/Rancid butter- Hypermethioninemia

pH

- Concentration ability of kidney to maintain normal hydrogen ion concentration
- Normal pH – 4.6 to 8.0
- Average- 6.0

- **PROCEDURE**
- Dip the litmus paper strips in the urine, remove and read the color change immediately.
- Blue litmus turns red – acid
- Red litmus turns blue – alkaline

- **Decrease in pH**

- High protein intake
- Ingestion of cranberries
- Respiratory acidosis
- Metabolic acidosis
- Uremia
- Severe diarrhoea
- Starvation
- UTI caused by E.coli

- **Increase in pH**

- Diet high in vegetables and citrus fruits
- Respiratory alkalosis
- Metabolic alkalosis
- Vomiting
- UTI caused by Proteus and Pseudomonas

pH

- Reflects ability of kidney to maintain normal hydrogen ion concentration in plasma & ECF
- Urine pH ranges from 4.5 to 8
- Normally it is slightly acidic lying between 6 – 6.5.
- Tested by:
  - litmus paper
  - pH paper
  - dipsticks
- Acidic Urine –Ketosis (diabetes, starvation, fever),systemic acidosis, UTI- E.coli, acidification therapy
- Alkaline Urine – after meal, systemic alkalosis, UTI – proteus, alkalization therapy

Specific gravity

- It is measurement of urine density which reflects the ability of the kidney to concentrate or dilute the urine relative to the plasma from which it is filtered.
- Measured by:
  - urinometer
  - refractometer
  - dipsticks

Specific gravity

- Normal :- 1.001- 1.040.

S.G	Osmolality (mosm/kg)
1.001	100
1.010	300
1.020	800
1.025	1000
1.030	1200
1.040	1400

- Increase in Specific Gravity - Low water intake, Diabetes mellitus, Albuminuruia, Acute nephritis.
- Decrease in Specific Gravity - Absence of ADH, Renal Tubular damage.
- Fixed specific gravity (isosthenuria)=1.010

Microscopic examination of urine

- A sample of well-mixed urine (usually 10-15 ml) is centrifuged in a test tube at relatively low speed (about 2000-3,000 rpm) for 5-10 minutes which produces a concentration of sediment (cellular matter) at the bottom of the tube.
- A drop of sediment is poured onto a glass slide, a thin slice of glass (a coverslip) is place over it ond observed under microscope

Microscopic examination of urine

- A variety of normal and abnormal cellular elements may be seen in urine sediment such as:
  - Red blood cells
  - White blood cells
  - Mucus
  - Various epithelial cells
  - Various crystals
  - Bacteria
  - Casts

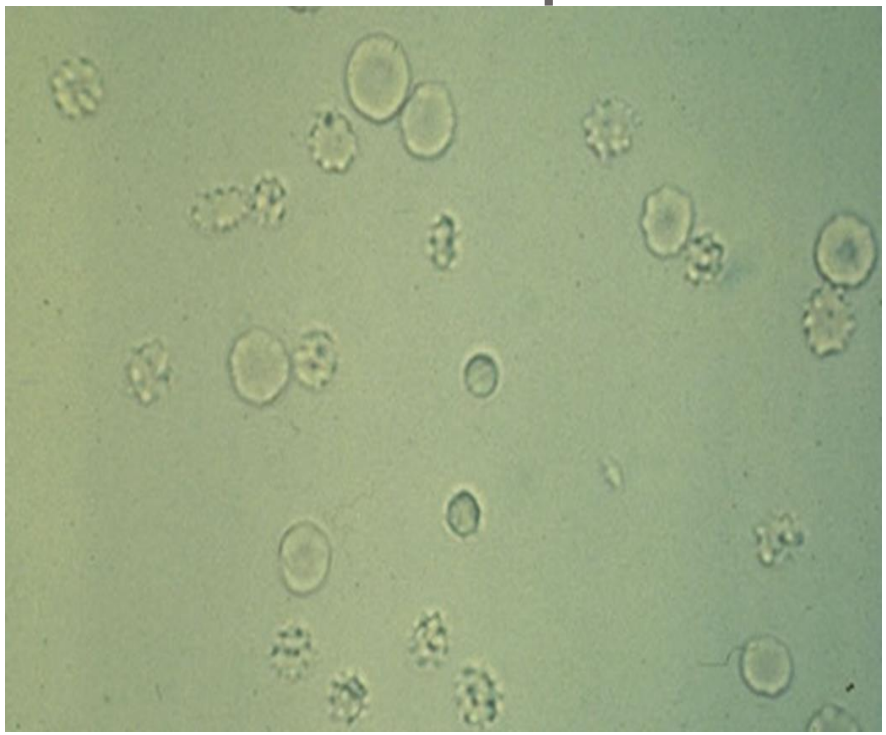
Abnormal findings

- Per High Power Field (HPF) (400x)
  - > 3 erythrocytes
  - > 5 leukocytes
  - > 2 renal tubular cells
  - > 10 bacteria
- Per Low Power Field (LPF) (200x)
  - > 3 hyaline casts or > 1 granular cast
  - > 10 squamous cells (indicative of contaminated specim
  - Any other cast (RBCs, WBCs)
- Presence of:
  - Fungal hyphae or yeast, parasite, viral inclusions
  - Pathological crystals (cystine, leucine, tyrosine)
  - Large number of uric acid or calcium oxalate crystals

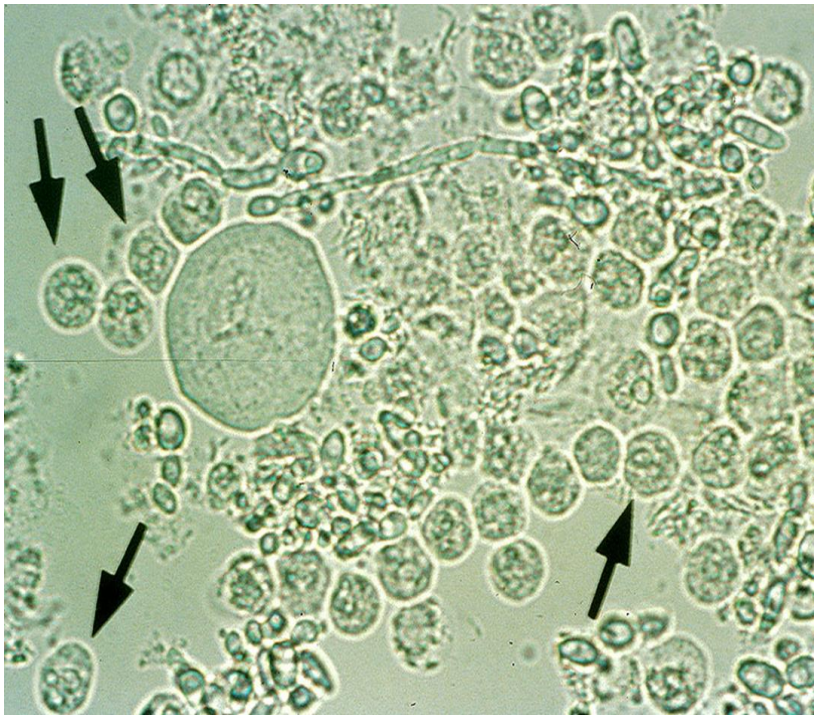
Microscopic examination of urine

- Hematuria is the presence of abnormal numbers of red cells in urine due to any of several possible causes.
  - glomerular damage,
  - tumors which erode the urinary tract anywhere along its length,
  - kidney trauma,
  - urinary tract stones,
  - acute tubular necrosis,
  - upper and lower urinary tract infections,
  - nephrotoxins
- WBC in high numbers indicate inflammation or infection somewhere along the urinary or genital tract

Microscopic examination of urine



Red blood cells in urine appear as refractile disks



White blood cells in urine

Microscopic examination of urine

Casts

- Urinary casts are cylindrical aggregations of particles that form in the distal nephron, dislodge, and pass into the urine. In urinalysis they indicate kidney disease.
- They form via precipitation of Tamm-Horsfall mucoprotein which is secreted by renal tubule cells.

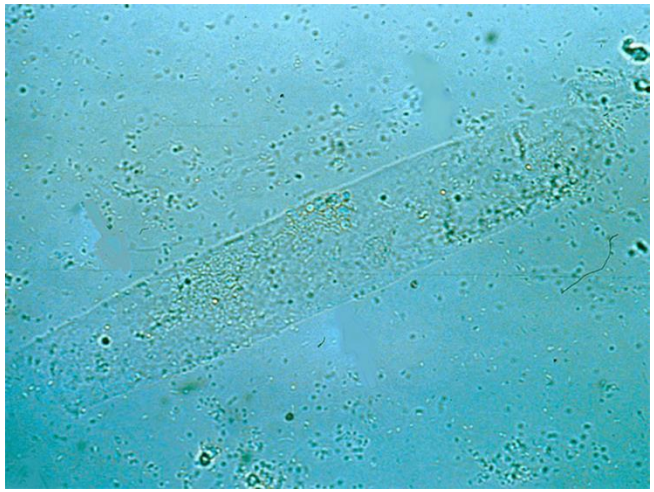
Microscopic examination of urine

Types of cast seen :

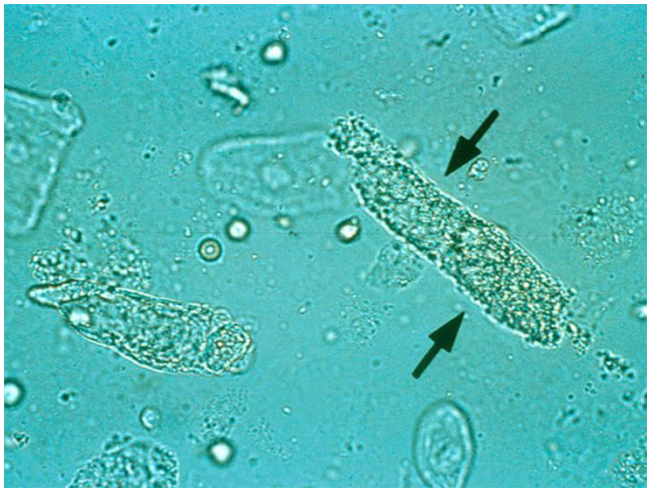
- Acellular cast: Hyaline casts, Granular casts, Waxy casts, Fatty casts, Pigment casts, Crystal casts.
- Cellular cast: Red cell casts, White cell casts, Epithelial cell cast

- The most common type of cast- hyaline casts are solidified Tamm-Horsfall mucoprotein secreted from the tubular epithelial cells and seen in fever, strenuous exercise, damage to the glomerular capillary.
- Red blood cells may stick together and form red blood cell casts. Such casts are indicative of glomerulonephritis, with leakage of RBC's from glomeruli, or severe tubular damage
- White blood cell casts are most typical for acute pyelonephritis, but they may also be present with glomerulonephritis. Their presence indicates inflammation of the kidney.

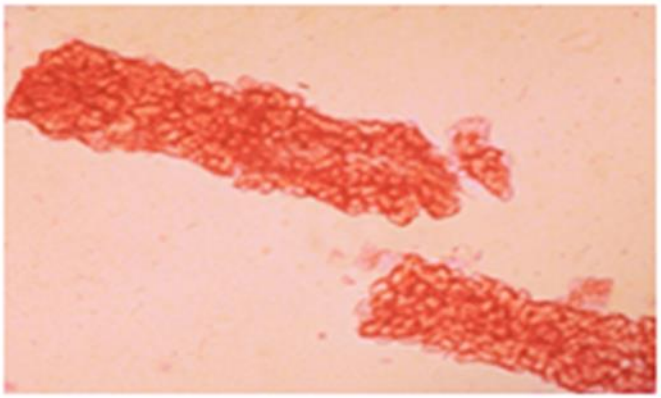
Microscopic examination of urine



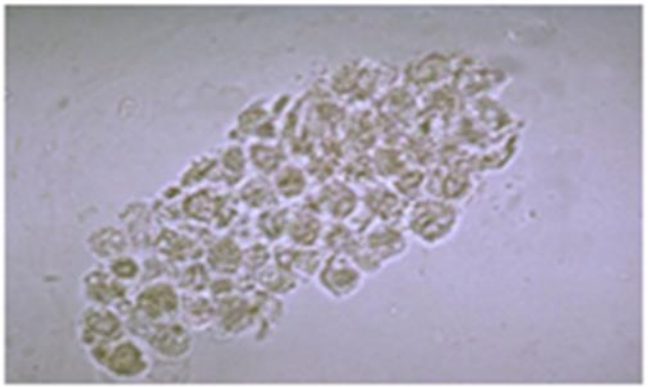
Hyaline Cast



Granular Cast

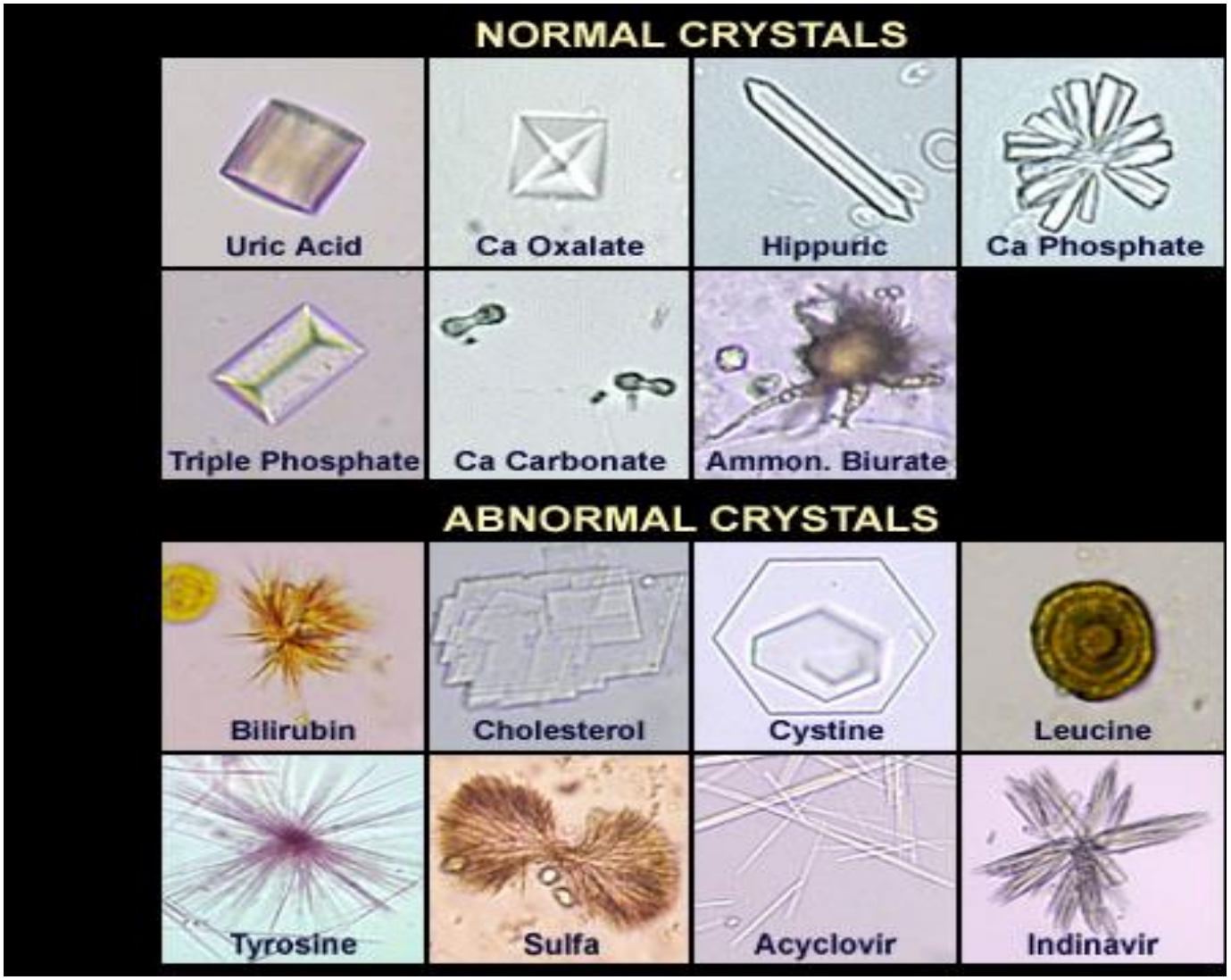


Red blood cell cast in urine



White blood cell cast in urine

Microscopic examination of urine



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

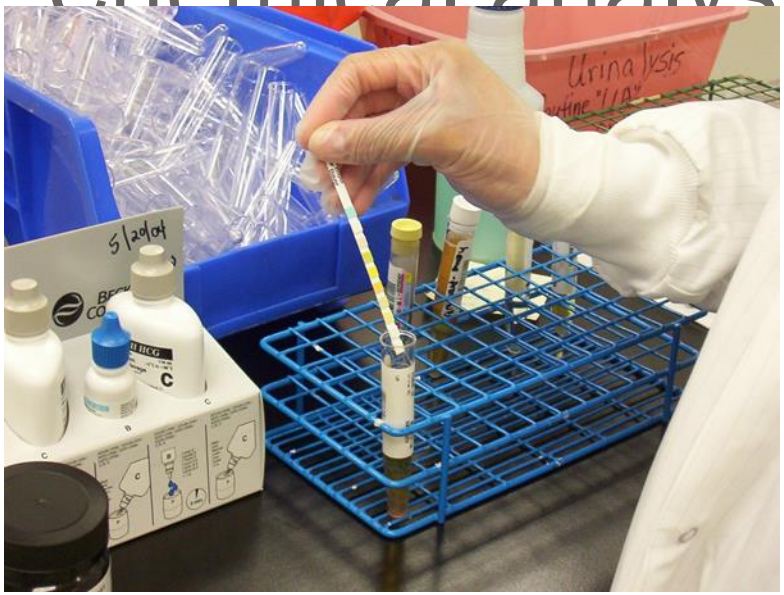
Chemical analysis of urine

- The chemical analysis of urine us undertaken to evaluate the levels of the following componen:
  - Protein
  - Glucose
  - Ketones
  - Occult blood
  - Bilirubin
  - Urobilinogen
  - Bile salts

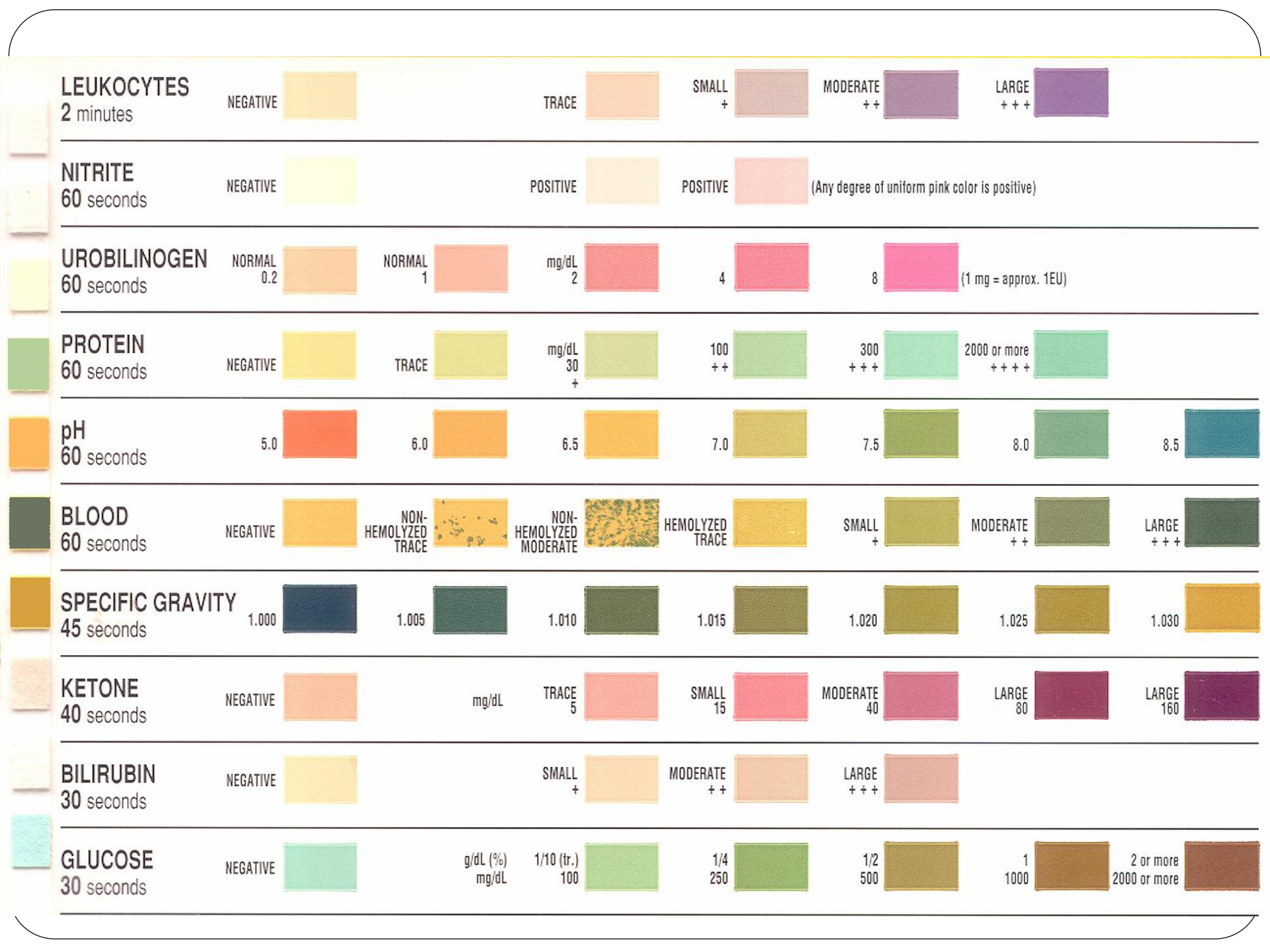
Chemical analysis of urine

- The presence of normal and abnormal chemical elements in the urine are detected using dry reagent strips called dipsticks.
- 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 elapse, this color change is compared against a reference color chart provided by the manufacturer of the strips.

Chemical analysis of urine



The dipstick method of chemical analysis of urine

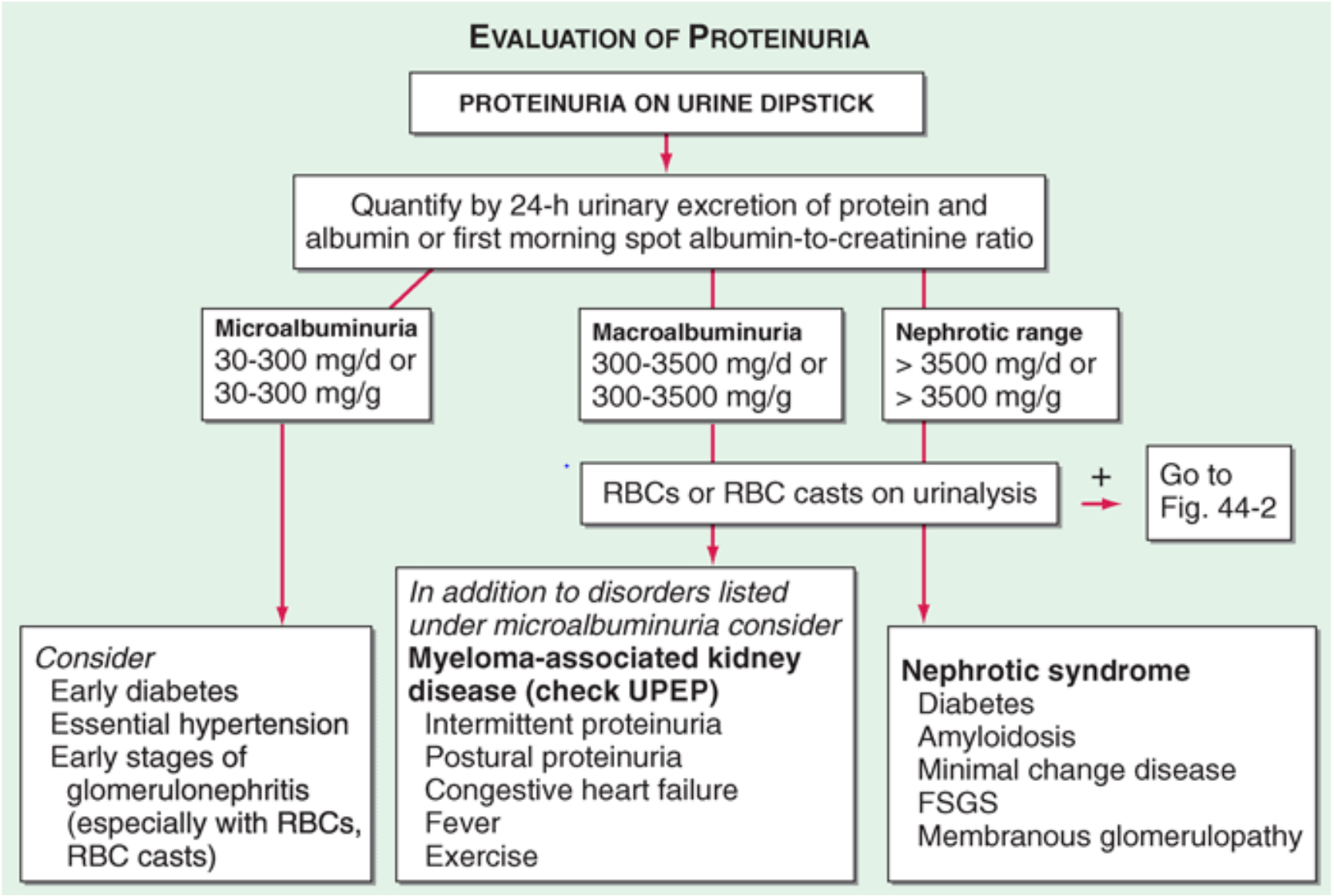

LEUKOCYTES
2 minutes
NEGATIVE
TRACE
SMALL +
MODERATE + +
LARGE + + +
NITRITE
60 seconds
NEGATIVE
POSITIVE
POSITIVE
(Any degree of uniform pink color is positive)
UROBILINOGEN
60 seconds
NORMAL 0.2
NORMAL 1
mg/dL 2
4
8
(1 mg = approx. 1EU)
PROTEIN
60 seconds
NEGATIVE
TRACE
mg/dL 30 +
100 + +
300 + + +
2000 or more + + + +
pH
60 seconds
5.0
6.0
6.5
7.0
7.5
8.0
8.5
BLOOD
60 seconds
NEGATIVE
NON-HEMOLYZED TRACE
NON-HEMOLYZED MODERATE
HEMOLYZED TRACE
SMALL +
MODERATE + +
LARGE + + +
SPECIFIC GRAVITY
45 seconds
1.000
1.005
1.010
1.015
1.020
1.025
1.030
KETONE
40 seconds
NEGATIVE
mg/dL
TRACE 5
SMALL 15
MODERATE 40
LARGE 80
LARGE 160
BILIRUBIN
30 seconds
NEGATIVE
SMALL +
MODERATE + +
LARGE + + +
GLUCOSE
30 seconds
NEGATIVE
g/dL (%) mg/dL
1/10 (tr.) 100
1/4 250
1/2 500
1 1000
2 or more 2000 or more

Chemical analysis of urine

Proteins in urine:

- Detected by heat coagulation or dipstick method
- Urine proteins come from plasma protein and Tomm-Horsfall (T-H) glycoprotein
- healthy individuals excrete <150 mg/d of total protein and <30 mg/d of albumin.
- Plasma cell dyscrasias (multiple myeloma) can be associated with large amounts of excreted light chains in the urine, which may not be detected by dipstick. The light chains produced from these disorders are filtered by the glomerulus and overwhelm the reabsorptive capacity of the proximal tubule and Bence Jones proteinuria occurs

Chemical analysis of urine



Proteins in urine

- Normal- upto 150 mg/24 hours or 10mg/100ml in single sample.
- **Methods-**
- **Heat and acetic acid test-** The test is based on the principle of heat coagulation and precipitation of proteins by acetic acid.
- **Sulphosalicylic acid test-** Sulphosalicylic acid neutralizes protein cation, resulting in precipitation of protein.

Causes of proteinuria

- **Pre-renal**
  - Addison's disease
  - Fever
  - Eclampsia
  - Hypertension
  - Haemoglobinuria
  - Rhabdomyolysis

Renal

- All cases of glomerulonephritis
- Nephrotic syndrome
- Pyelonephritis

- **Post renal**
  - Lesions of renal pelvis, urethra (cystitis, prostatitis)
  - Severe UTI

Cause of Proteinuria as Related to Quantity

DAILY PROTEIN EXCRETION	CAUSE
0.15 to 2.0 g	Mild glomerulopathies
	Tubular proteinuria
	Overflow proteinuria
2.0 to 4.0 g	Usually glomerular
> 4.0 g	Always glomerular

- **MINIMAL PROTEINURIA (<0.5 gm/day)**

- Exercise
- Fever
- Emotional stress
- HTN
- Renal tubular dysfunction
- Polycystic kidneys
- Lower UTI

- **MODERATE PROTEINURIA (0.5-3 gm/day)**

- Chronic glomerulonephritis
- CCF
- Pyelonephritis
- Pre-eclampsia
- Multiple myeloma

- **MARKED PROTEINURIA (> 3gm/day)**

- Acute glomerulonephritis
- Chronic glomerulonephritis, severe
- Nephrotic syndrome
- Diabetic nephropathy, severe
- Renal amyloidosis
- Lupus nephritis

Classification of Proteinuria

TYPE	PATHOPHYSIOLOGIC FEATURES	CAUSE
Glomerular	Increased glomerular capillary permeability to protein	Primary or secondary glomerulopathy
Tubular	Decreased tubular reabsorption of proteins in glomerular filtrate	Tubular or interstitial disease
Overflow	Increased production of low-molecular-weight proteins	

Selected Causes of Proteinuria by Type

Glomerular

Primary glomerulonephropathy

- Minimal change disease
- Idiopathic membranous glomerulonephritis
- Focal segmental glomerulonephritis
- Membranoproliferative glomerulonephritis
- IgA nephropathy

Secondary glomerulonephropathy

- ✓Diabetes mellitus
- ✓Collagen vascular disorders (e.g., lupus nephritis)
- ✓Amyloidosis
- ✓Preeclampsia
- ✓Infection (e.g., HIV, hepatitis B and C, poststreptococcal illness, syphilis, malaria and endocarditis)
- ✓Gastrointestinal and lung cancers
- ✓Lymphoma, chronic renal transplant rejection

Glomerulonephropathy associated with the following drugs:

- ➢Heroin
- ➢NSAIDs
- ➢Gold components
- ➢Penicillamine
- ➢Lithium
- ➢Heavy metals

Tubular

Hypertensive nephrosclerosis

Tubulointerstitial disease due to:

- ❑Uric acid nephropathy
- ❑Acute hypersensitivity interstitial nephritis
- ❑Fanconi syndrome
- ❑Heavy metals
- ❑Sickle cell disease
- ❑NSAIDs, antibiotics

Overflow

- ❑Hemoglobinuria
- ❑Myoglobinuria
- ❑Multiple myeloma
- ❑Amyloidosis

- **Selective proteinuria**

➢ When LMW proteins like albumin (MW- 66000) or transferrin (MW-76000) are selectively excreted through kidney.

➢ Eg- all causes of nephrotic syndrome.

- **Non-selective proteinuria**

➢ When HMW protein like globulin, fibrinogen in addition to LMW protein are excreted through kidney

Proteins in urine

- **Heat and Acetic Acid Method**
- **Procedure :**
- Take a long test tube and fill ¾ the tube with clear urine.
- Boil the upper portion over a flame, the lower portion serves as the control.
- If proteins, phosphates or carbonates are present in the urine a turbidity develops.
- Add 1-3 drops of 10% glacial acetic acid.
- Any turbidity due to phosphate precipitation will clear or if it is due to carbonates they disappear with effervescence.
- If it persists, it is due to albumin.

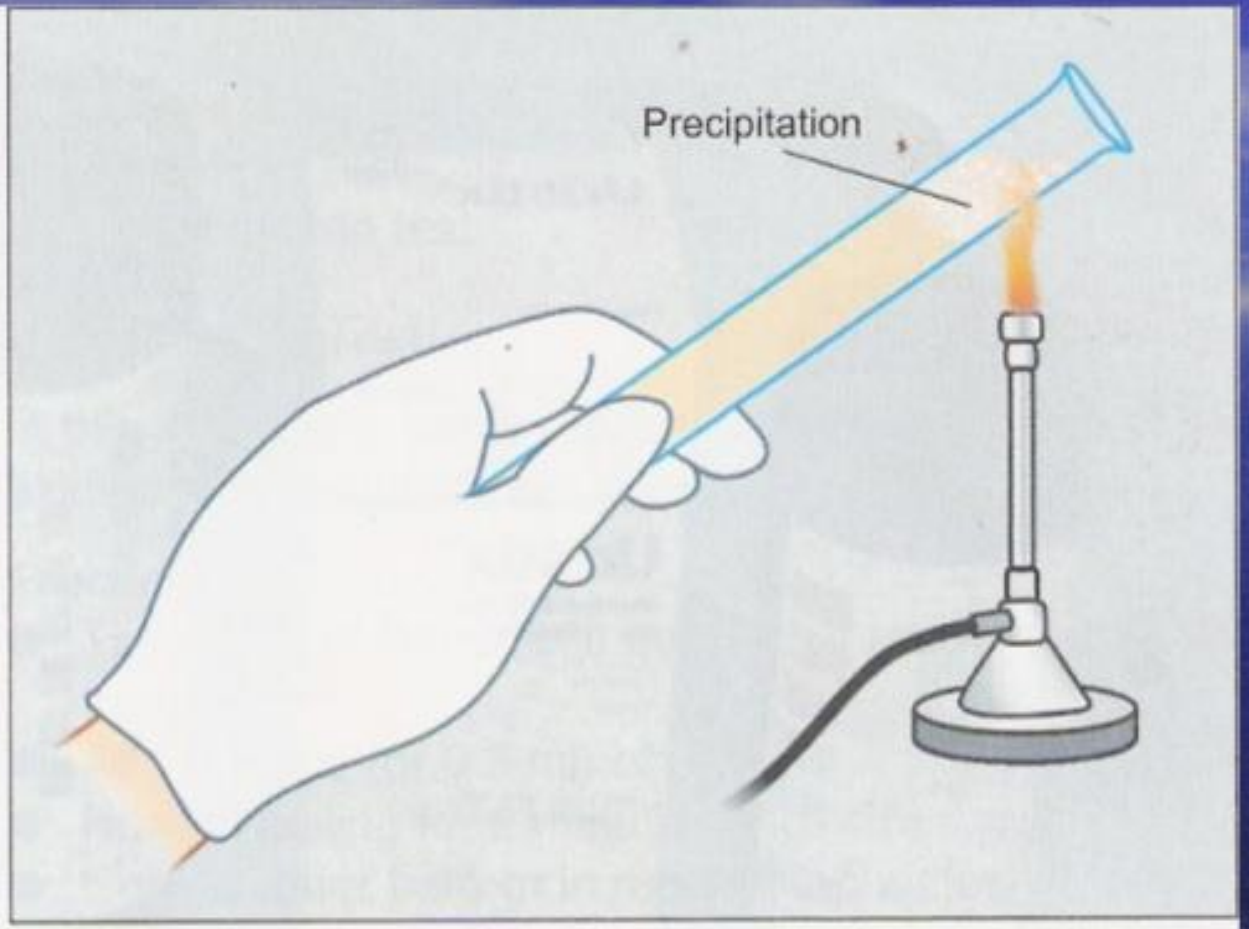
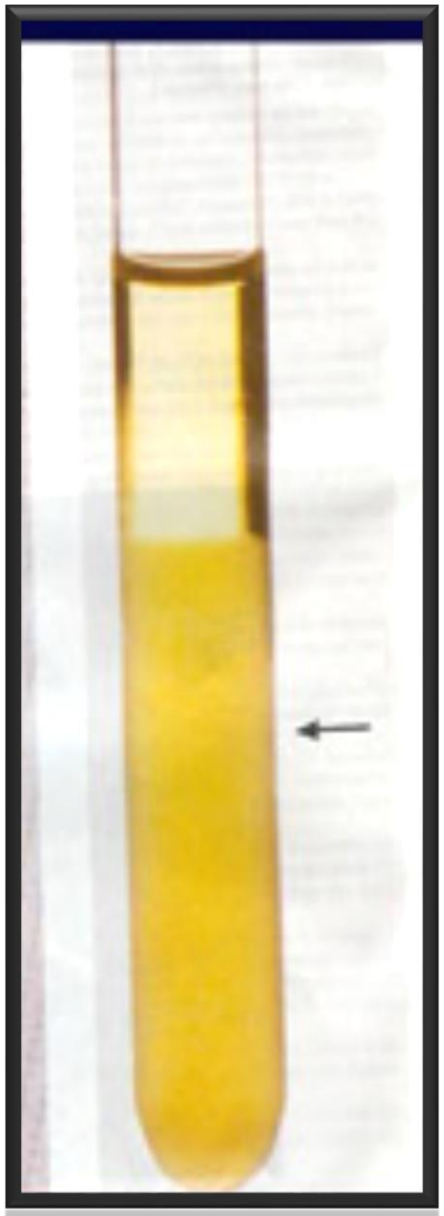


FIGURE 4.2: Heat and acetic acid test for proteinuria. Note the method of holding the tube from the bottom while heating the upper part.

Interpretation

- Negative – No turbidity or cloudiness.
- Trace – Cloudiness visible against a black background (~ 5 mg / dl).
- 1+ - Definite cloudiness without flocculation and granularity (~ 10 – 30 mg / dl).
- 2+ - Heavy and granular cloudiness without flocculation. (~ 40 – 100 mg / dl).
- 3+ - Dense opaque cloud with marked flocculation (~ 200 – 500 mg / dl) .
- 4+ - Thick cloudiness with precipitation (≥ 500 mg / dl).



Sulphosalicylic acid test

- If urine is alkaline, it should be acidified.

- **<u>Procedure:</u>**
- 2ml of acidic urine taken in test tube.
- Add an equal volume of 20% Sulphosalicylic acid.
- Mix thoroughly, allow it to stand for 10 minutes and estimate the amount of turbidity.
- Absence of cloudiness- Absence of protein.
- **If turbidity persists after boiling- Positive for protein.**

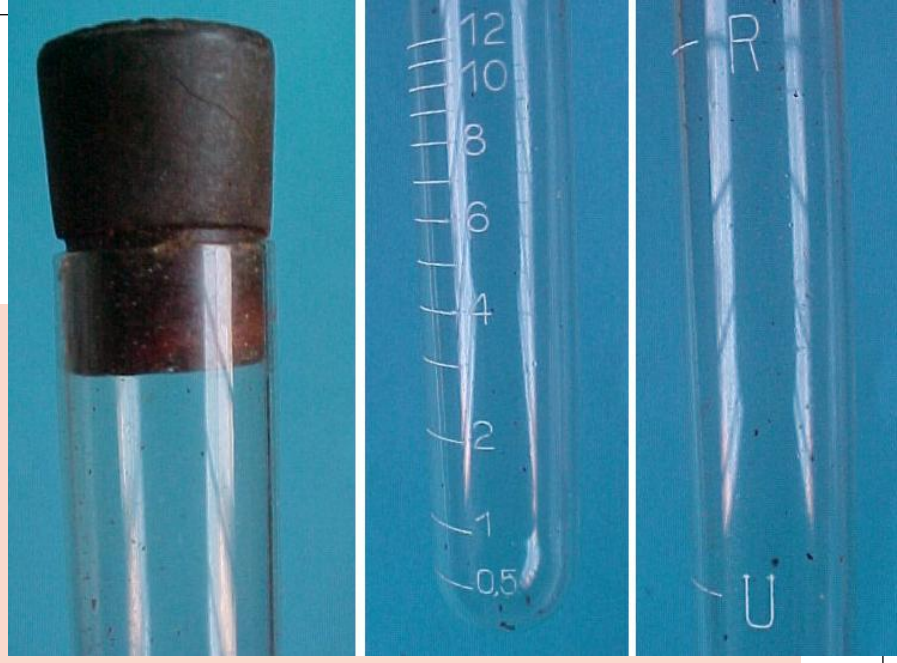
- Negative : No cloudiness
- Trace: Barely visible cloudiness.
- 1+ : definite cloud without granular flocculation
- 2+ : heavy and granular cloud without granular flocculation
- 3+ : dense cloud with marked flocculation.
- 4+ : Cloudiness with precipitation

Quantitative estimation of protein

- **Esbach's method using albuminometer**.

- **Reagents-**
  - Esbach's reagent
    - Picric acid
    - Citric acid
    - Water
  - Acetic acid
  - pH paper

- **Instrument**
- Esbach's albuminometer



- **Procedure-**
- Fill Esbach's albuminometer with acidic urine upto mark U and reagent is added upto mark R.
- Tube is shaken well by inversion.
- Stopper the tube.
- Keep in standing erect position for 18-24 hours for the precipitate to settle down.
- Reading of the length of ppt is taken indicated by markings present over the tube.
- Albumin is expressed in gm/L of urine.

- When test done on 24 hours urine sample, quantity of urine passed per day may be calculated by

- Dividing quantity of albumin per litre by total quantity of urine passed in 24 hours in litre.

Microalbuminuria

- Urinary albumin excretion between 30-300 mg/day.
- Cannot be detected by dipstick methods.
- Strong predictor of development of diabetic nephropathy.
- Can be detected 10-15 years before development of diabetic nephropathy.
- Significant risk marker of cardiovascular ds.
- Measured by nephelometry and radioimmunoassay

Diagnostic relevance microalbuminuria

- In diabetic patients for early diagnosis of nephropathy.
- In hypertensive patients as indicator of end organ damage

Bence Jones proteins

- BJ protein is abnormal LMW globulin consisting of light chains of Ig either Lambda or Kappa chains.

- Characteristic feature- PPT at 40^0 C to 60^0 C and redissolves at higher temperature (100^0c) & reappears when the urine is cooled.

- **<u>Conditions a/w BJ proteinuria:</u>**
- Multiple myeloma
- Plasmacytoma
- Waldesnstrom macroglobinaemia

Detection of Bence-Jones protein

- Take 5ml urine in a test tube.
- If the urine is cloudy, than filter it with filter paper.
- If the reaction is alkaline of urine than do it acidic by adding a few drops of 25% acetic acid.
- Than set the test tube in a water bath.
- Heat in water bath for 15 minutes.
- If the Bence -Jones Protein is present in urine then precipitate forms between temperature of 40°C -60°C.
- But when temperature is raised to 85^0 -100°C, precipitate disappears.
- When the temperature is decreased to 60°C, precipitate reappears.
- It again disappears when temperature goes below 40^0C.

SUGARS IN URINE

- This is a non-specific test useful for semiquantitation of marked glucosuria.

- **Benedict's qualitative test**

- Principle- Aldehyde group of reducing sugar reduces Cupric ions in Benedict's reagent to cuprous oxide.

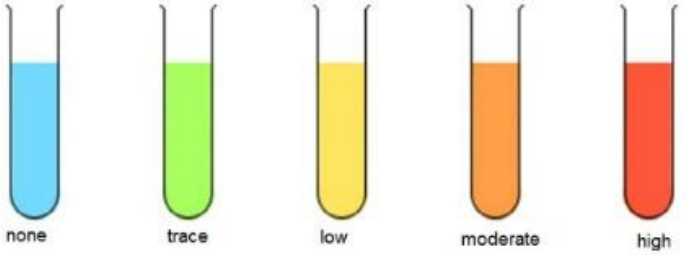
- Detects all sugars except sucrose.

- The final color of the solution depends on how much of this precipitate was formed, and therefore the color gives an indication of how much reducing sugar was present.

- **Increasing amounts of reducing sugar**
- **Green yellow orange red**

Components of Benedict's reagent

- **Sodium carbonate-** 100 gm (Provides alkaline conditions which are required for the redox reaction)
- **Sodium citrate-** 173 gm (complexes with the copper (II) ions so that they do not deteriorate to copper(I) ions during storage)
- **Copper sulphate-** 17.3 gm



- **<u>Procedure</u>**

- Take 5ml of Benedict's reagent
- Boil for 3 – 5 minutes
- Add 0.5ml (8 drops)of urine.
- Boil for 2 minutes.
- Cool and note the colour.

- **Recording results**
- The color varies from blue through green – yellow- orange- brick red.

Negative	No change in color.
Trace	Greenish blue
1+	Greenish yellow (0.5% sugar)
2+	Yellow (1% sugar)
3+	Orange precipitate (1.5% sugar)
4+	Brick red precipitate (2% sugar)

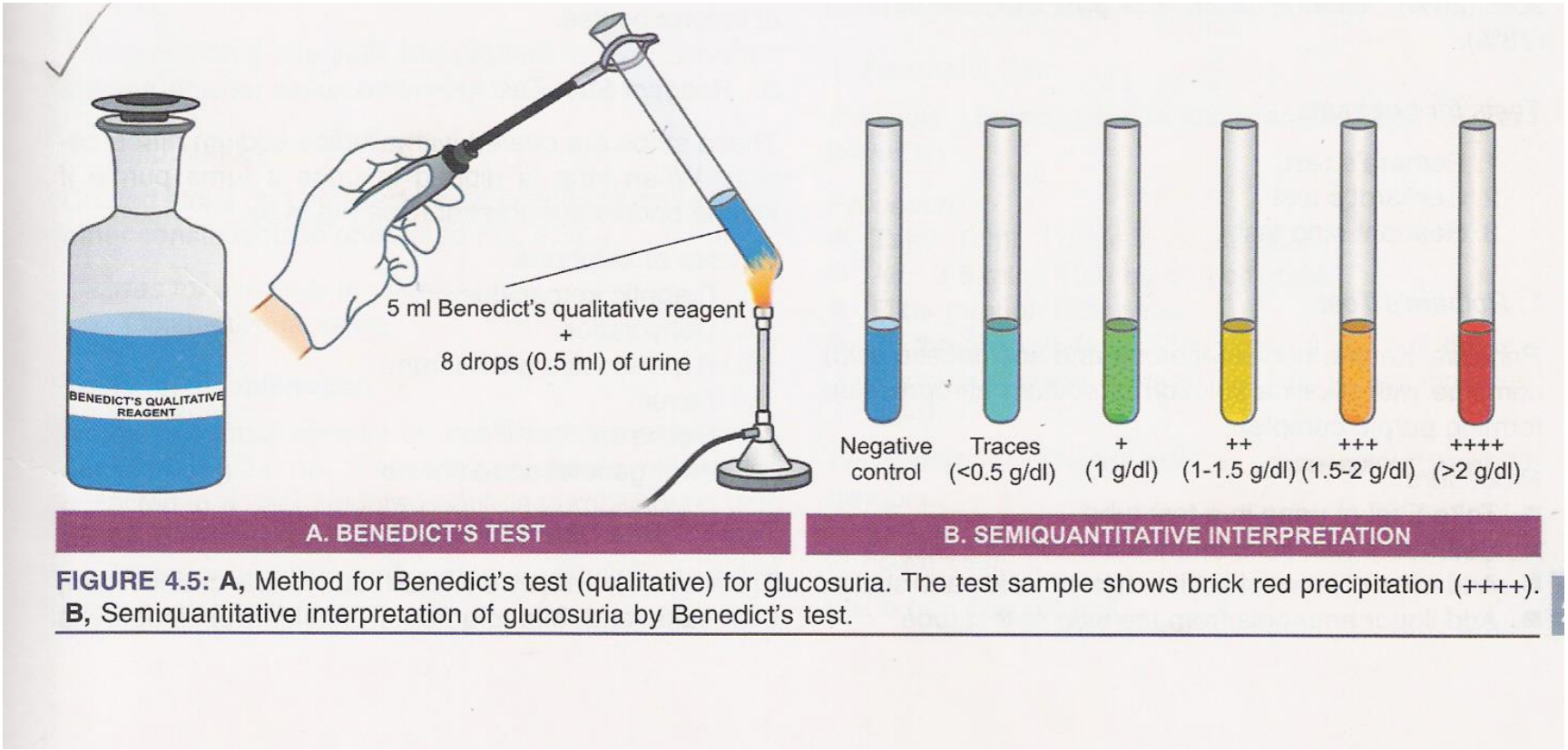


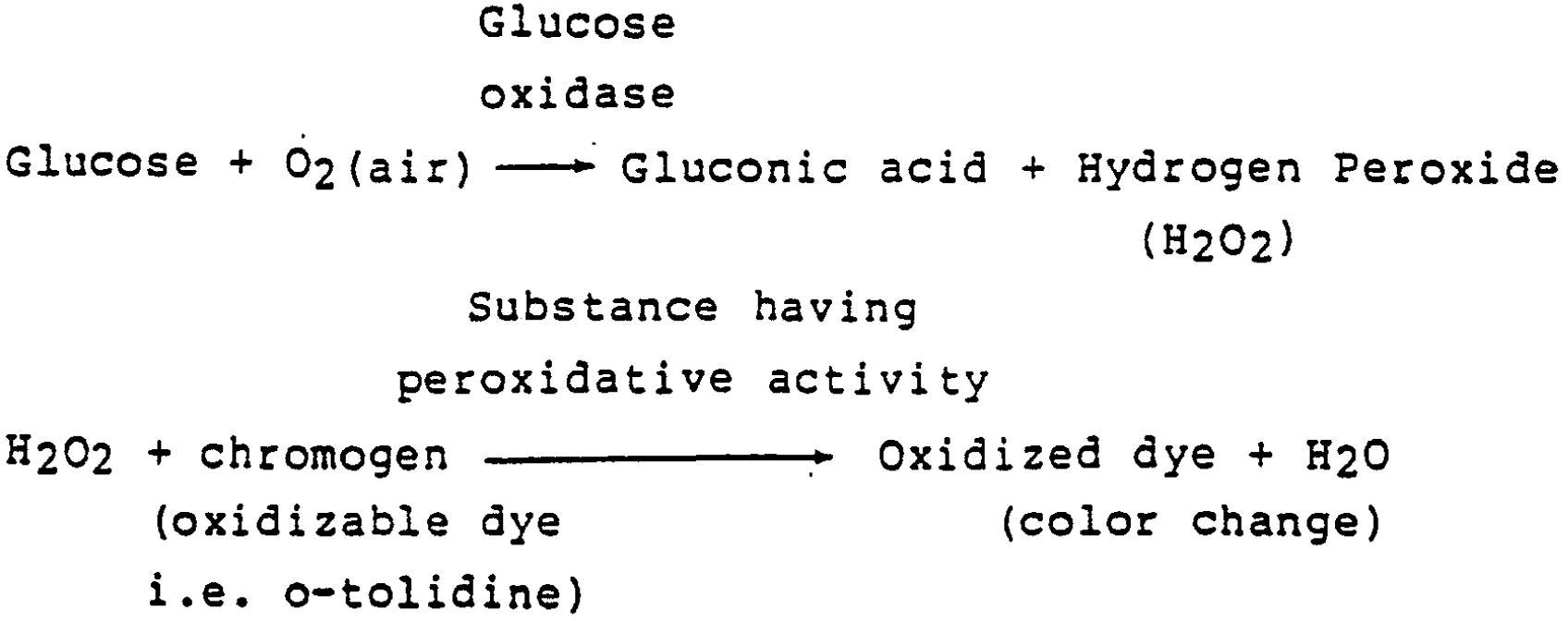
FIGURE 4.5: A, Method for Benedict's test (qualitative) for glucosuria. The test sample shows brick red precipitation (++++). **B,** Semiquantitative interpretation of glucosuria by Benedict's test.

- **Sugars detected by Benedict's test-**
  - Glucose
  - Galactose
  - Lactose
  - Fructose
  - Maltose
  - Pentose

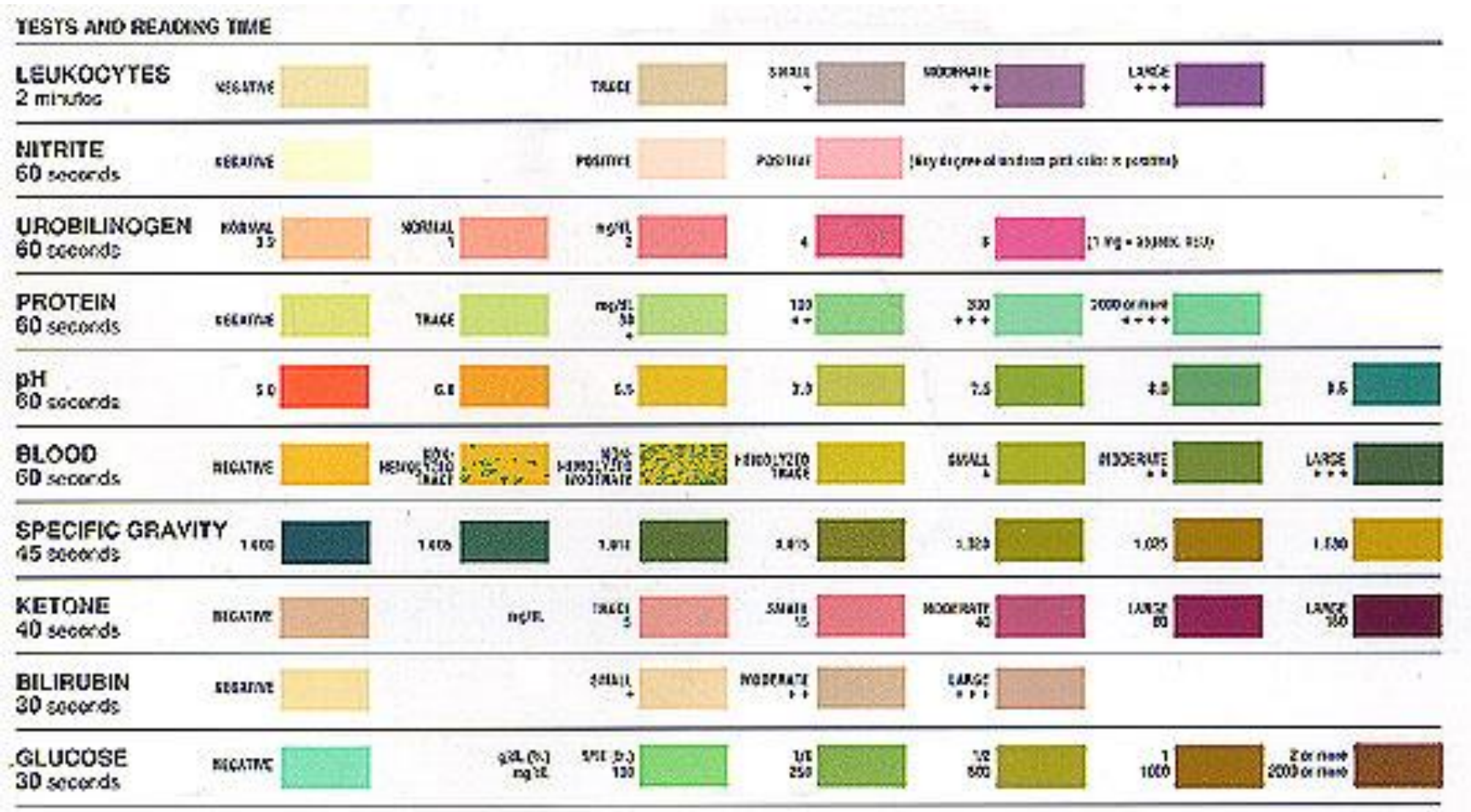
- **False +**
  - Ascorbic acid, Creatinine, Uric acid
  - Salicylates
  - X-ray contrast

COLORIMETRIC REAGENT STRIP TEST

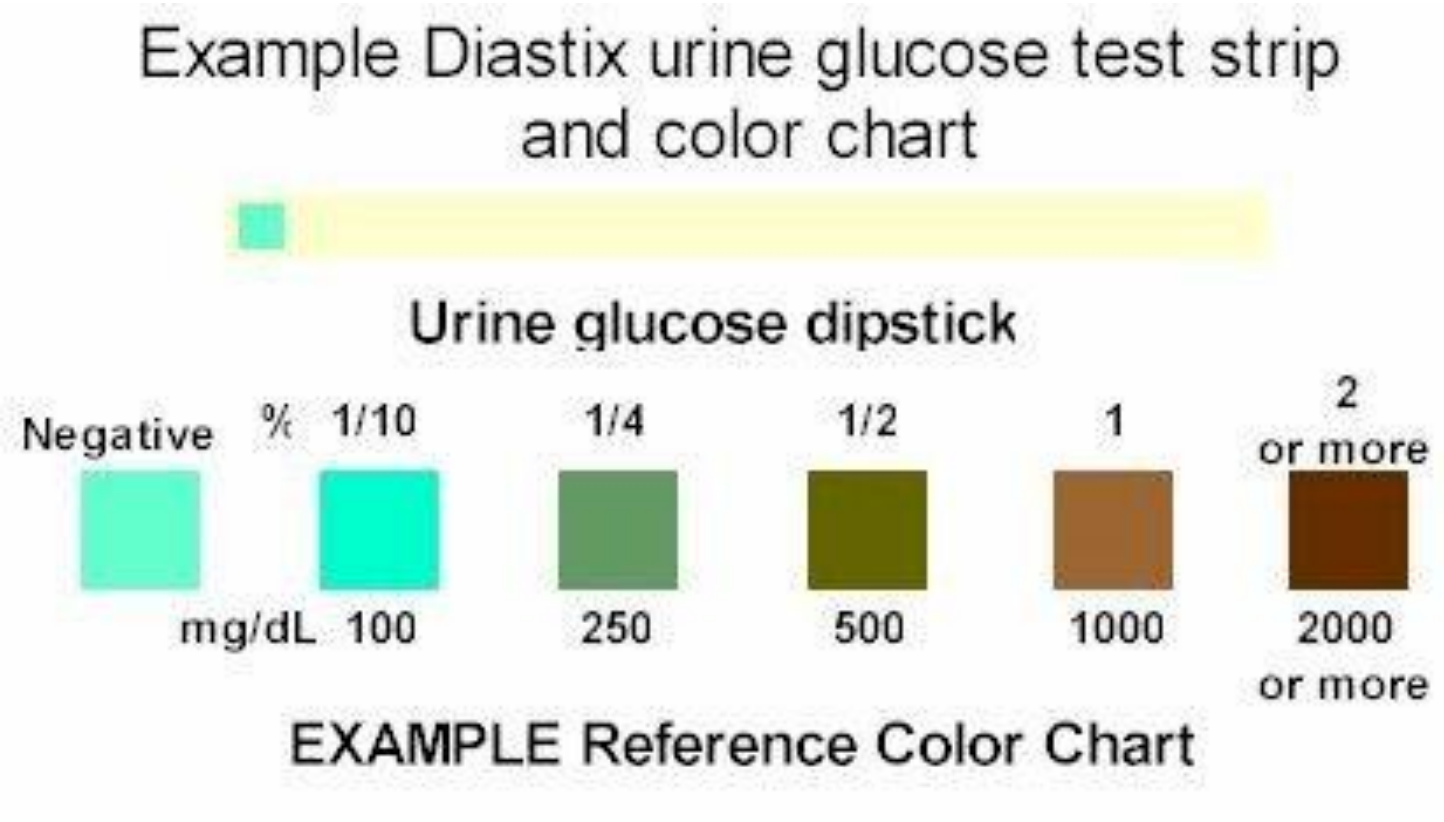
- **Principle**: this test is based on a double sequential enzyme reaction.
- One enzyme, glucose oxidase, catalyzes the formation of gluconic acid and hydrogen peroxide from the oxidation of glucose.
- A second enzyme, peroxides catalyzes the reaction of hydrogen peroxide with potassium iodide chromogen to oxidize the chromogen to colors ranging from green to brown.


Glucose
oxidase
Glucose + O_2(air) ⟶ Gluconic acid + Hydrogen Peroxide
(H_2O_2)
Substance having
peroxidative activity
H_2O_2 + chromogen ⟶ Oxidized dye + H_2O
(oxidizable dye
i.e. o-tolidine)
(color change)


WANCHENG
URS–5K
WANCHENG
URS–5K
Reagent Strips for Urinalysis
Glucose
pH
Protein
Ketone
Blood
WANCHENG
URS–5K
Reagent Strips for Urinalysis
Glucose
pH
Protein
Ketone
Blood

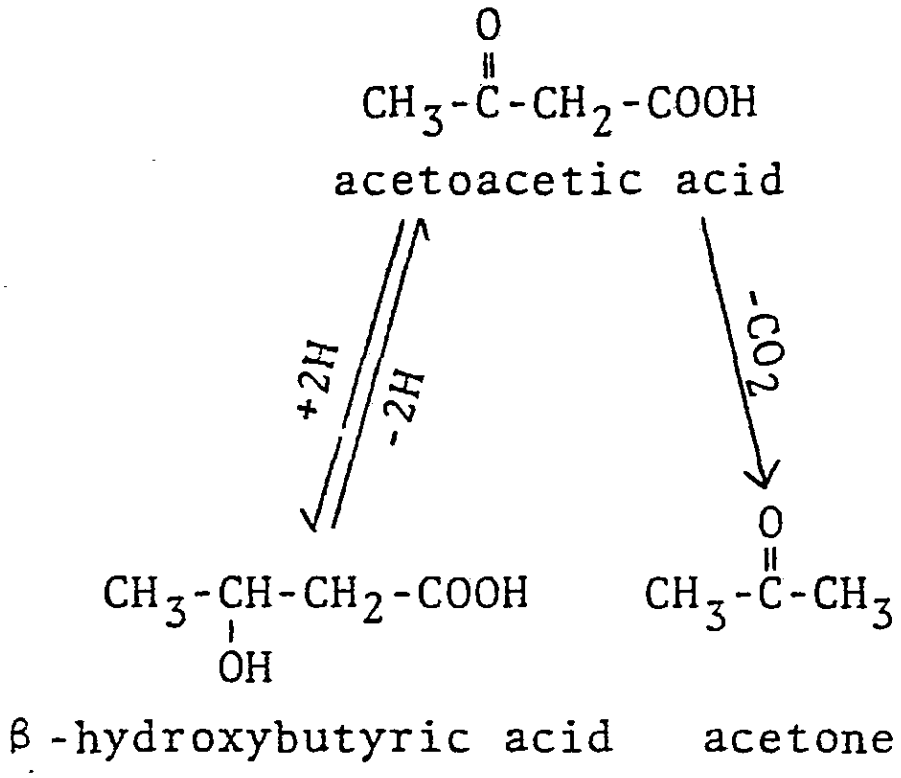

TESTS AND READING TIME
LEUKOCYTES
2 minutes
NEGATIVE
TRACE
SMALL
+
MODERATE
++
LARGE
+++
NITRITE
60 seconds
NEGATIVE
POSITIVE
POSITIVE
UROBILINOGEN
60 seconds
NORMAL
NORMAL
1
4
8
PROTEIN
60 seconds
NEGATIVE
TRACE
100
++
300
+++
2000 or more
++++
pH
60 seconds
5.0
6.0
6.5
7.0
7.5
8.0
8.5
BLOOD
60 seconds
NEGATIVE
TRACE
MODERATE
HEMOLYZED TRACE
SMALL
MODERATE
++
LARGE
+++
SPECIFIC GRAVITY
45 seconds
1.000
1.005
1.010
1.015
1.020
1.025
1.030
KETONE
40 seconds
NEGATIVE
TRACE
5
SMALL
15
MODERATE
40
LARGE
80
LARGE
160
BILIRUBIN
30 seconds
NEGATIVE
SMALL
+
MODERATE
++
LARGE
+++
GLUCOSE
30 seconds
NEGATIVE
100
1/2
250
1/2
500
1
1000
2 or more
2000 or more

Example Diastix urine glucose test strip and color chart



KETONES IN URINE (ketonuria):

- **<u>TYPES</u>**
- Acetone, diacetic acid (acetoacetic acid), betahydroxybutyric acid.



- **Causes of Ketonuria:**

- DKA
- Fever
- Anorexia
- Gastrointestinal disturbances
- Fasting
- Starvation
- Severe vomiting

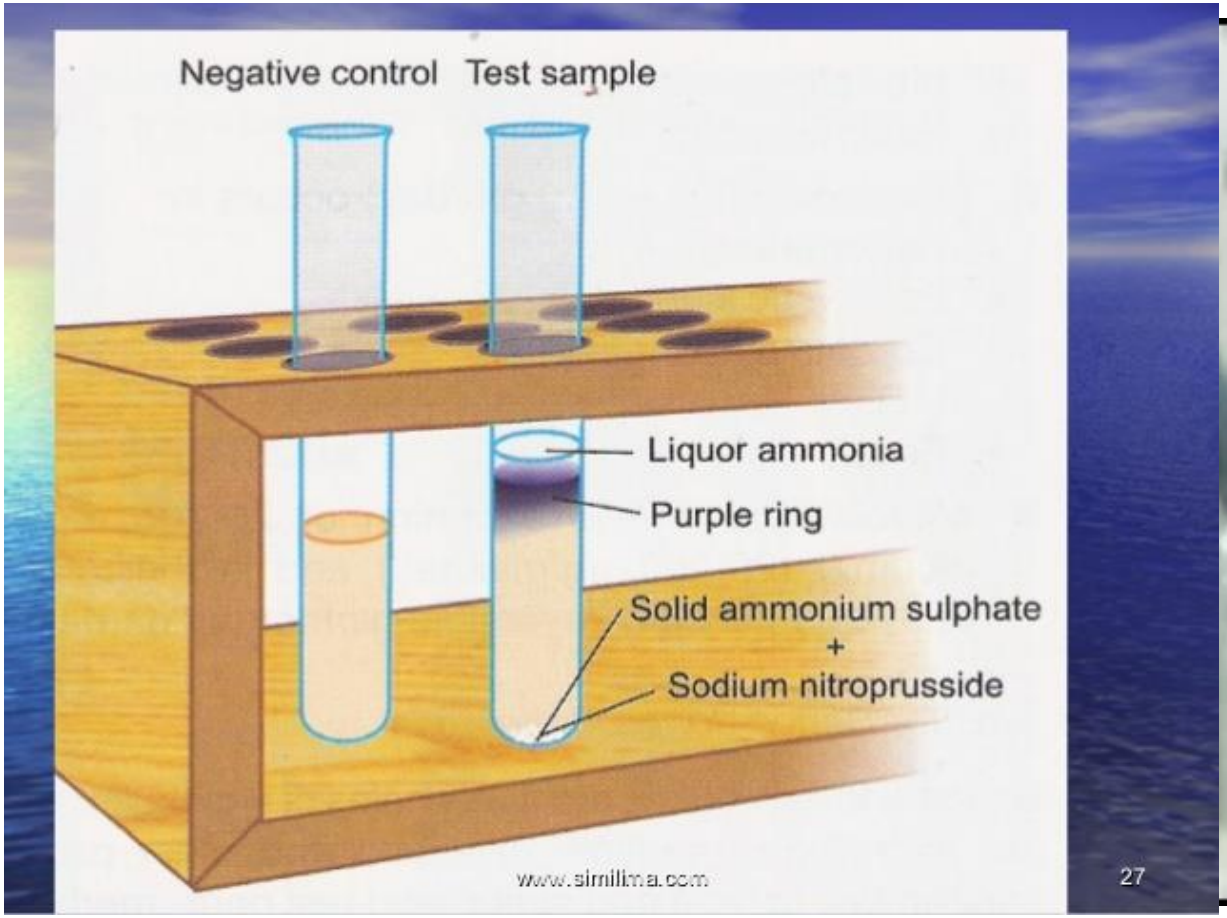
- **Rothera's Test for Acetone and Acetoacetic Acid:**

- **Principle:**
- Acetone and acetoacetic acid develops purple coloured complex with sodium nitroprusside in alkaline medium.

- **Hart's test**
- For detection of beta-hydroxybutiric acid.

- **Rothera's Test for Acetone and Acetoacetic Acid:**

- **Procedure:**
- Take 5ml of urine in a test tube and saturate it with ammonium sulphate.
- Add 1 crystal of sodium nitroprusside.
- Mix.
- Run liquid ammonia carefully at the side of the tube so as to form a layer on top of the saturated urine.
- **POSITIVE**- Formation of purple ring at junction of two fluids.


Negative control
Test sample
Liquor ammonia
Purple ring
Solid ammonium sulphate
+
Sodium nitroprusside
www.similima.com

OCCULT BLOOD IN URINE:

- Red blood cells / haemoglobin.
- Haematuria- when 5 or more intact RBCs/HPF.

Causes of Haemoglobinuria

- Malaria- black water fever.
- Hemolytic streptococcal septicaemia.
- Incompatible blood transfusion.
- Drugs- Sulphonamides, phenylhydralazine.
- PNH

Causes of Haematuria

- **Renal**
  - Neoplasms
  - Calculi
  - TB
  - Pyelonephritis
  - Hydronephrosis
  - Oxaluria
  - Acute GN
  - Polycystic kidney ds

- **Post-Renal**
  - Ureter- calculus, neoplasm
  - Urinary bladder- neoplasm, TB, Cystitis, calculus.
  - Prostate- BPH, Neoplasm

- **General**
  - Embolism of kidney from SBE.
  - Malignant HTN kidney
  - Haemophilia
  - Leukemia

Benzidine Test

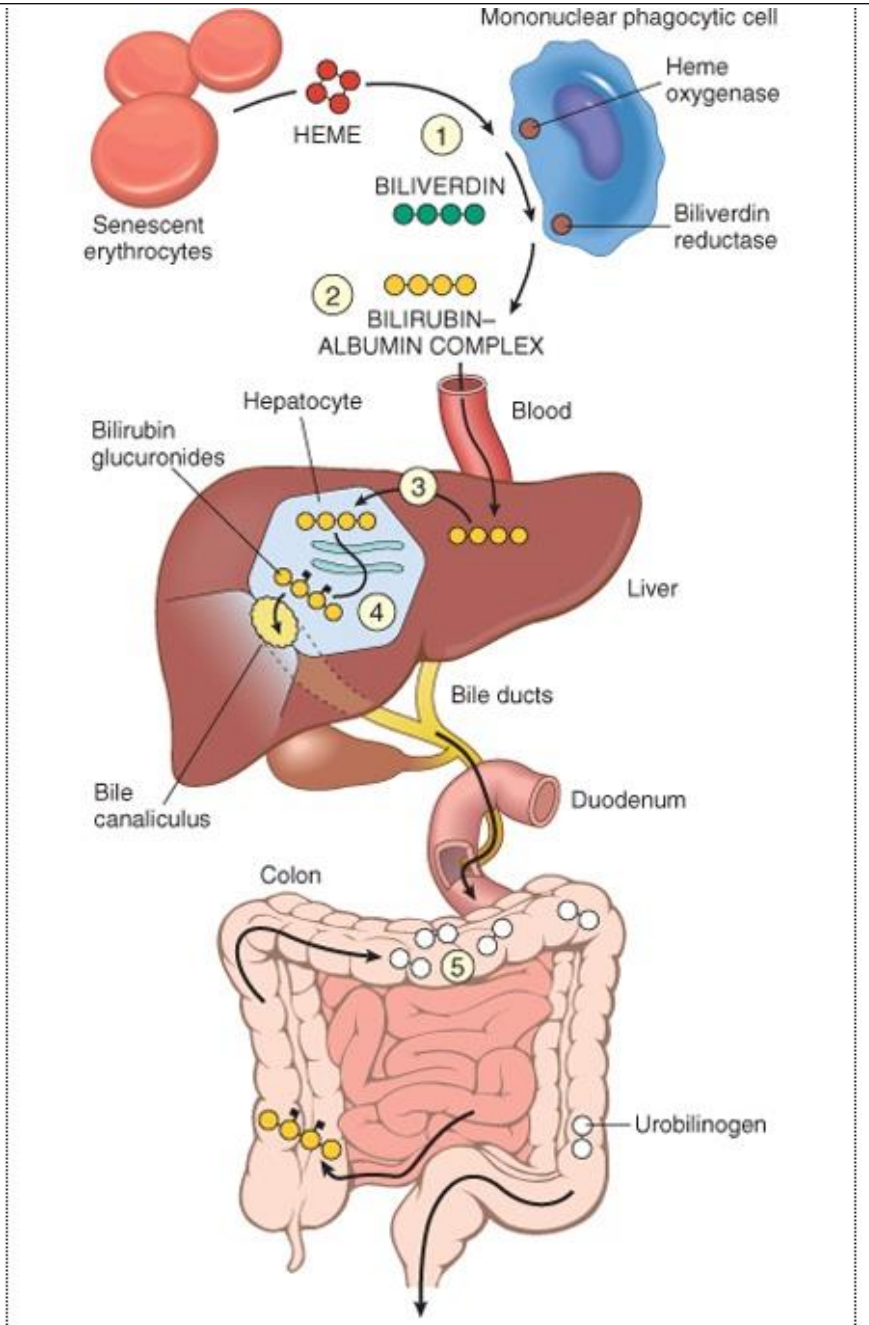
- **PRINCIPLE**
- The peroxidase activity of hemoglobin decomposes hydrogen peroxide releasing nascent oxygen which in turn oxidizes benzidine to give blue color.

- **REAGENTS**
- A: Saturated solution of benzidine in glacial acetic acid
- B: Hydrogen peroxide

Benzidine Test

- **PROCEDURE**
  - Add 2 ml of urine in test tube.
  - Add 2ml of 1% Benzidine solution in acetic acid.
  - Shake well.
  - Add 2ml of hydrogen peroxide.
  - Mix and observe for a change in color.
    - **Positive result:** Green or blue color. (Hematuria)

BILIRUBIN METABOLISM



Bile salts

- **Primary bile acids**
- Cholic acid and chenodeoxycholic acid (CDCA)- synthesized from cholesterol in the liver, conjugated with glycine or taurine, and secreted into the bile.
- **Secondary bile acids**
- Deoxycholate and lithocholate, are formed in the colon as bacterial metabolites of the primary bile acids.

- Sodium taurocholate and sodium glycocholate are found in urine.

Tests for detection of bile salts

- **Hay Test**

- **Principle:**
  - Bile salts when present decreases surface tension of urine.

- **Procedure:**
  - Take 10 ml of urine in beaker.
  - Sprinkle dry sulphur powder on the surface of the urine

- **Results:**
- If bile salts are present they sink to the bottom.
- Otherwise they float on the surface.

Bile pigments

- **Normal urine-**
  - Urochrome
  - Traces of Urobilin

- **Abnormal urine**
  - Bilirubin
  - Urobilinogen
  - Biliverdin
  - Urobilin

Fouchets Test/Harrison's spot test

- **FOUCHETS REAGENT**

- Trichloroacetic acid – 25 gms
- Distilled water - 100 ml
- 10% Ferric chloride solution – 10 ml.

Principle:

- Barium chloride added to urine combines with sulphate radicals in urine to form precipitate of barium phosphate. If bile pigments are present in urine, they will adhere to these large molecules. Ferric chloride present in fouchet reagent then oxidizes yellow bilirubin in presence of trichloroacetic acid to green bilverdin.

Fouchets Test/Harrison's spot test:

- **PROCEDURE**
- Place 5 ml of acidified urine in a test tube.
- Add 5ml of 10 % barium chloride.
- Mix and filter through filter paper.
- Let the paper dry.
- Add 1-2 drops of Fouchet's reagent to the ppt on filter paper.
- **RESULT**: A green color indicates the presence of bilirubin.

Ehrlich's test for urobilinogen

- **Principle-**
- Urobilinogen reacts with p-dimethylamino-benzaldehyde to form red colour.
- Intensity of red colour is proportional to the concentration of urobilinogen in urine.

- **Reagents-**
- P-dimethylaminobenzaldehyde
- HCL
- DW

Ehrlich's test for urobilinogen

- **Procedure**
- Add 1ml of Ehrlich's reagent to 10 ml of urine in test tube.
- Mix by inversion.
- Let stand for 5 minutes.

- **RESULT**
- Pink- Normal
- Dark red colour- Positive for urobilinogen.

Causes of increased urobilinogen

- Cirrhosis
- Haemolytic jaundice
- Paralytic enterocolitis
- Hepatic congestion

LABELLING

- **Sample container-for identification of sample.**
- **Cytology requisition form-for identification of individual patient sample.**

CENTRIFUGATION

Basically of 2 types-

- **I Normal.**

- **II Cytospin-A device that spins cells in a fluid suspension .**

- **Drawbacks-distortion of cellular morphology due to air drying artifacts and loss of cells by absorption of fluid into the filter card.**

- **In this process, urine sample is taken in a conical tube and centrifuge at a rate of 2000rpm for 10-15 minutes.**

PAPANICOLAOU STAIN

- Done by two methods-
  1. Automated stainer-- large scale slides.
     Takes 30 minutes for staining.
  2. Manual staining using copplin jar-
     For small scale slides.
     Takes less than 7 minutes to stain.
- Staining objectives-
  I. Well stained nuclear chromatin.
  II. Differential counterstaining i.e. staining the cytoplasm of different cell types into different colours and intensity.
  III. Retaining cytoplasmic transparency.

Urine Cytology

- INTERPRETATION-

1 Normal-Normal constituents of urine.

2 Abnormal-Any variation from normal-

I cellular components

II Acellular components.

CELLS derived from-

- Urothelial and its variants.
- Renal tubules.
- Adjacent organs-like prostrate.
- Cells extragenous to the urinary tract-RBCs.

Urine Cytology....

A Cells-

-Erythrocytes-Hematuria

-Leucocytes-Infective etiology

-Epithelial cells

B Casts-

-Hyaline cast

-Red cell cast

-Granular cast

-Epithelial cast

-waxy cast

-Fatty cast

C Crystals-Some are common in Acidic urine

-Some are common in Alkaline urine

D Bacteria-Normal urine is free from bacteria.

E Yeast.

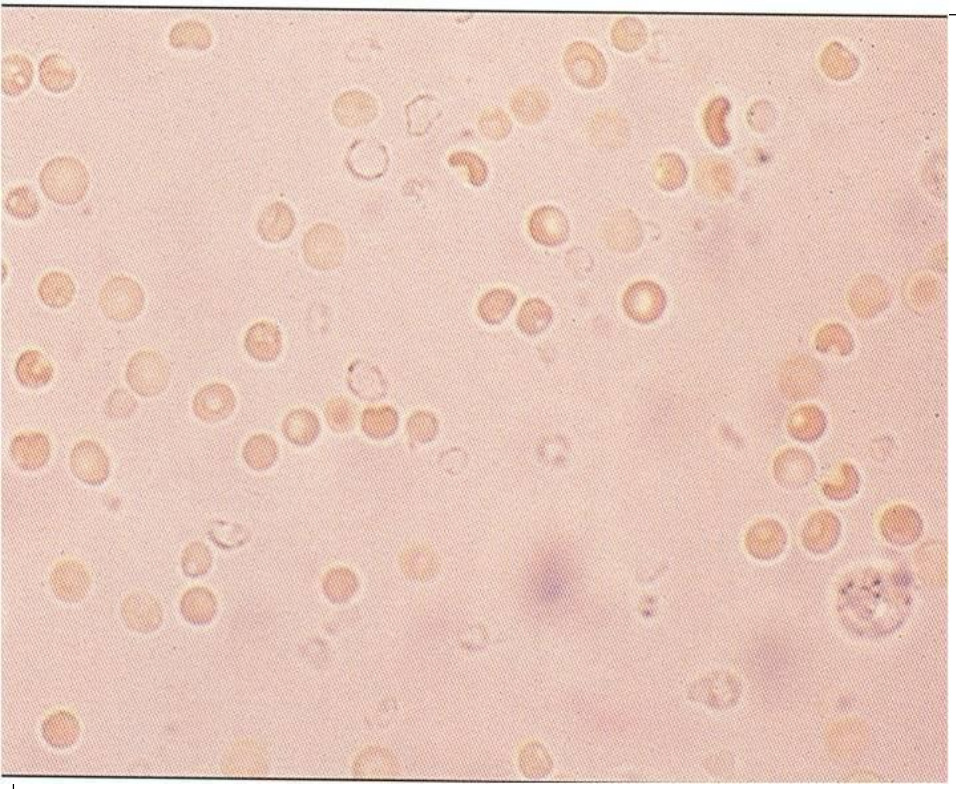
F Malignant cells.

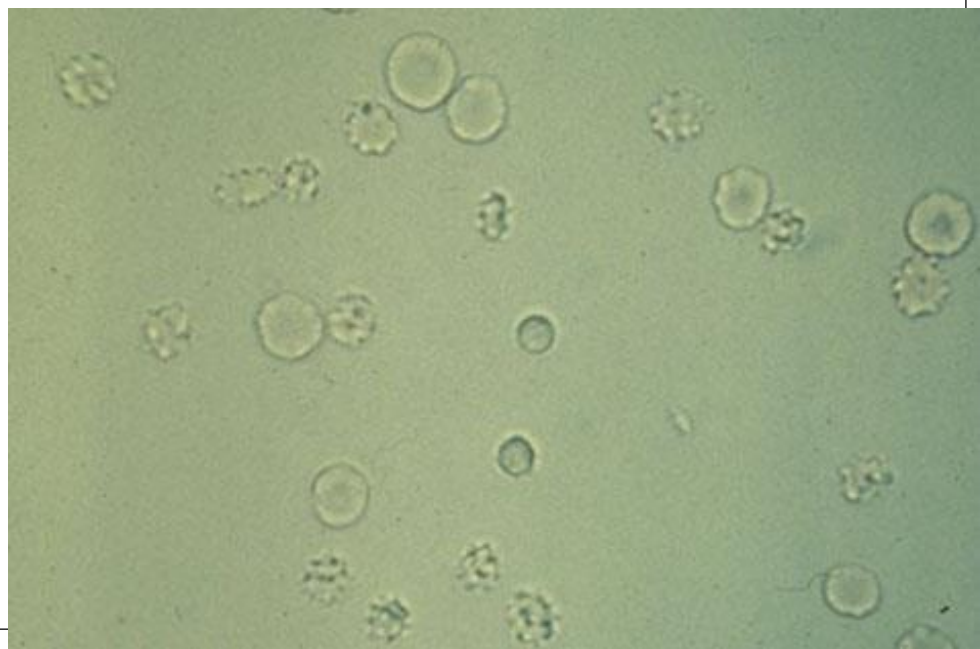
G Artifacts.

CELLS

Erythrocytes

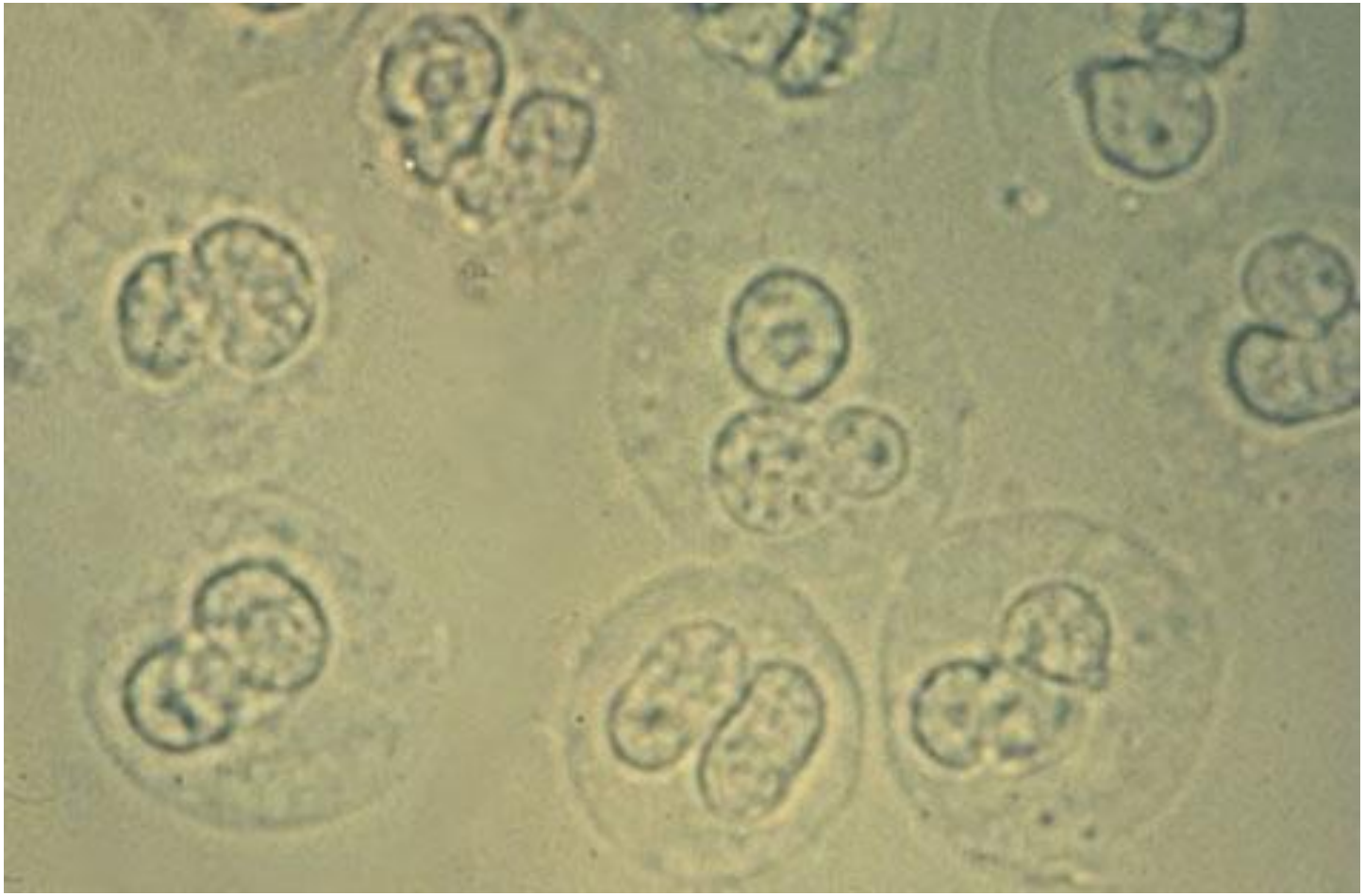
- usually appear as hourglass appearance.
- presence of red cells 1-2RBCs/HPF is not considered abnormal.
- in hypotonic urine red cells swell up causing lysis -releasing Hb in urine-lysed cells are referred as *ghost cells.*
- when the red cells are swollen/crenated sometimes mistaken for WBCs and yeast cells





Leucocytes

- Normal up to 1-2 WBCs/HPF.
- Larger than red cells and smaller than renal epithelial cells.
- Usually spherical- singly/clumps.
- Mostly neutrophils-presence of characteristics granules and lobulation.
- Addition of 2%acetic acid to slide accentuated the nuclei of cells.
- Presence of many white cells in clumps is strongly suggestive of acute urinary tract infection.



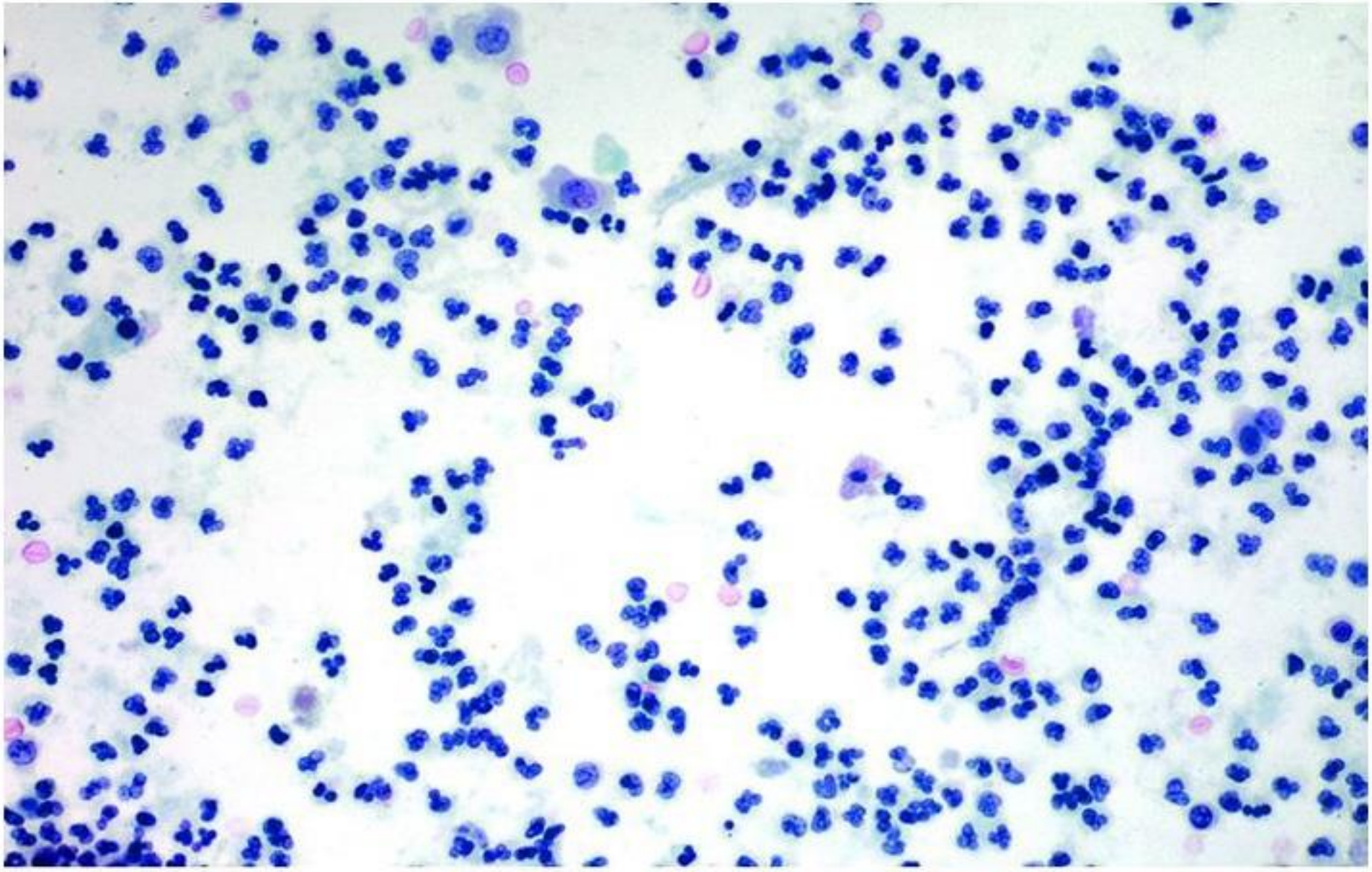


Figure 22-14A Urine sediment in inflammation. A low-power view of urine sediment containing numerous leukocytes.

Epithelial cells

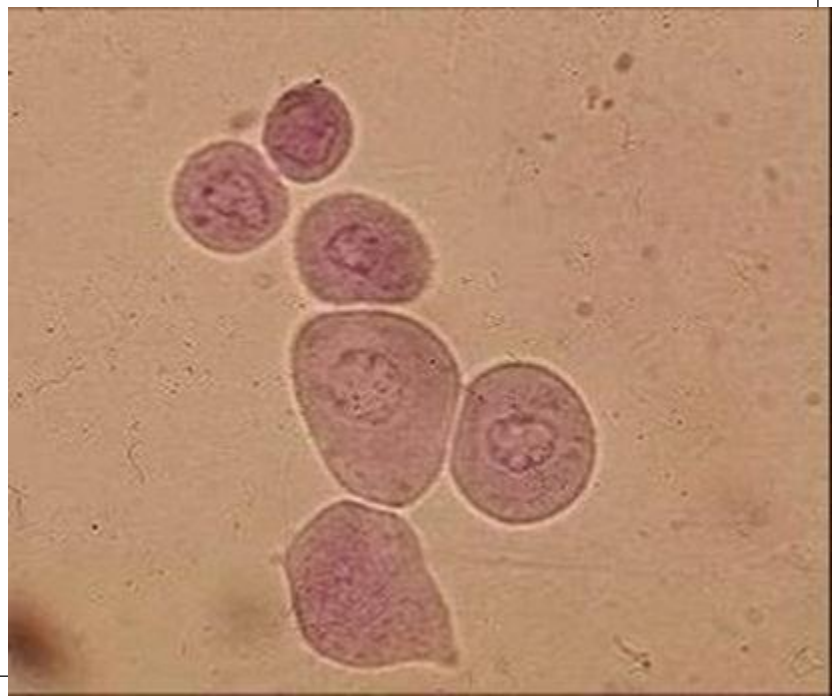
- **Any site in genitourinary tract from PCT to the urethra or from the vagina.**
- **Normally a few cells from these sites can be found.**
- **A marked increase indicates *inflammation* of that proportion of urinary tract from which the cell is derived.**

TYPES

- ***Renal tubular***
- ***Transitional***
- ***Squamous***

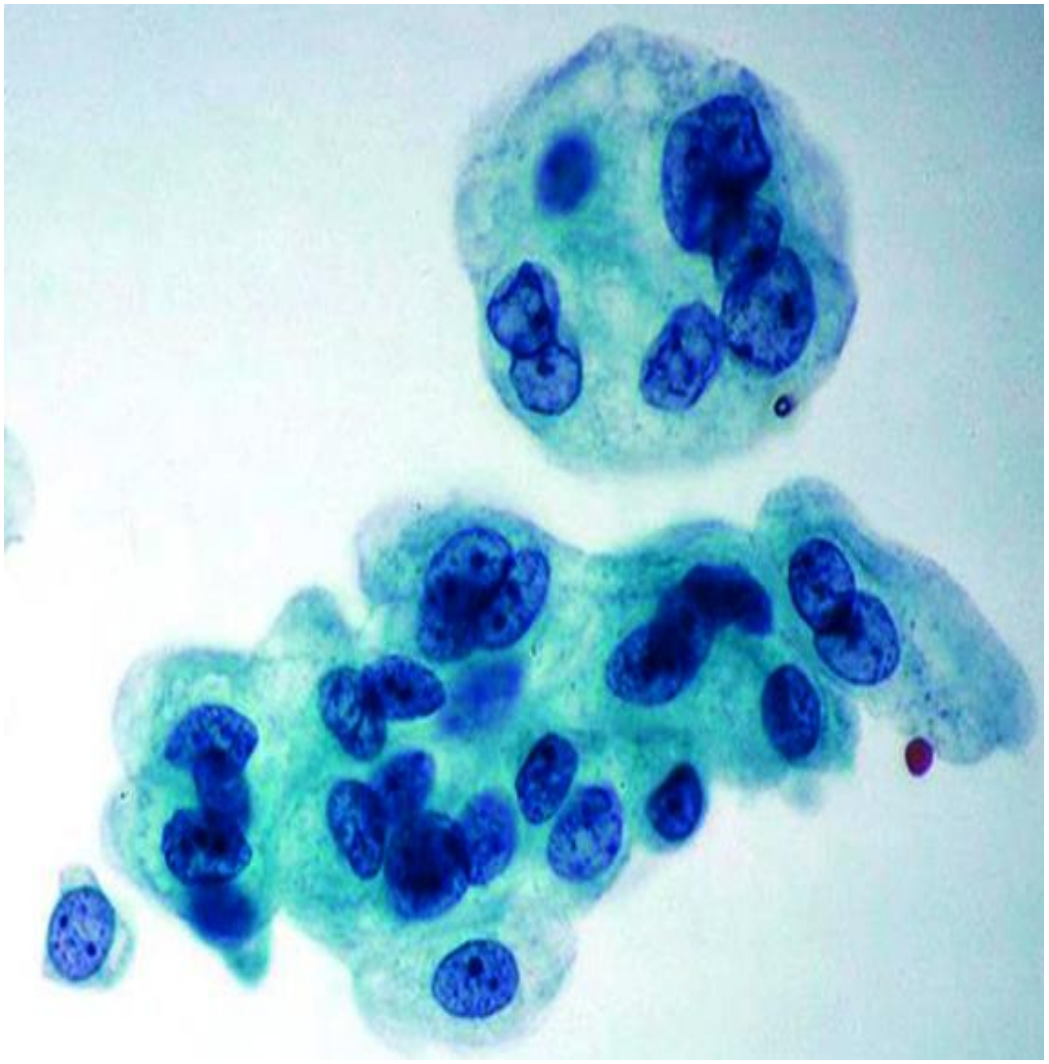
Renal tubular epi. cells

- Larger than white cells
- Large round nucleus.
- May flat/ cuboidal/ columnar.
- Increase no indicates tubular damage.



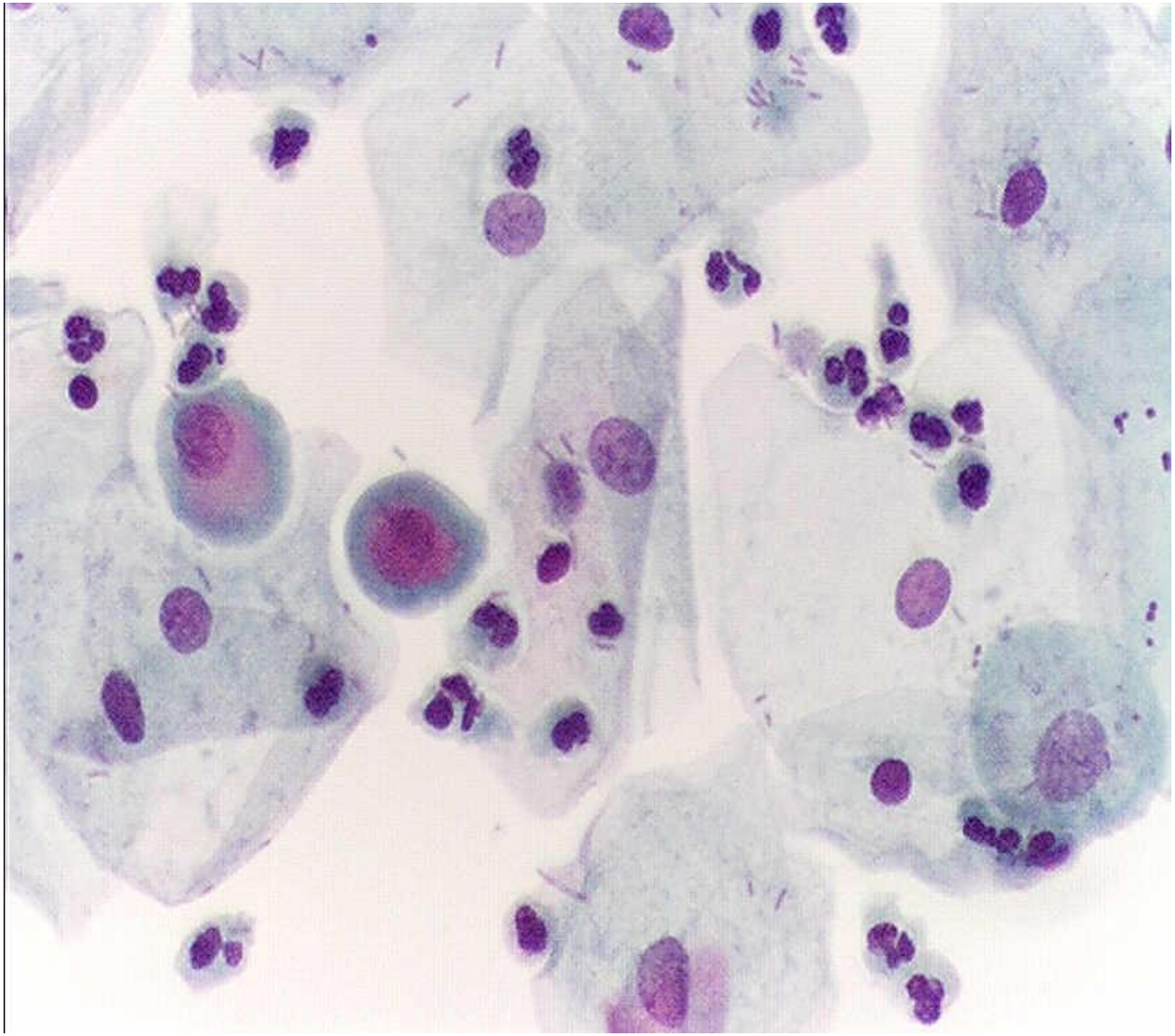
Transitional epithelial cells

- 2 to 4 times larger than white cells.
- Round/ pear shaped/ may have tail like projection.
- Line the urinary tract from pelvis of kidney to upper portion of urethra.



Squamous epithelial cells

- Line urethra and vagina.
- Have little diagnostic significance.



Crystals

- **Usually not found in fresh urine but appear when urine strands for a while.**
- **Many of crystal found in urine have little clinical significance except in case of metabolic disorders.**
- **Crystals are identified by their appearance and their solubility characteristics.**

TYPES-

- ***Acidic urine crystals***
- ***Alkaline urine crystals***

CRYSTALS IN ACIDIC URINE

- URIC ACID.
- AMORPHOUS URATES
- CALCIUM OXALATE
- CYSTINE
- LEUCINE
- TYROSINE
- SULPHA

CRYSTALS IN ALKALINE URINE

- TRIPLE PHOSPHATE
- CALCIUM CARBONATE
- CHOLESTROL
- Amorphous phosphates
- Ammonium biurate

Uric acid crystals

- Most characteristics form are diamond or rhombic prism.
- Presence of uric acid crystals in urine is a normal appearance.
- Increase in-gout

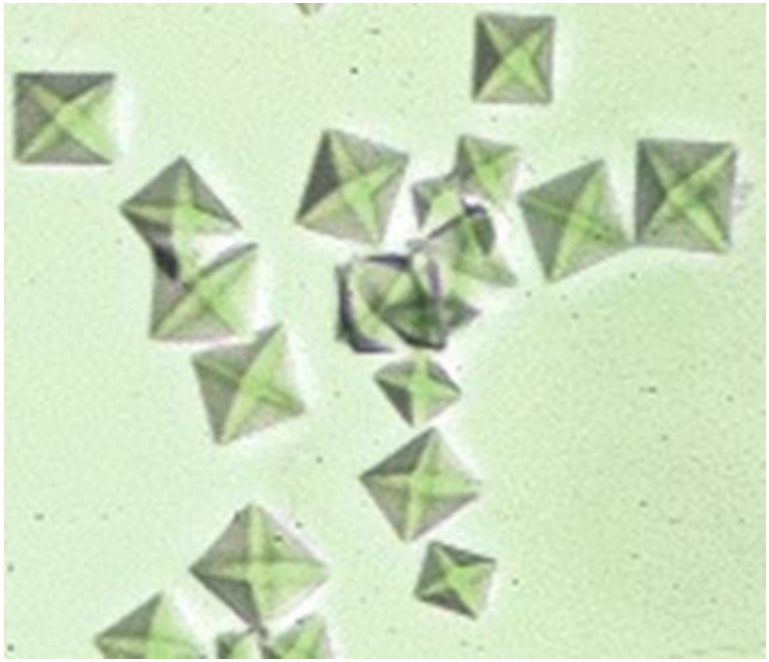
  -AFI

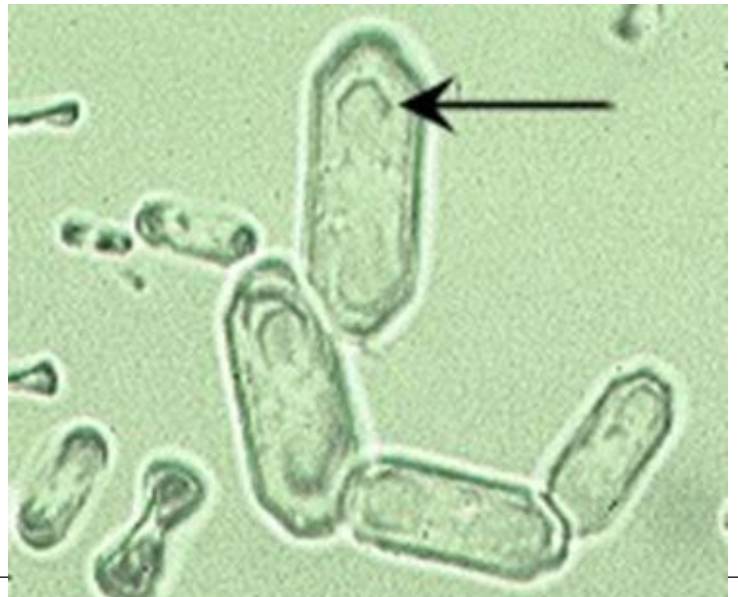
  -Chronic nephritis

  -high purine metabolism

Calcium oxalate crystals

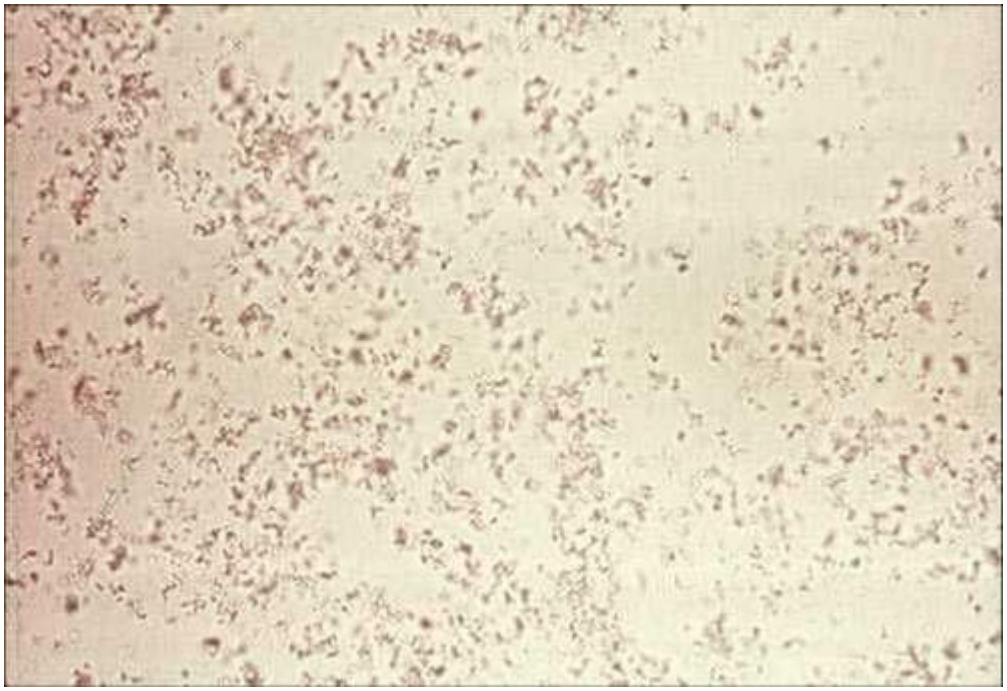
- Octahedral or envelope shaped crystal
- Can be present in normal urine after ingestion of various oxalate rich foods.
- Pathological- DM
  -Liver disease
  -Severe chronic renal disease





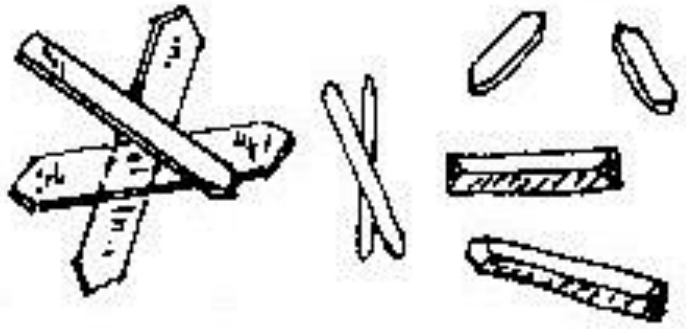
Amorphous urates

- Urates salts of sod, pot. and calcium
- Having a granular appearance
- Present in urine as non crystalline amorphous forms.
- No clinical significance.



Hippuric acid crystals

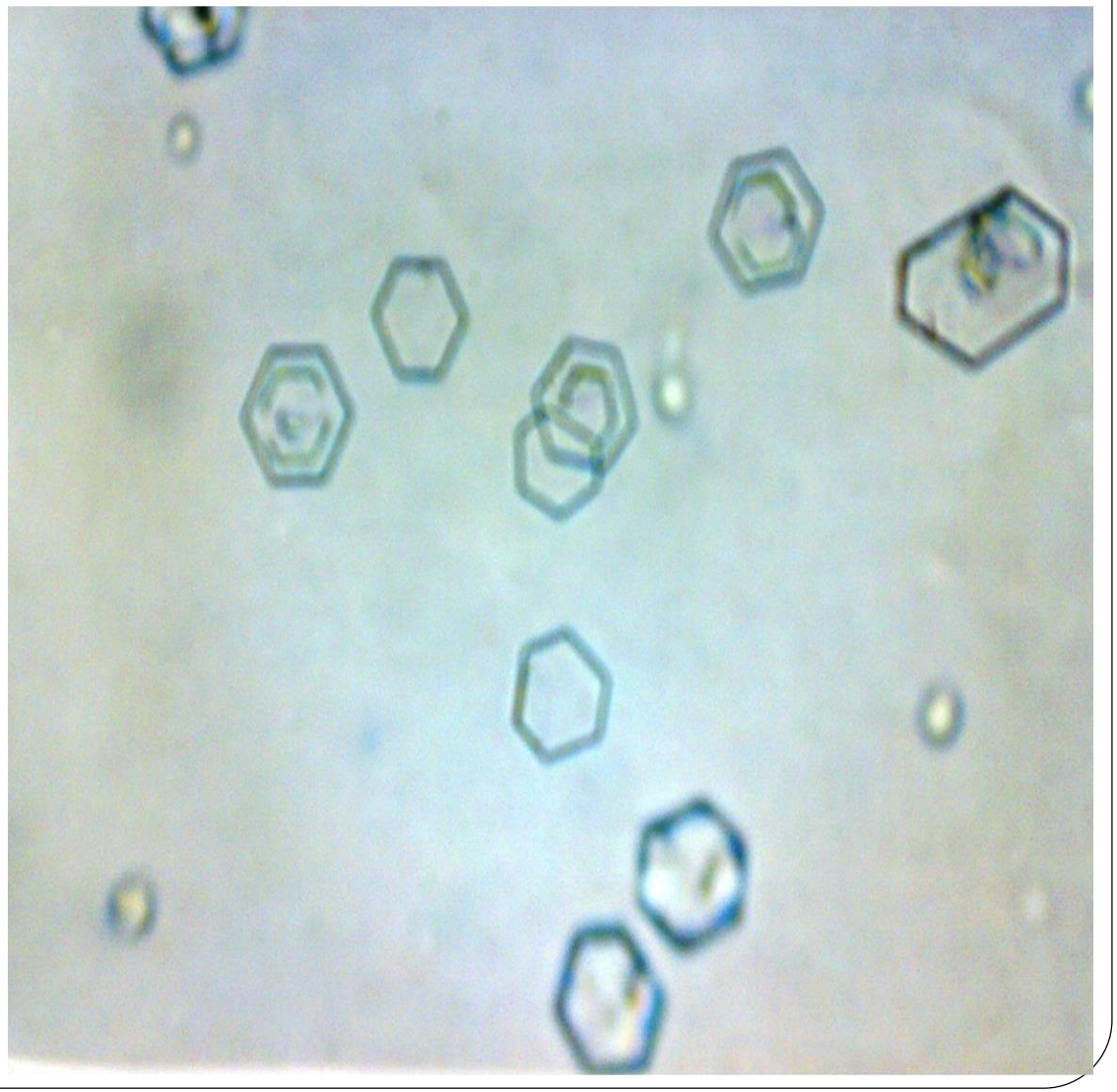
- Elongated prism like.
- Rarely seen in urine.
- No clinical significance.



Hippuric acid

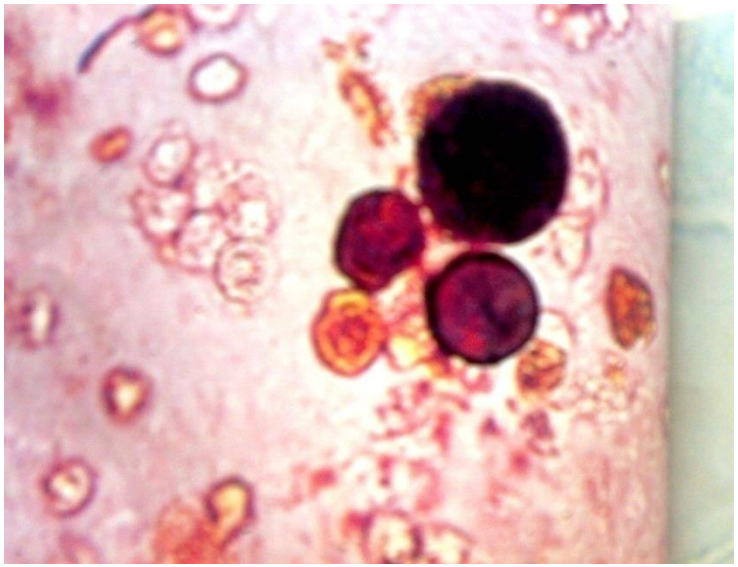
Cystine crystals

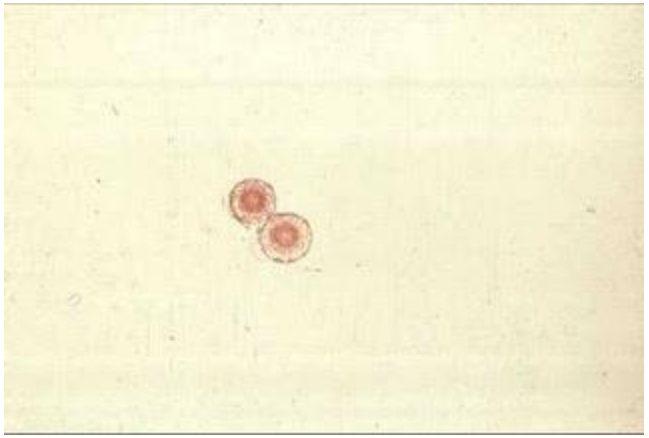
- Refractile hexagonal plate swith equal or unequal sides.
- Frequently have layered or laminated appearance.
- Soluble in ammonia.
- Can be detected chemically by Sod-cyanide-sod. Nitropruside test.
- Always Pathological (Cystinosis).



Leucine crystals

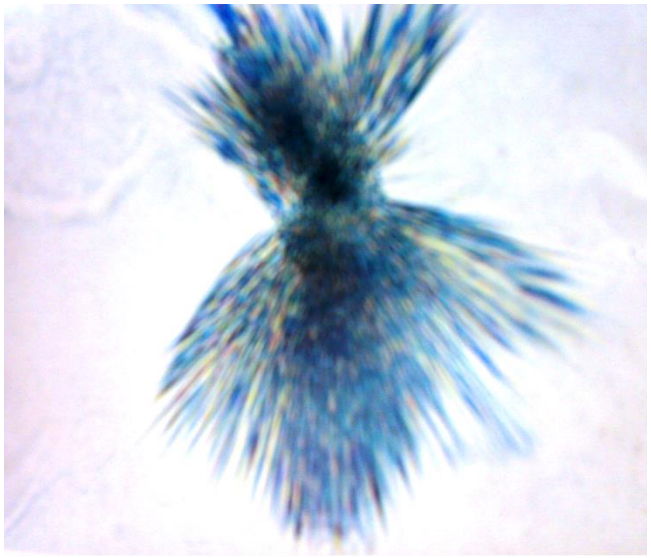
- Highly refractile having spheroid with radical and concentric striations.
- Clinically very significant.
- Maple syrup disease
- Serious liver disease





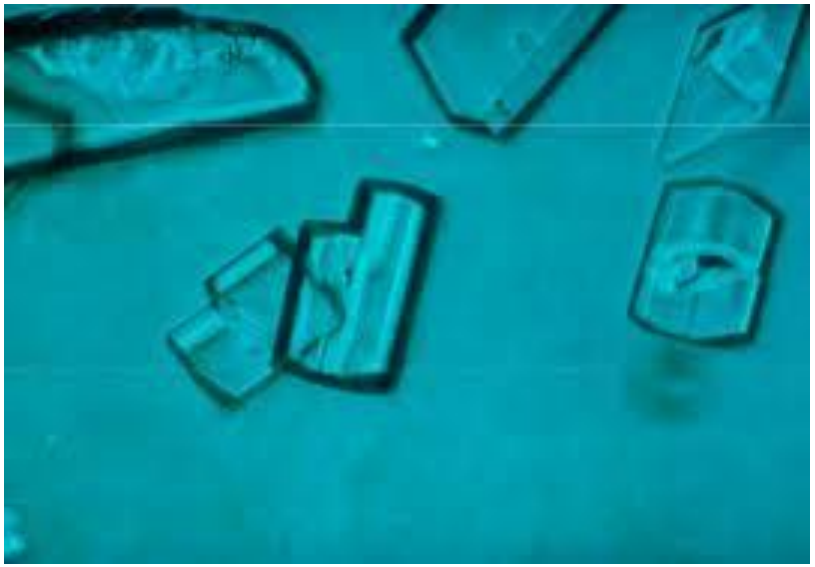
Tyrosine crystals

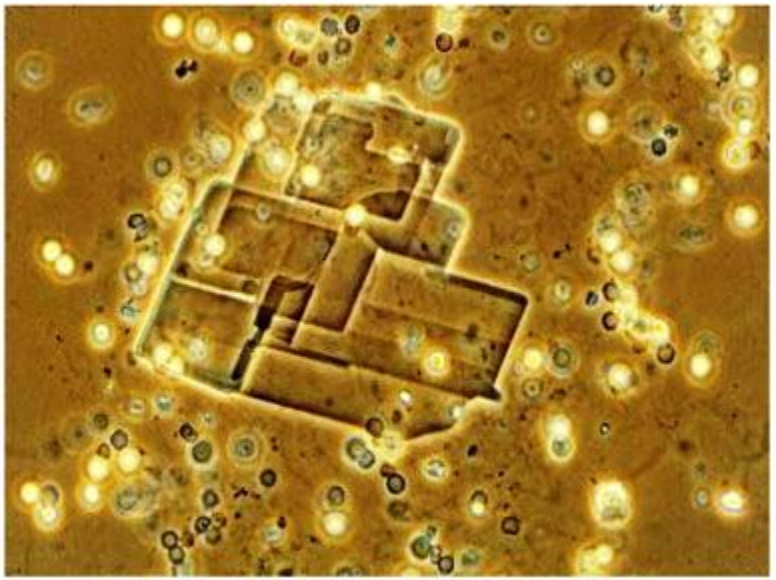
- very fine needle likes occurring in sheaves or clusters.
- Clinically significance
- Severe liver disease
- tyrosinosis



Cholesterol crystals

- large or flat plates with notched corners
- Presence of excessive in urine indicates tissue breakdown

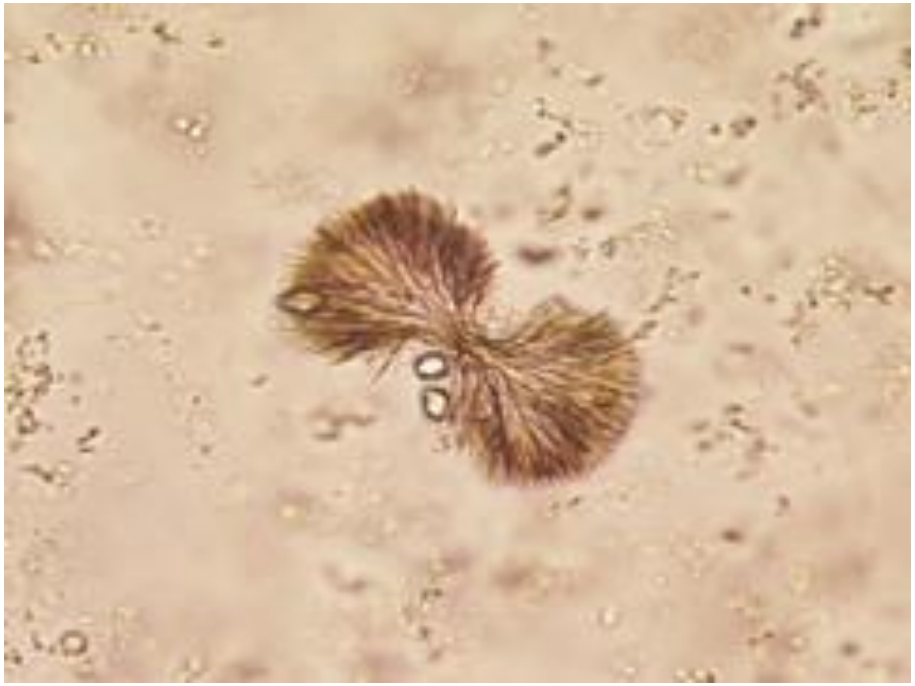




CHOLESTEROL CRYSTAL

Sulfa drugs crystals

- precipitate as sheets of needles usually with eccentric binding
- May be history of sulfa drugs medication



Alkaline urine crystals

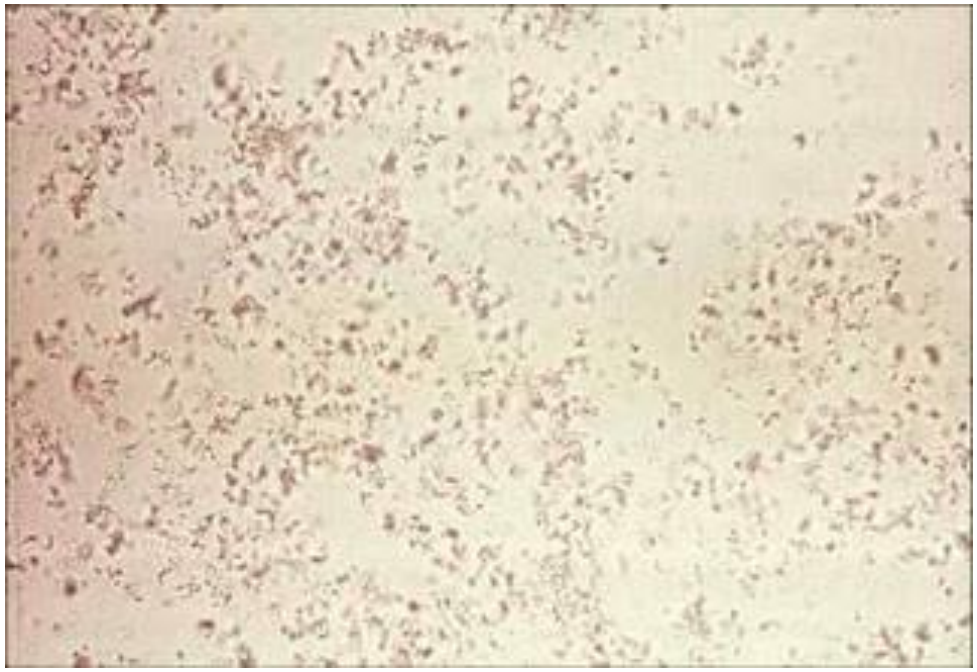
Triple phosphates crystals-

- prism like with three to six sides
- Frequently found in normal urine
- Pathological
  - -chronic pyelitis
  - -cystitis
  - -enlarged prostate

Amorphous phosphates

- granular particles with no definite shape

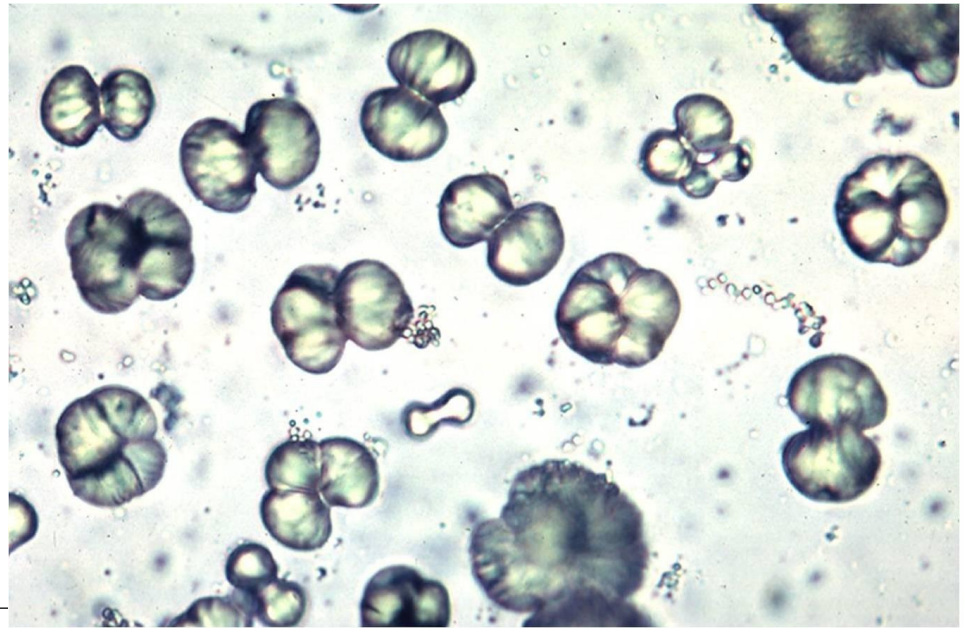
- No clinical significance.

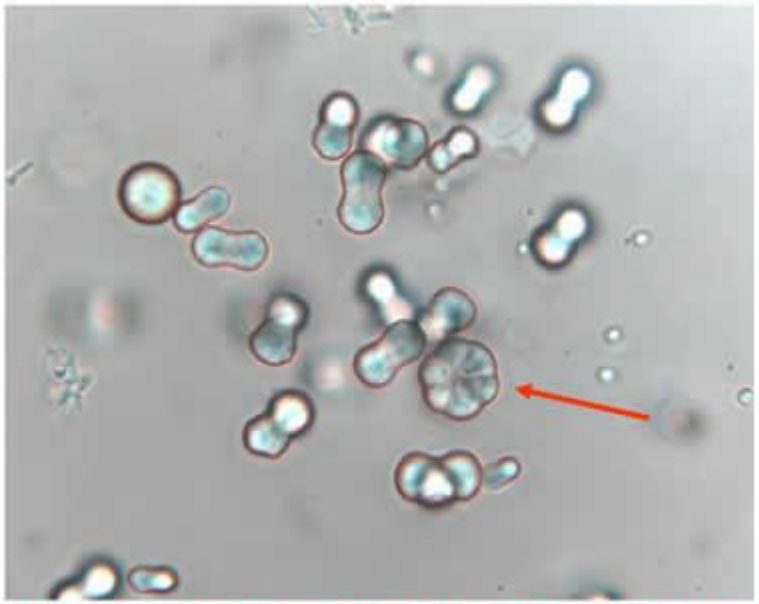


Calcium carbonate

- appearing as dumbbell or spherical or large granular mass.

- No clinical significance.





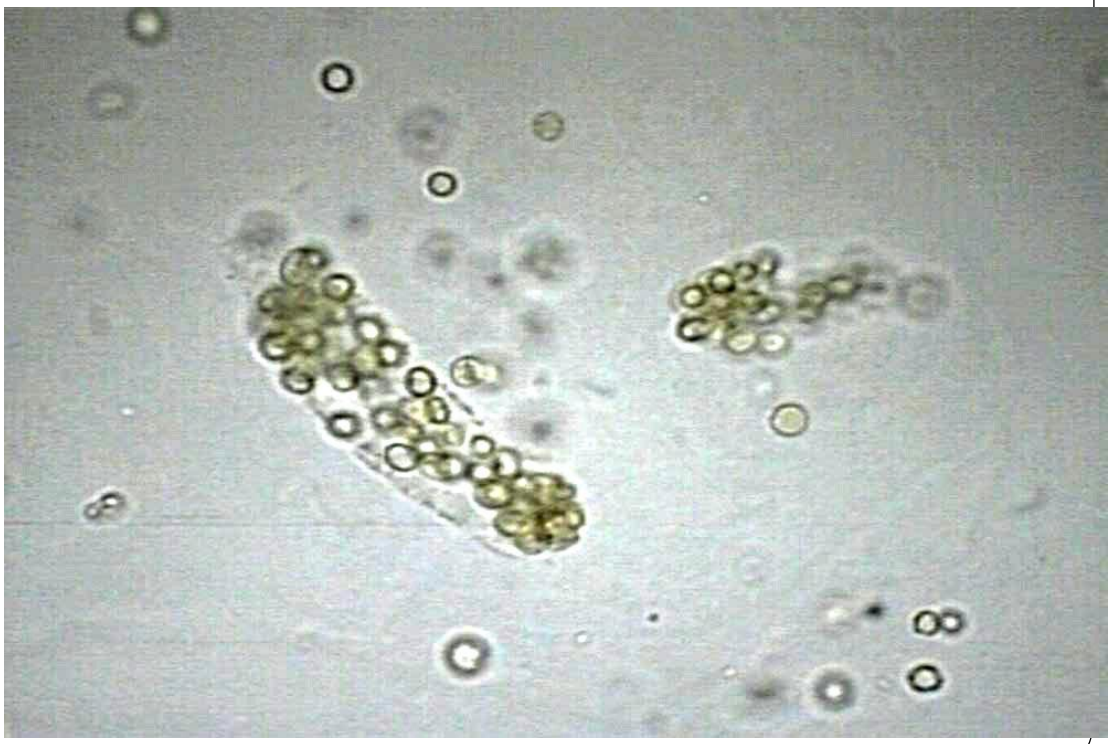
CALCIUM CARBONATE IN THE URINE OF A HORSE (BF)

Casts

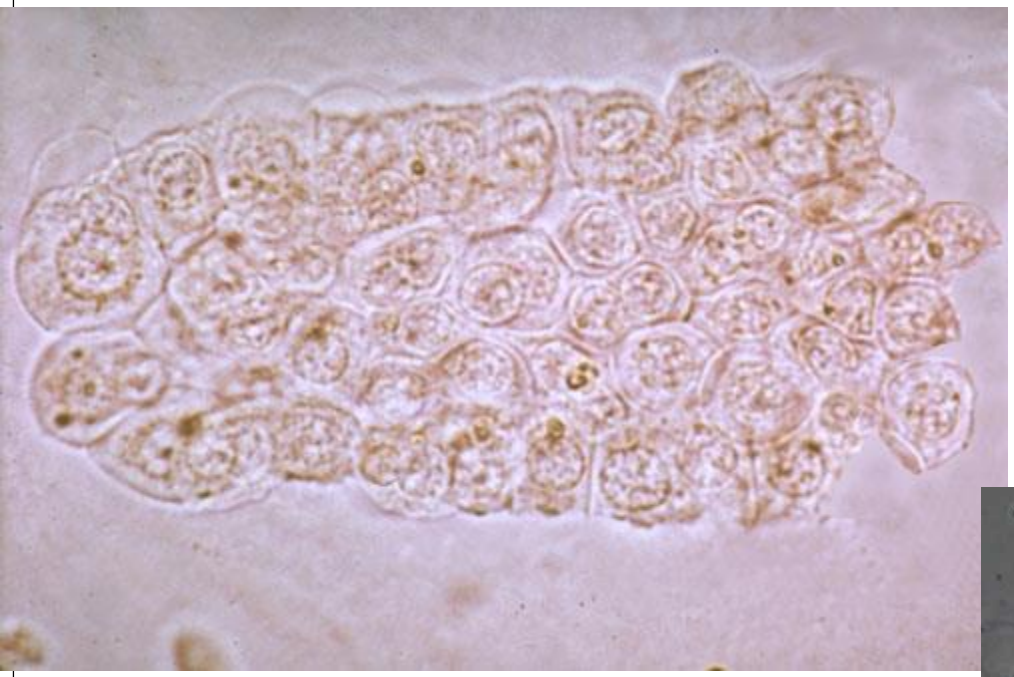
- Presence of casts are frequently associated with proteinuria.
- Have nearly parallel sides with rounded or blunts ends.
- Always renal in origin and indicates intrinsic renal disease
- Casts are more or less circular with thicker in middle.

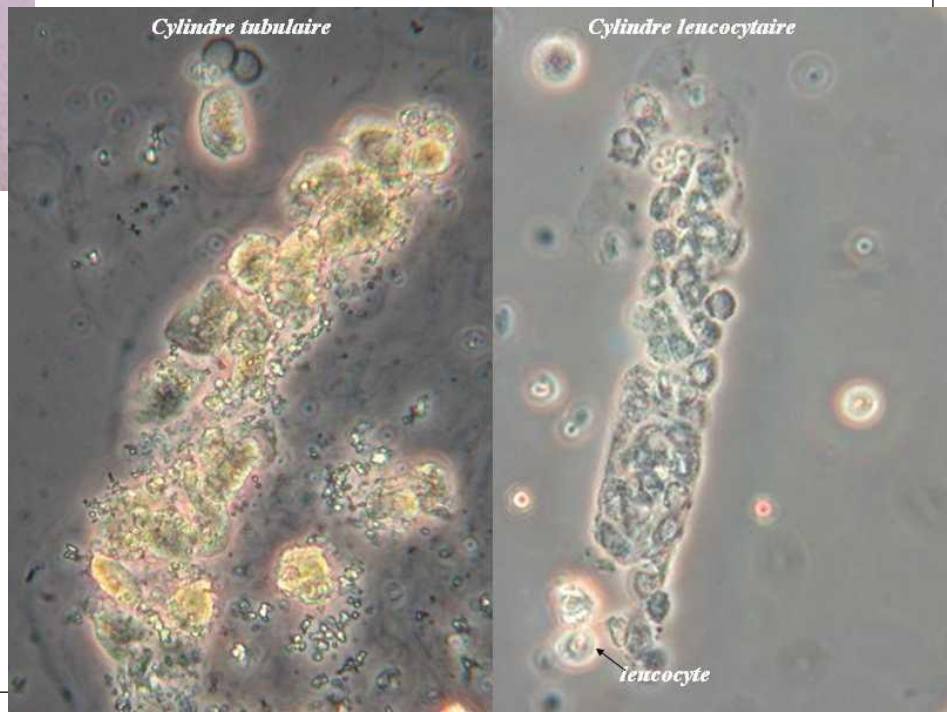
Red cells cast

- meaning renal hematuria
- Always pathological.



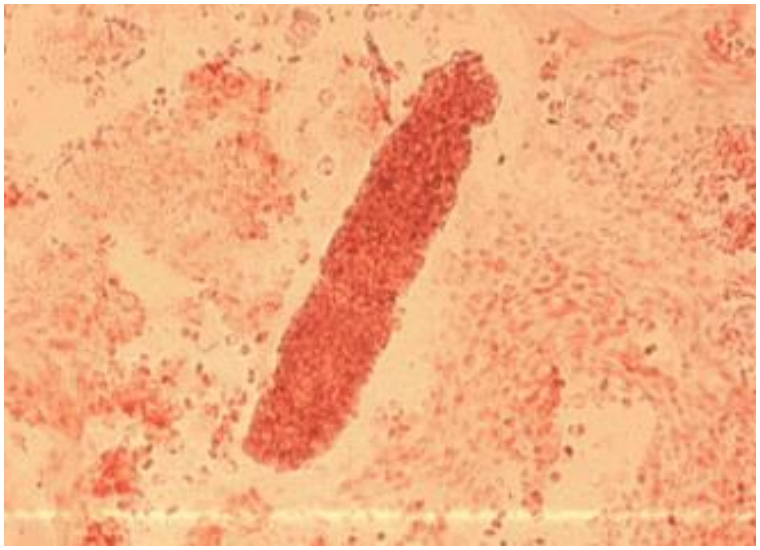
White cell cast

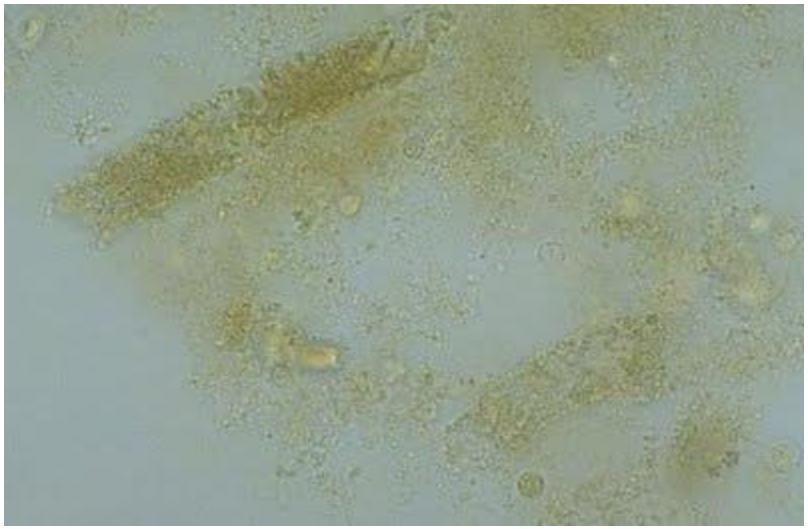




Granular casts

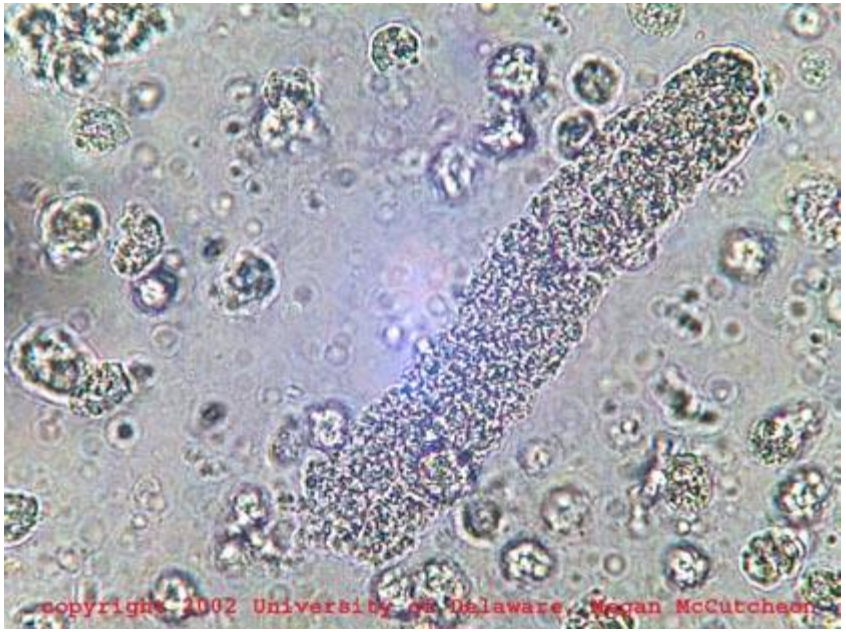
- degeneration of cellular casts or direct aggregation of serum proteins.
- Almost always indicate significant renal disease.
- May be fine granular or coarse granular casts.

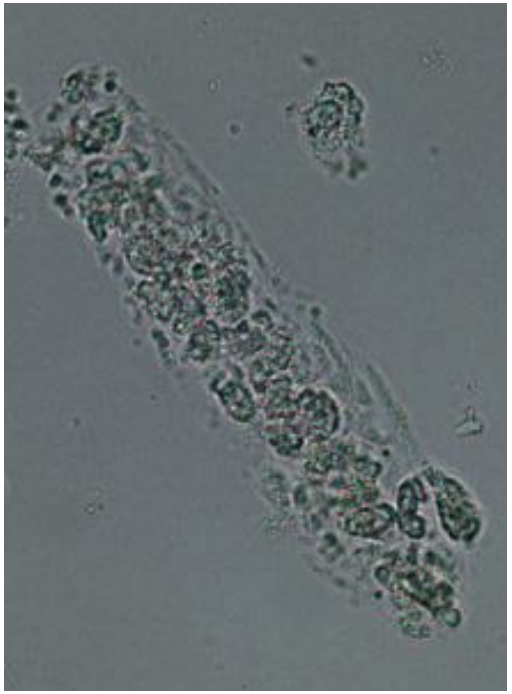




Epithelial cells casts

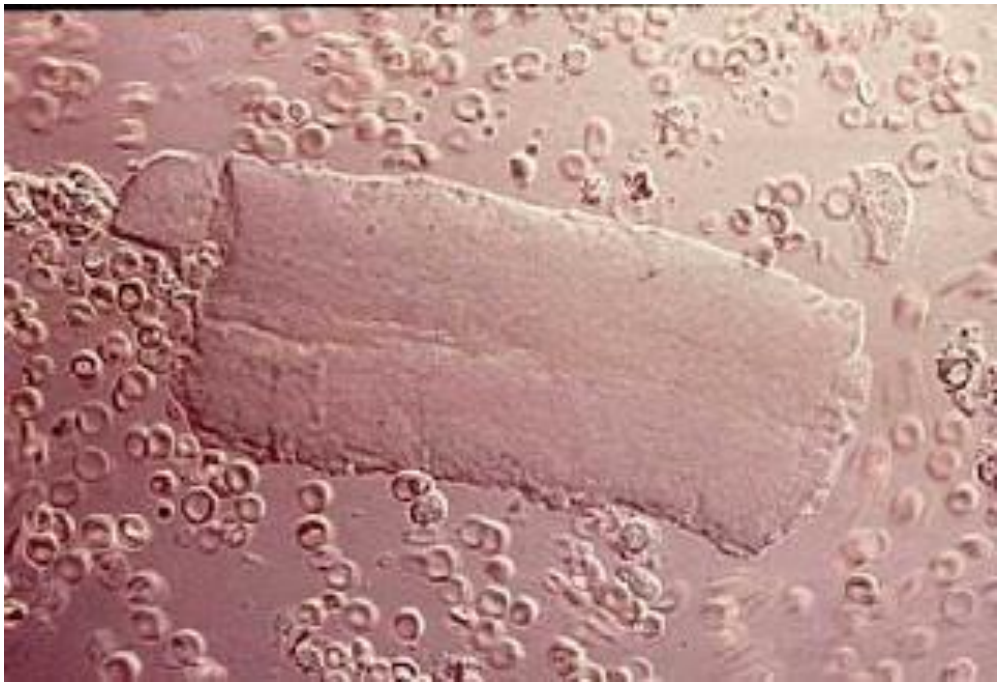
- result as statis and desquamation of renal tubular epithelial cells.
- Indicates tubular injury.





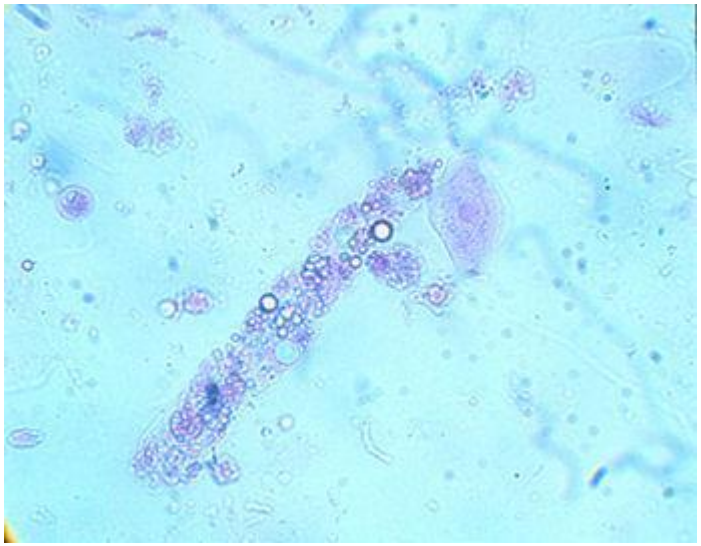
Waxy casts

- smooth homogenous appearance.
- Results from degeneration of granular casts.
- Found in acute and chronic renal disease.



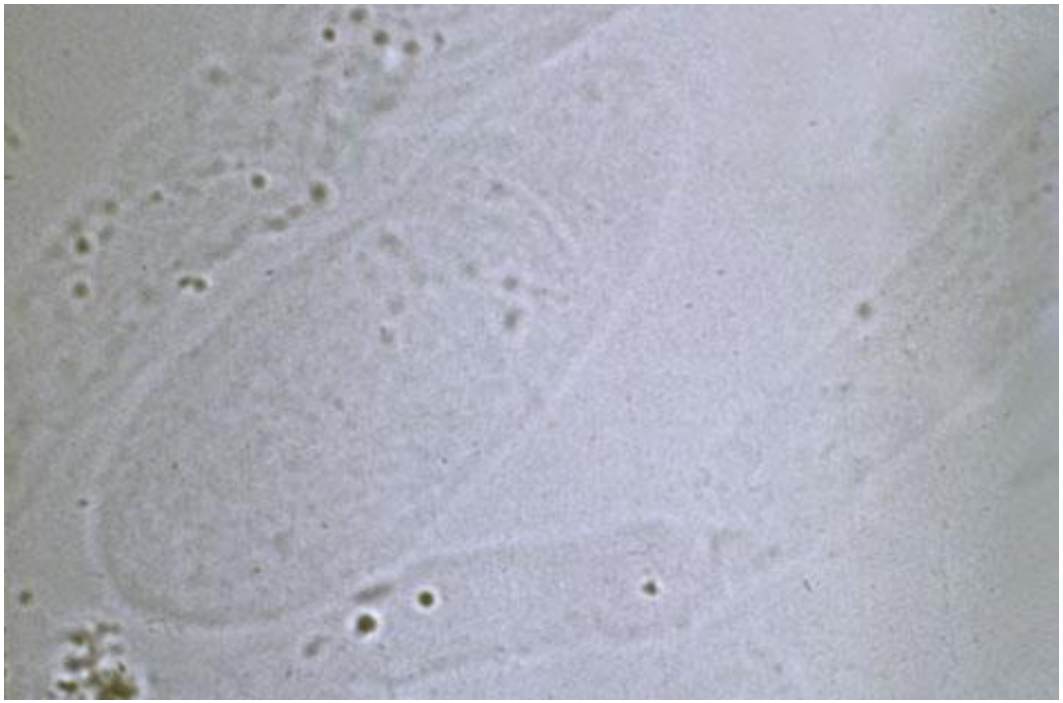
Fatty casts

- Appear as a few fat droplets or compose almost entirely of fat droplets of various sizes.
- Found in fatty degeneration of tubular epithelial.



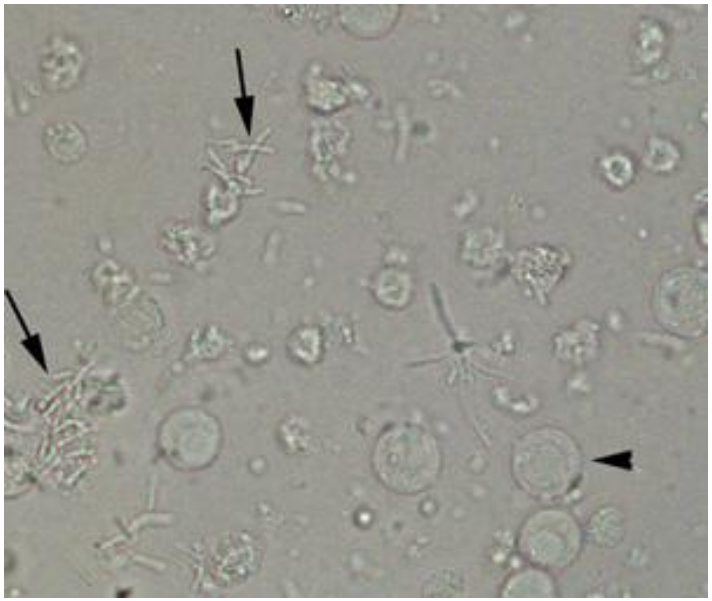
Hyaline cast

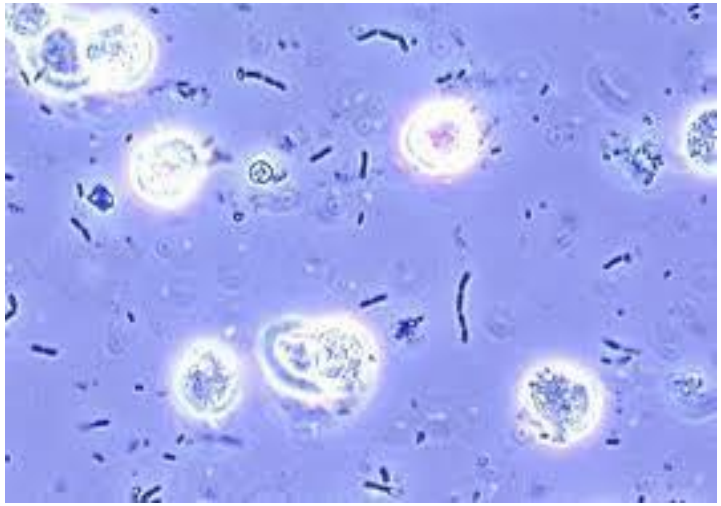
- Damage to glomerular capillary membrane, fever, orthostatic proteinuria, and emotional stress or strenuous exercise.



Bacteria

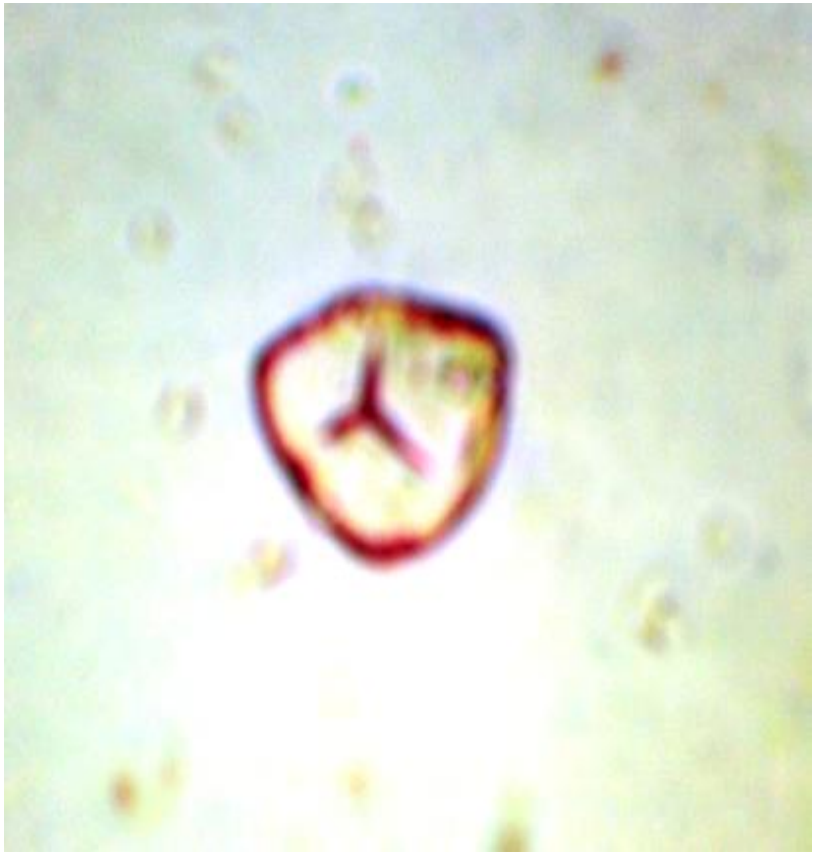
- When accompanied with white cells usually indicates UTI.
- Occurs as rod or chains or cocci.



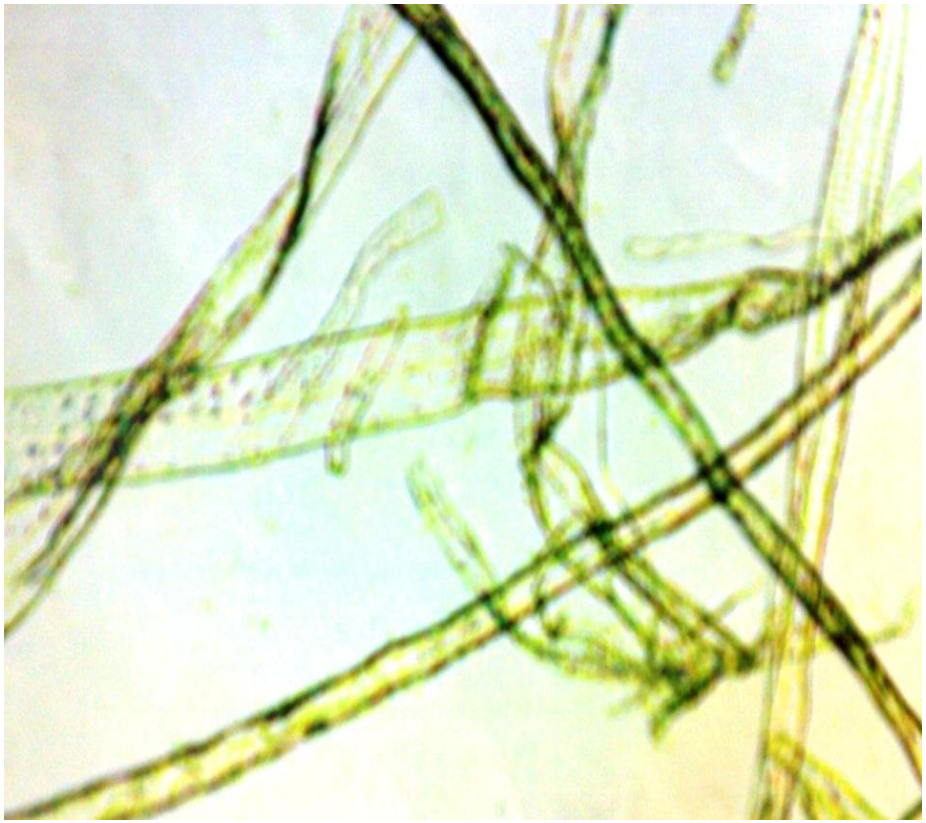


Artifacts

Starch crystals

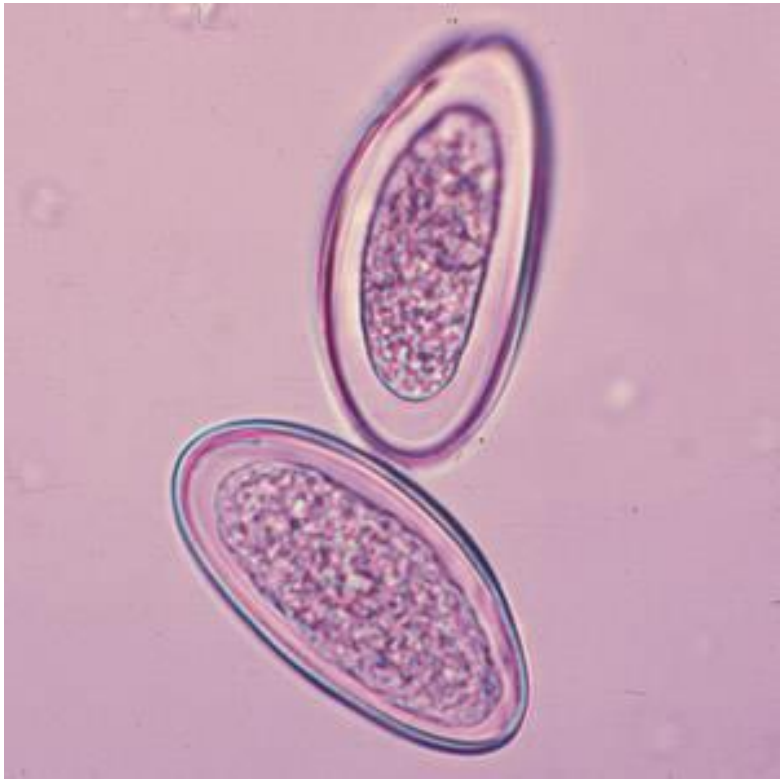


Fibres



Parasites

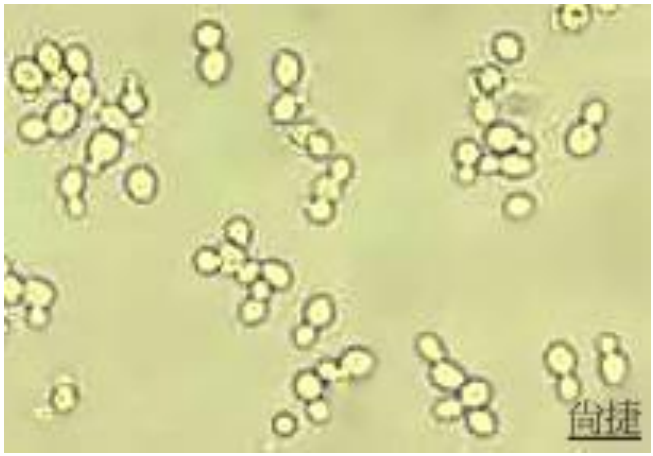
Enterobius vermicularis

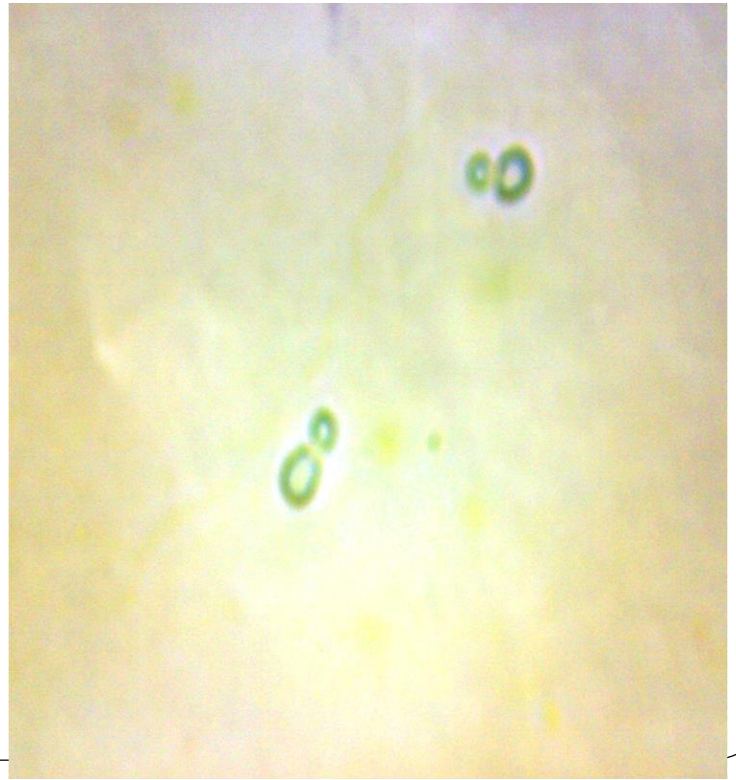


Schistosoma Haematobioum

Yeast

- Usually ovoid cells with budding.
- Not dissolve in 2%acetic acid solution and not stained with Eosin.



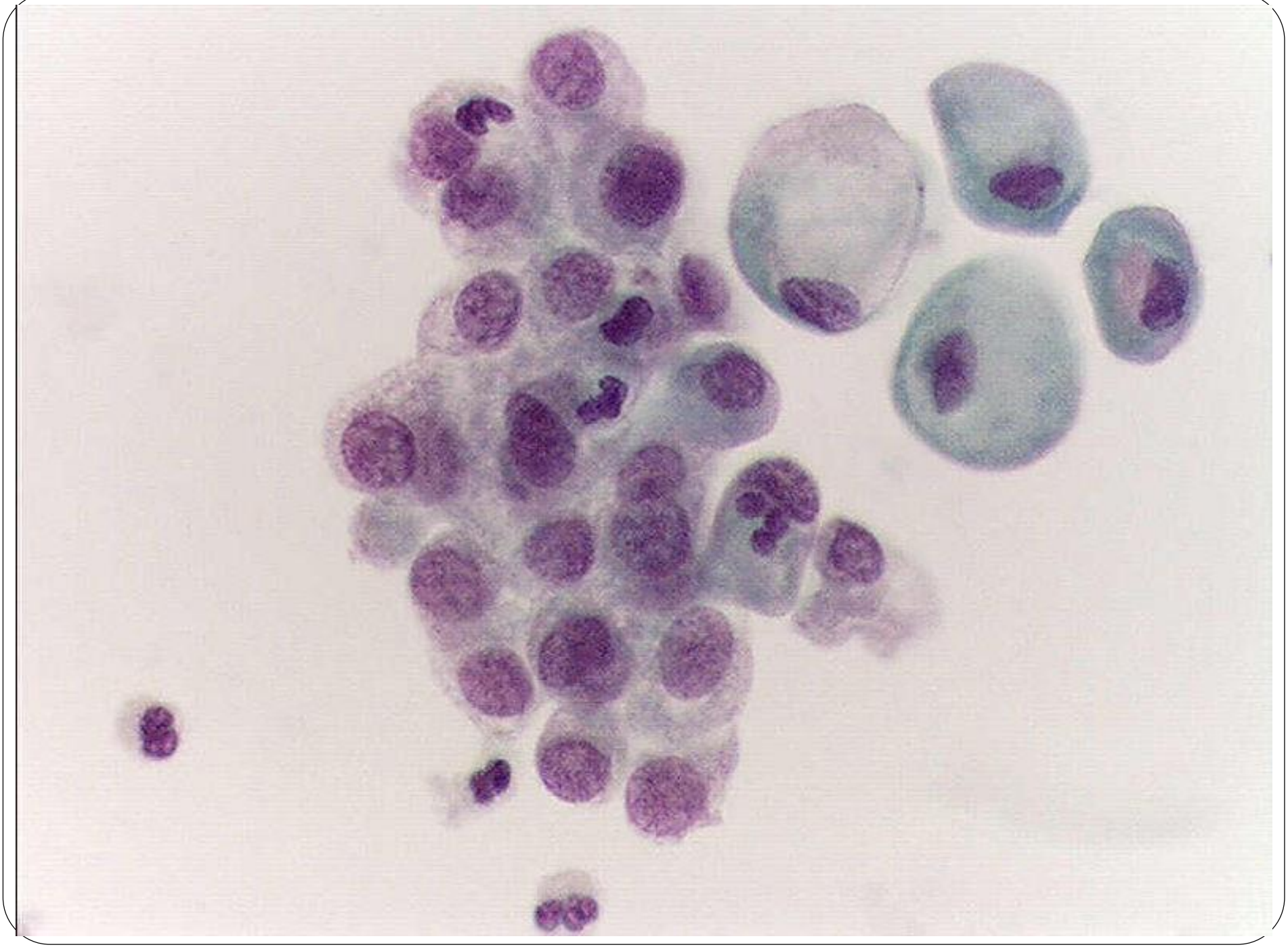


Malignancy

- **Urinary bladder**
- **Renal pelvis**
- **Kidney**
- **Ureter**
- Adjacent organs.

Types

- Low grade papillary tumours.
- High grade papillary tumours.



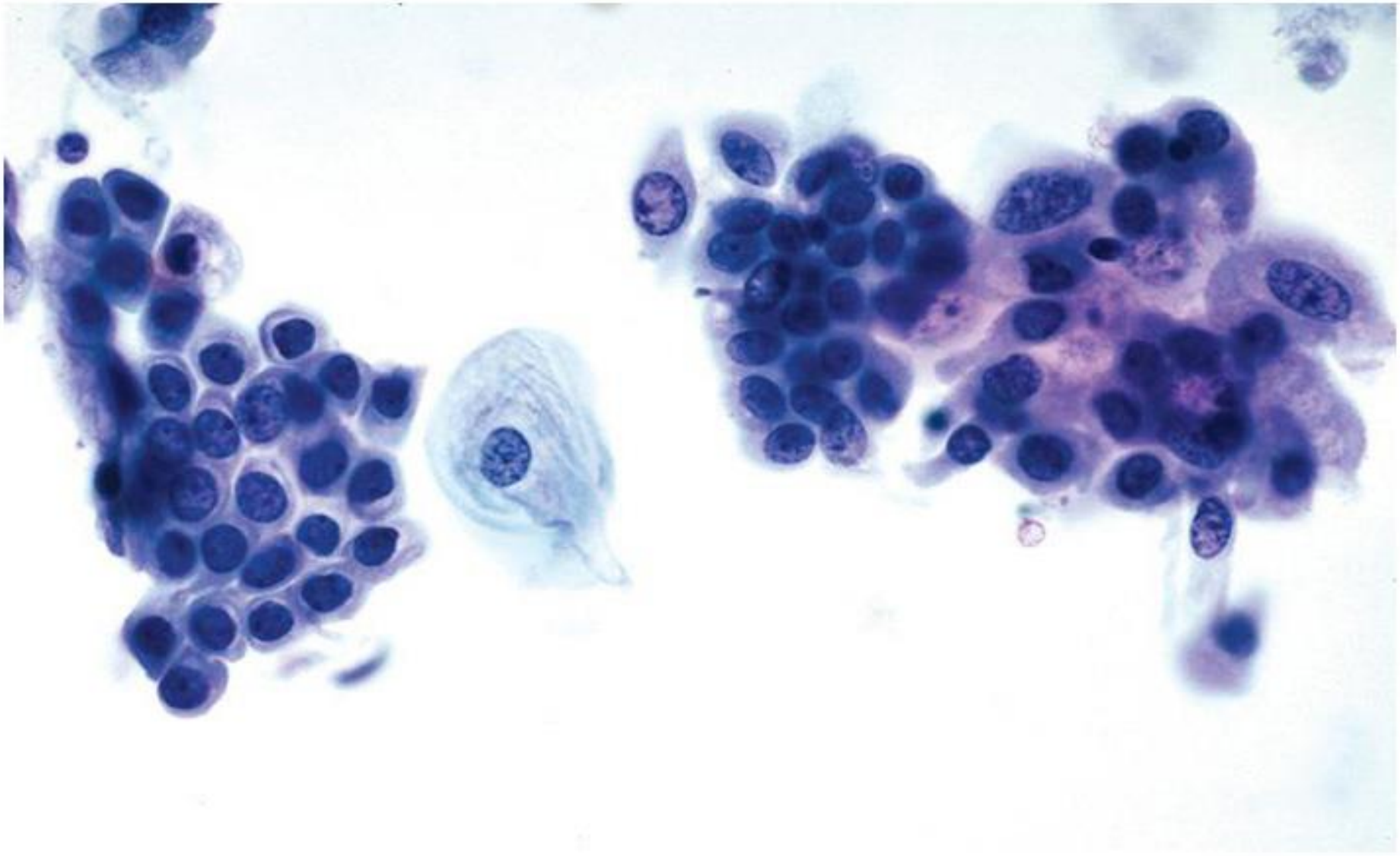


Figure 23-18C Various aspects of low-grade papillary tumors (tumors with low malignant potential) in voided urine sediment. Comparison between two clusters of urothelial cells, one showing normal configuration (left) and one showing slight nuclear enlargement and hyperchromasia (right). (*A–C:* High magnification.)

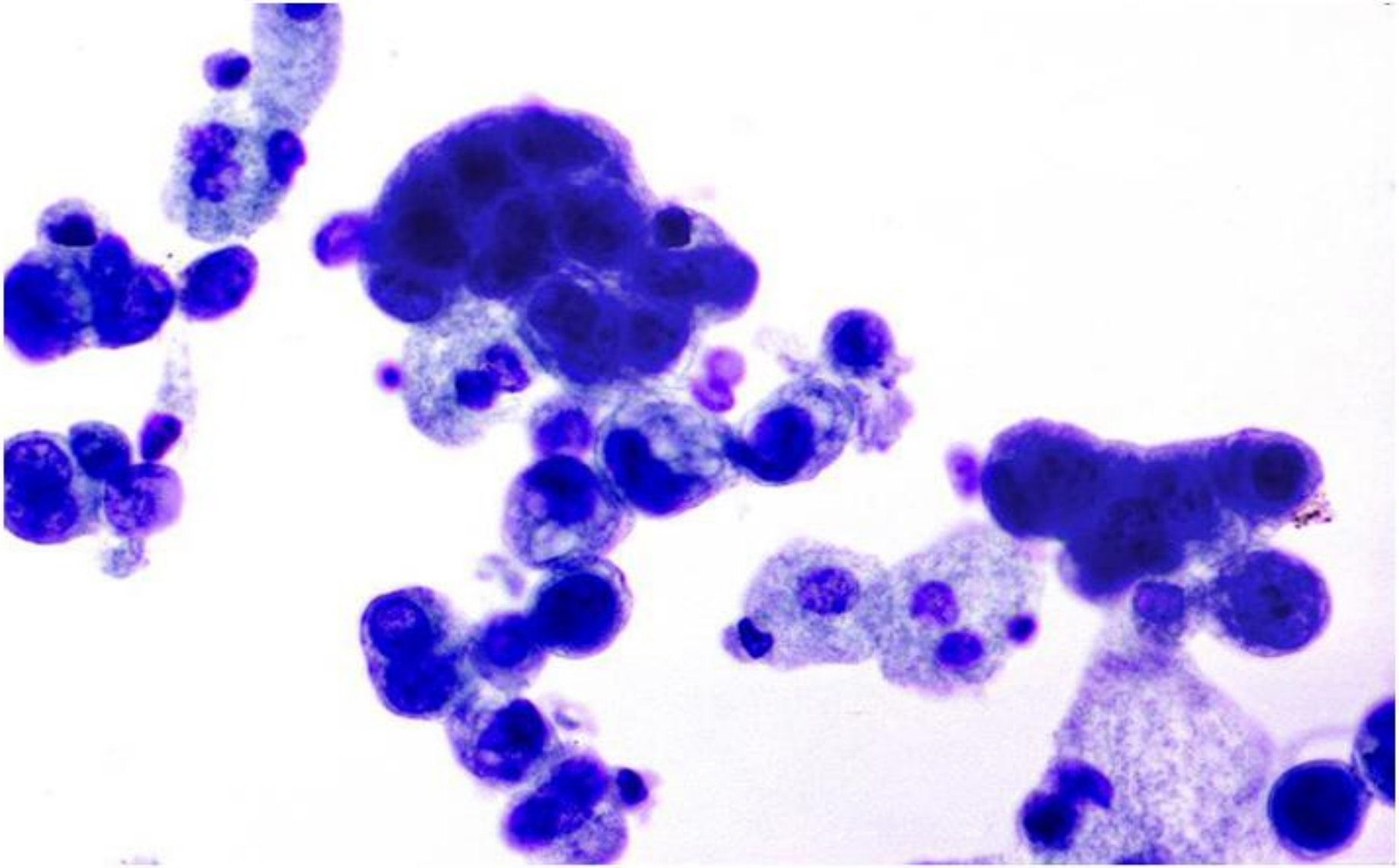


Figure 23-14B Cancer cells in urinary sediment. Cancer cells, appearing singly and in clusters, in voided urine sediment (ThinPrep).

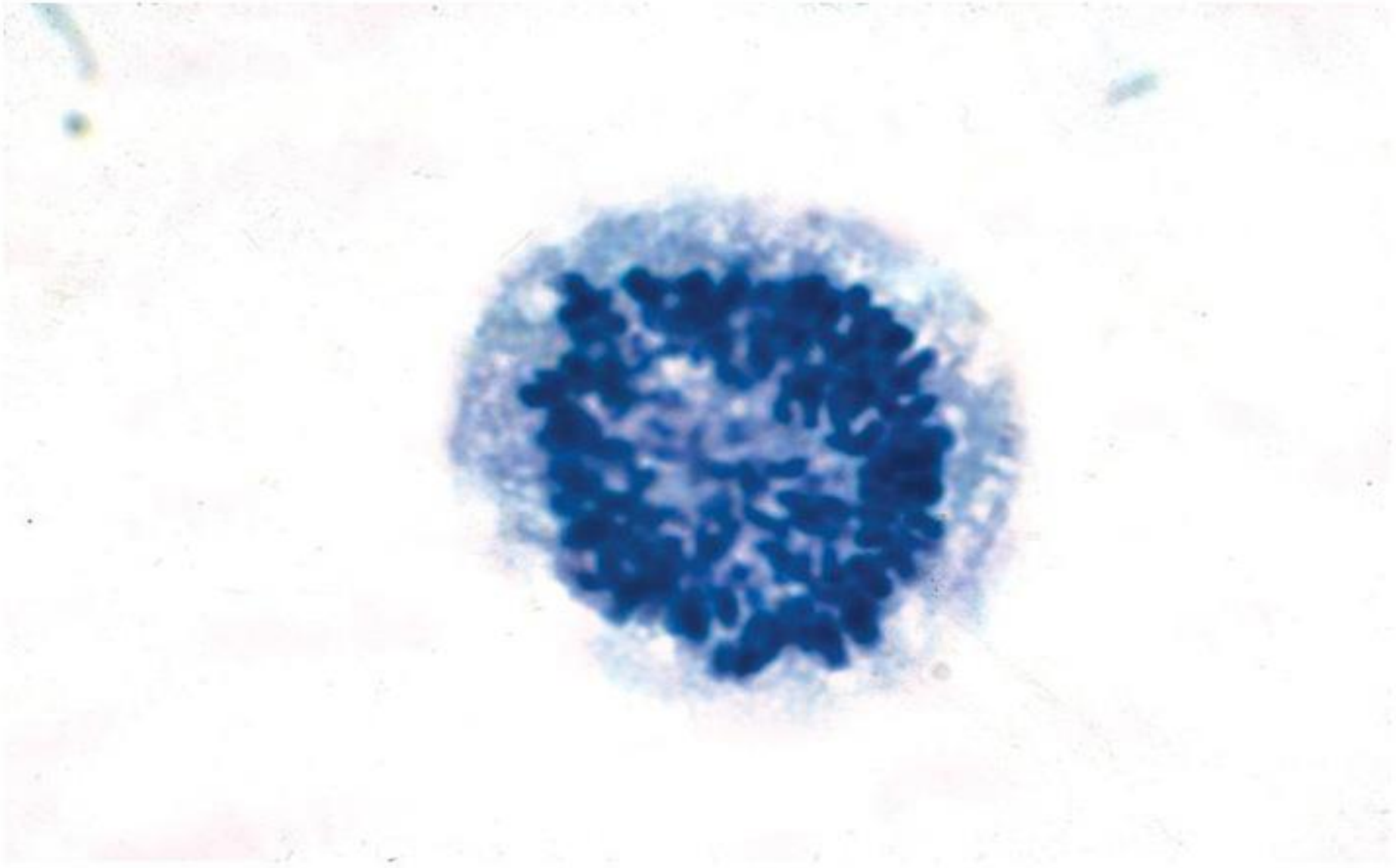


Figure 7-23D Mitotic abnormalities in cancer cells. Carcinoma of bladder, voided urine sediment with a tumor cell metaphase containing numerous chromosomes. (*A*,*B*: High magnification; *D*: oil immersion.) (*A* and *B* Courtesy of Dr. Carlos Rodriguez, TucumaAAn, Argentina.)

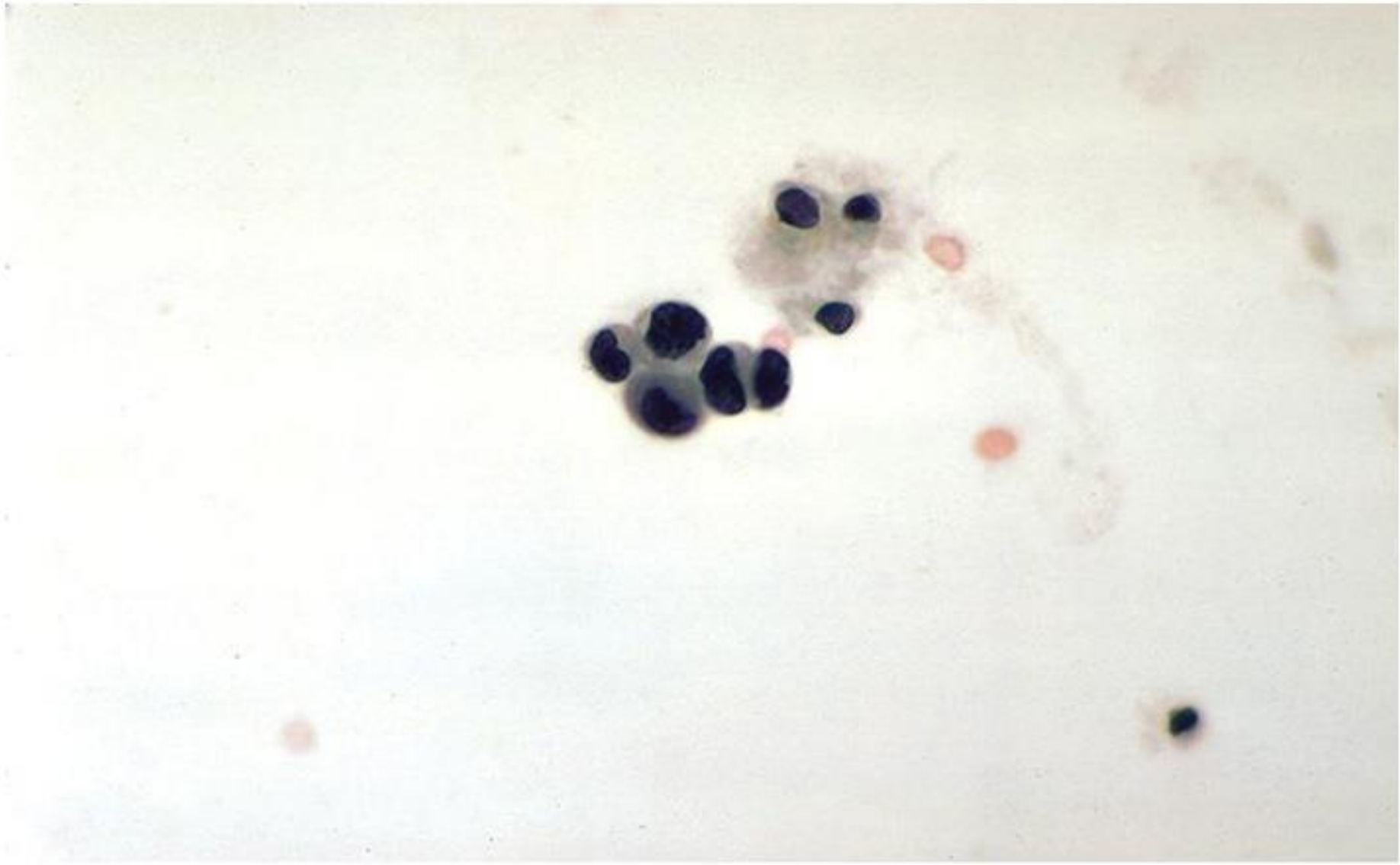


Figure 33-10 Prostatic carcinoma in voided urine. A small cluster of small cancer cells with relatively large, hyperchromatic nuclei. The details of the nuclear structure are not visible. The prostatic origin of the cluster is not secure.

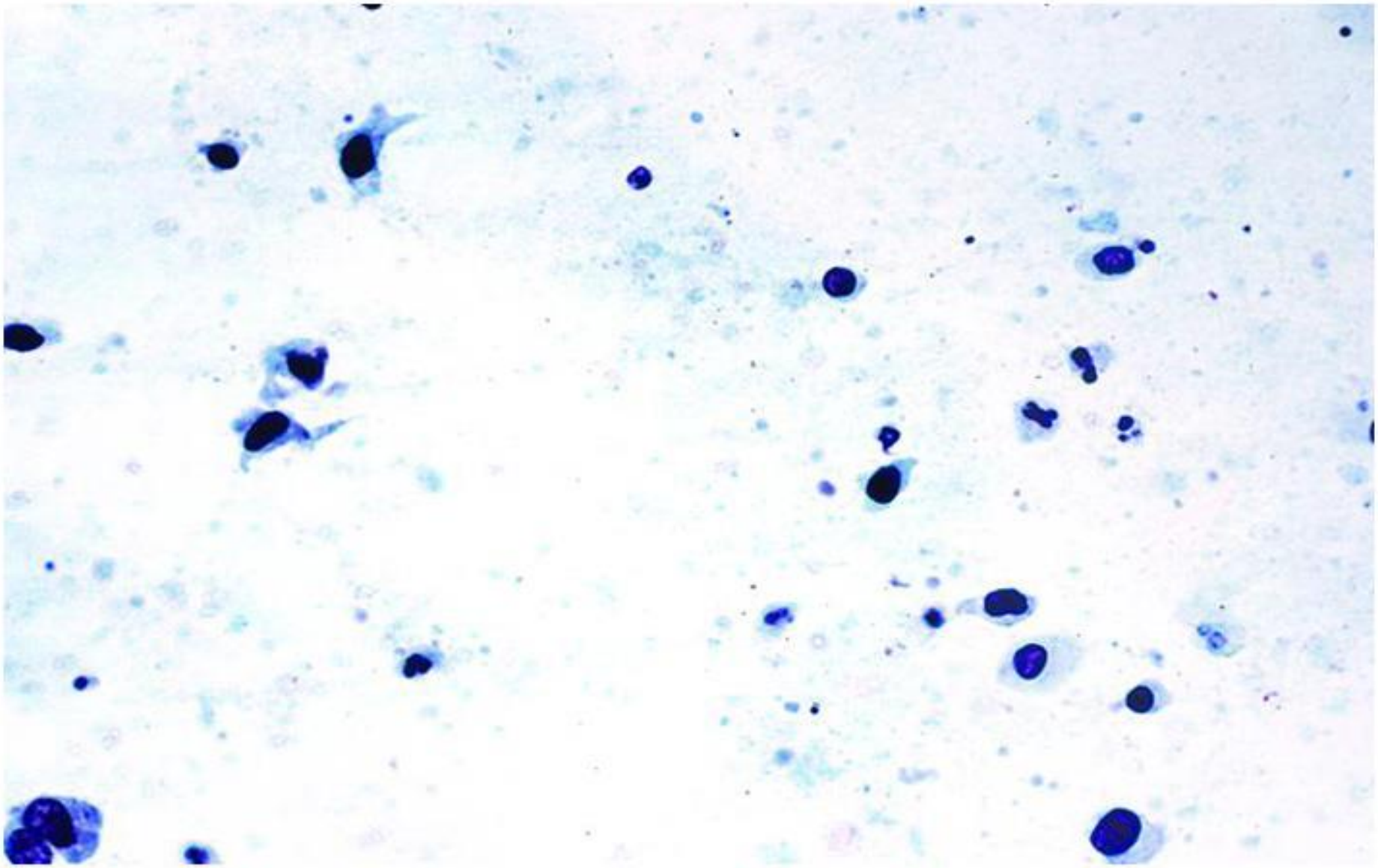


Figure 23-40C Renal pelvic carcinoma. Voided urine sediment (scanning power) containing small cancer cells corresponding to the tumor of renal pelvis shown in *D*.

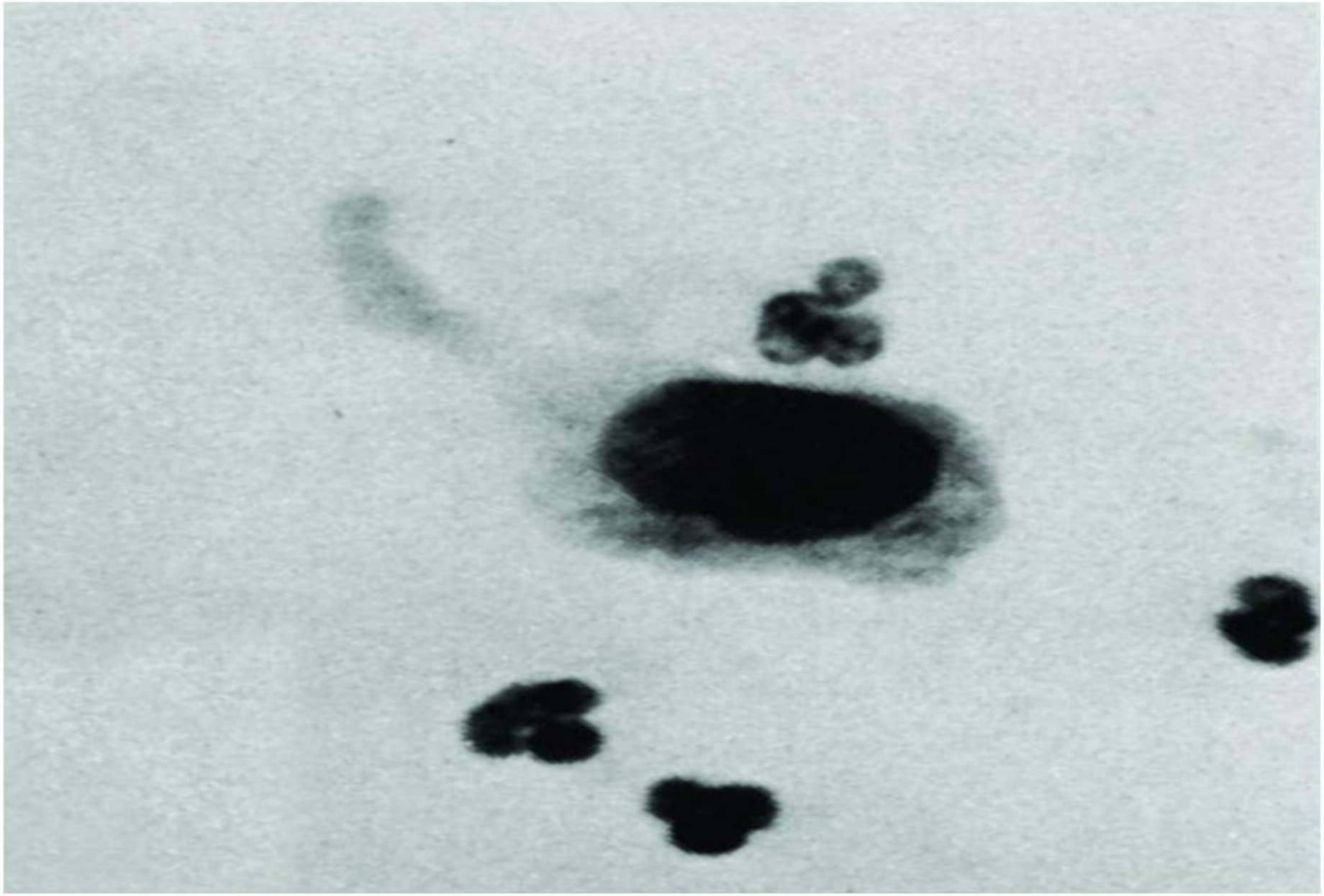


Figure 23-38A Choriocarcinoma of urinary bladder, accompanied by a flat carcinoma in situ (not shown). Cancer cells in voided urine resembling cells of urothelial carcinoma.