Examination of Urine: Detection and Estimation of Some Abnormal Constituents

- Urinalysis:

- The urinalysis is one of the most commonly ordered clinical tests in pediatrics.
- This frequency is partly due to the ease of urine collection and testing.
- Two types: Physical and chemical analysis.

- Urine:

1- Physical Examination:

- Volume, odour, color, pH, and specific gravity.

2- Chemical Examination:

- Blood (RBC), leukocyte, ascorbic acid, glucose, nitrite, ketone bodies, proteins, amino acids, bilirubin, uroblinogen.

1- Physical Examination (abnormal):

PH

Acidic below 5:
- Diabetic Ketoacidosis.

Alkaline above 8:
- Due to bacteria infection.

Color

Dark yellow:
-Dehydration.
-Metabolic disorders.
-Medications.

Pink or Red color:
-Hematuria.
-Medications.

Orange:
-Presence of bilirubin.
-Medication.

Volume

Polyuria:
-Diabetes mellitus.

Oliguria:
-diarrhea or vomiting .

Anuria:
-Obstruction due to a stone or tumor.

Specific Gravity

High:
-Diarrhea that causes dehydration.
-Sugar, or glucose, in the urine.

Low:
-Diabetes insipidus.

Odor

Acetone:
-diabetes mellitus.

2- Chemical Examination:

- The following are some abnormal constituent that **not normally** found in **detectable** amount:

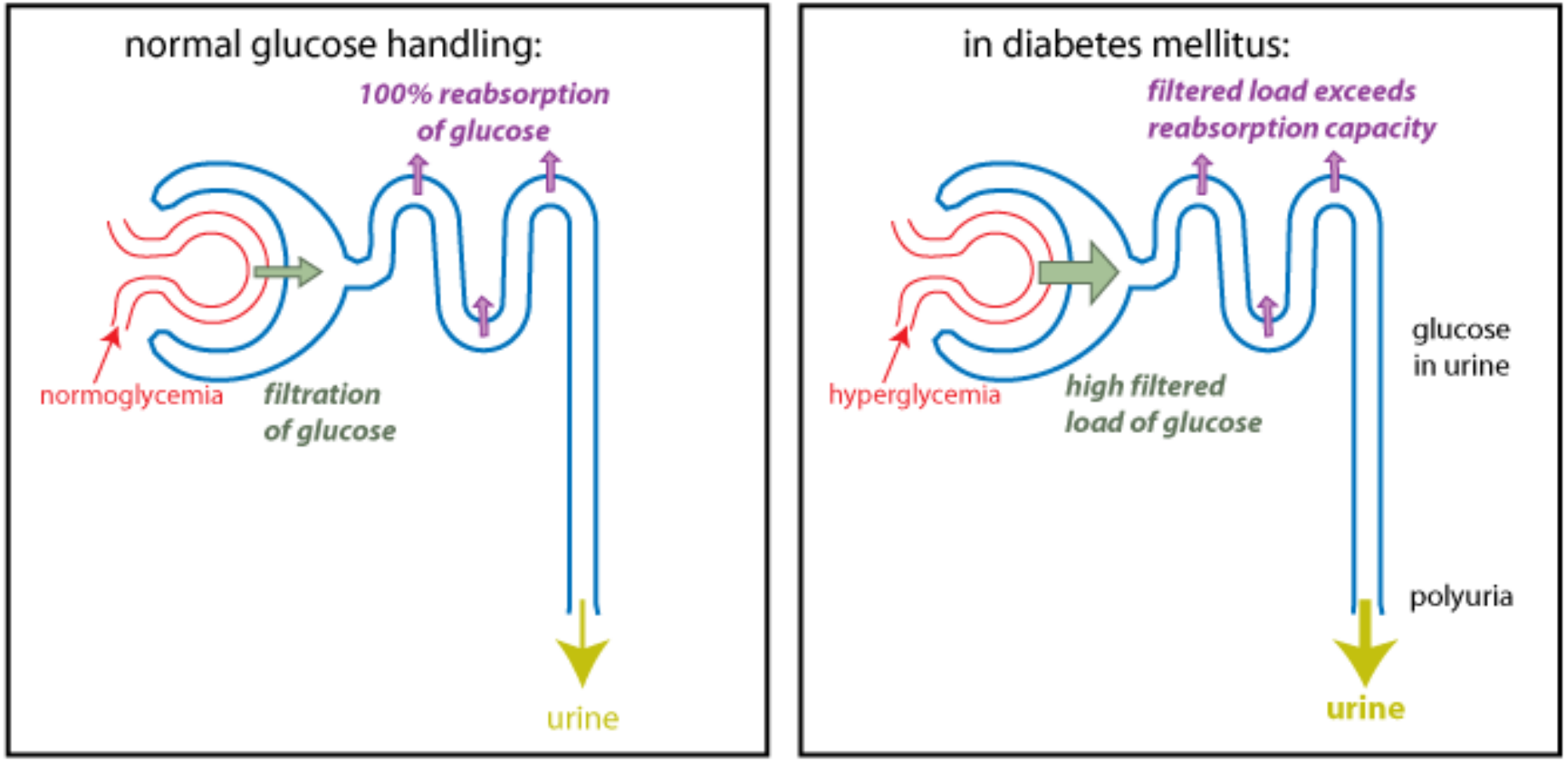
Positive in Urine	Cause	Notes
Blood (hematuria)	• Bleeding because of damage to kidney or genitourinary system,eg: Renal Calculi, Renal Tumor, Tauma to kidneys. • Urinary tract infection.	• Any pink, red or brown urine must be considered as bloody until proved otherwise. 
Haemoglobinuria	• Intravascular haemolysis due to haemolytic anemia.	
Leukocyte	• Urinary tract infection bacteria.	
Ascorbic acid	• Large urinary concentrations arise from therapeutic doses of vitamin C.	

2- Chemical Examination cont':

positive in Urine	Cause	Notes
Glucose (Glycosuria)	• Blood glucose level exceeds the reabsorption capacity of the tubules, eg, **Diabetes mellitus.** • Defect in the tubular reabsorption **eg. fanconi syndrome.**	- Normally, Glucose is present in the glomeroular filtrate and reabsorbed by the proximal tubules. (see next slide)
Ketone bodies	• Occur whenever increased amounts of fat are metabolized eg, **Diabetes mellitus, Starvation.**	- Urine may have a fruity (aceton) smell .
Nitrite	• Urinary tract infection Bacteria that can reduce the nitrate to nitrite.	- Bacteria that can reduce the nitrate to nitrite.

- Note:

- Glucose level exceeds the reabsorption in diabetes:



2- Chemical Examination cont':

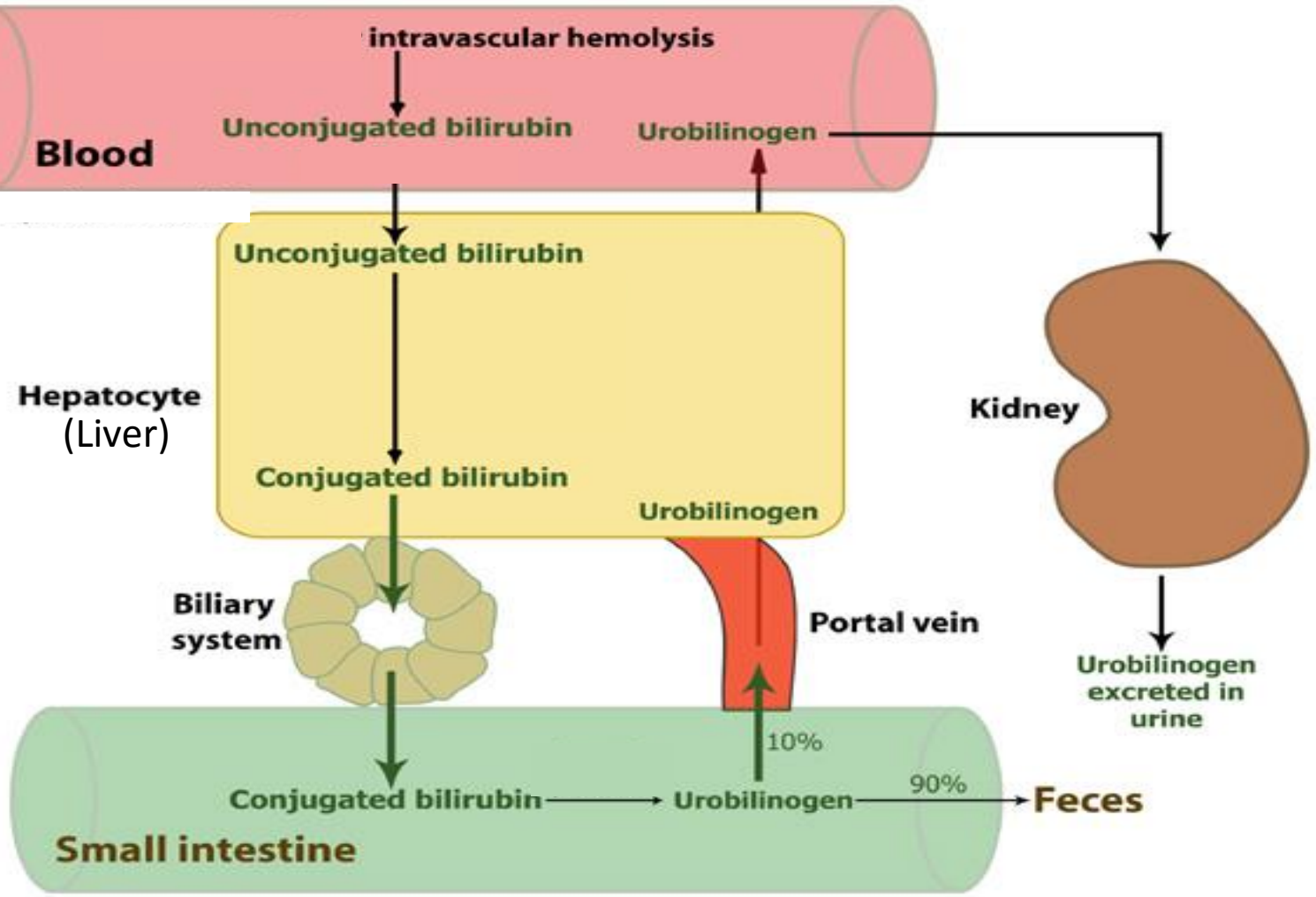
Positive in Urine	Cause	Notes
Bilirubin	• Elevated amount of bilirubin in the blood stream, eg, **Bile duct obstruction.**	• The urine may be dark with a yellow foam if much is present.
Uroblinogen	• Increased production eg, **hemolytic anemia.**	• Its presence does <u>not</u> give a colored foam.
Amino acid (aminoaciduria)	• Blood amino acid level exceeds the reabsorption capacity of the tubules eg, **Phenylketonuria, Alkaptouria** • Defect in the tubular reabsorption eg, **fanconi syndrome, cystinuria**	
Protein	• Acute infection. • Primary kidney disease. • Secondary kidney disease.	

- Aminoacidurias:

Aminoacidurias	Amino Acids Increased in Urine and Blood
Phenylketonuria	Phenylketonuria
Tyrosinosis	Tyrosine
Histidinemia	Histidine
Maple syrup urine disease	Valine, leucine, and isoleucine
Hypervalinemia	Valine
Hyperglycinemia	Glycine (lysine on high-protein diet)
Hyperprolinemia	
Type I Type II	Proline
Hydroxyprolinemia	Hydroxyproline

- Note:

- Bilirubin and Urobilinogen :



-Bilirubin and Urobilinogen:

TABLE 3.5 Comparison of Urine Urobilinogen and Urine Bilirubin Values

Test	In Health	In Hemolytic Disease	In Hepatic Disease	In Biliary Obstruction
Urine urobilinogen	Normal	Increased	Increased	Low or absent
Urine bilirubin	Negative	Negative	Positive or negative	Positive

NOTE: Biliary obstruction refers to the blockage of any duct that carries bile from the liver to the gallbladder or from the gallbladder to the small intestine.

- Notes in using test strip:

- Reagent strips should be stored in their original container.
- The lid should be kept tightly closed.
- Strips should **not** be used if expired or discoloured.
- Strips should not be exposed to sunlight, moisture, heat, or cold.
- The specific reagents should be read at the appropriate time after dipping in urine, as recommended by the manufacturer.
- The strip should **not** be dipped for more than a second in the urine, and excess urine should be blotted off on the edge of absorbent paper to prevent mixing of reagents.

-Types of urine specimens:

- Type of specimen and collection procedure are determined by physician and depend on the tests to be performed.

- There are basically four types of urine specimens:

Sample type	Sampling	Purpose
Random specimen	No specific time most common, taken anytime of day	Routine screening, chemical
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

Note : 24h sample is necessary for accurate quantitative results.

Practical Part

- Objectives:

1. The semi-quantitative detection of some abnormal constituents using test-strips.
2. The detection of amino-acids in urine sample using ninhydrine.
3. The effect of the type of urine collection in the detection of urine constituents.

1- Detection of some abnormal constituent of urine using test strip:

- Method:

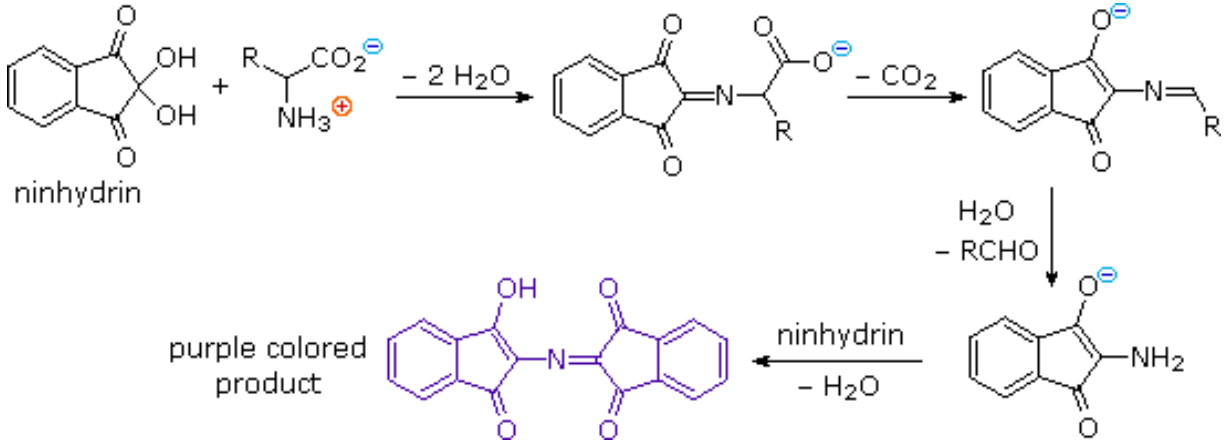
- You will have 2 different urine sample.
- You should fill the following information and then the probable diagnosis:

Test	Sample 1	Sample 2	Sample 3
Volume	3000 ml	900 ml	1000 ml
Color			
Odor			
pH			
Specific gravity			
Protein			
Blood			
Bilirubin			
Uroblinogen			
Glucose			
Ketone			
Nitrite			
Leukocyte			
Clinical Diagnosis for sample			

2- Detection of amino acid using ninhydrin:

- Principle :

- Ninhydrin reacts with all amino acids <u>except proline and hydorxyproline</u> at pH 3-4 to give a purple coloured compound.
- Proline will give **<u>a yellow color</u>**.
- Initially, the amino acid is oxidized to an aldehyde containing one carbon atom less together with the release of ammonia and carbon dioxide. Then the ammonia, ninhydrin and the reaction product hydrindantin react to form the purple product.



- Method:

- As standard, use proline and glycine as the following table:

Solution	
Glysine	Add 1 ml of Glysine
Proline	Add 1 ml of proline
Urine Sample	Add 1 ml of sample

- Add a few drops of ninhydrin solution to each test-urine.
- Boil the contents of each test tube for 2 minutes.
- Record your observations.

Solution	Observation
Glycine	
Proline	
Urine sample A	

3- The effect of the type of urine collection on the detection of Urine constituents:

-Method:

- You have two samples, one is random urine sample, the other is 24-hour urine sample from the same patient.
- Compare between the two samples using the test strip.

Test Parameter	24 hour Urine sample	Random urine Sample
Protein (positive or negative)		