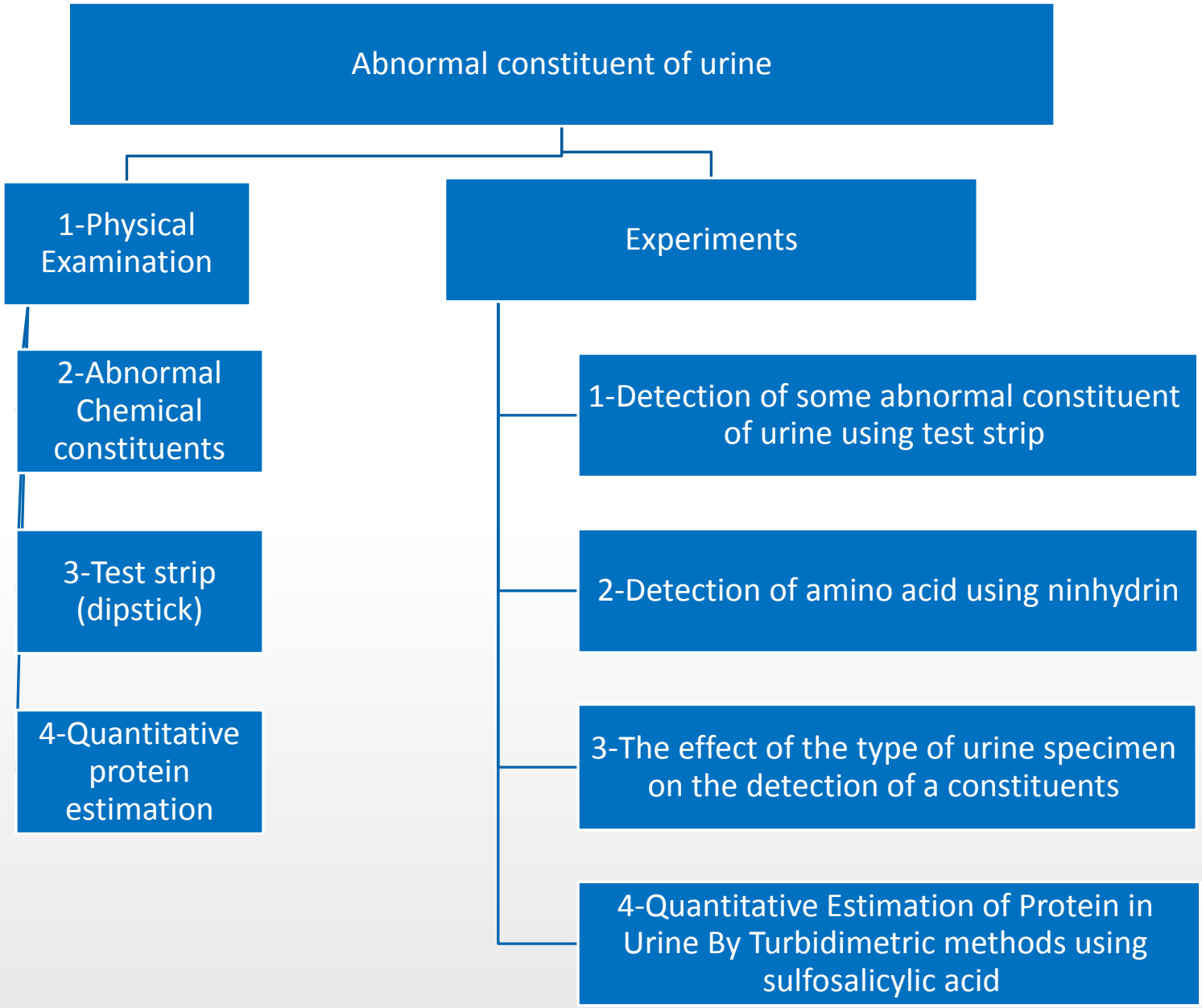
Examination of Urine:

Detection and Estimation of Some Abnormal Constituents.

Objectives

- The semi-quantitative detection of some abnormal constituents by means of test-strips.
- The detection of amino-acids in abnormal urine using ninhydrine.
- The effect of the type of urine collection in the detection of urine constituents.
- The quantitative estimation of protein in abnormal urine.

Summary


Abnormal constituent of urine
1-Physical Examination
2-Abnormal Chemical constituents
3-Test strip (dipstick)
4-Quantitative protein estimation
Experiments
1-Detection of some abnormal constituent of urine using test strip
2-Detection of amino acid using ninhydrin
3-The effect of the type of urine specimen on the detection of a constituents
4-Quantitative Estimation of Protein in Urine By Turbidimetric methods using sulfosalicylic acid

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.

Physical Analysis:

PH

Acidic below 5

-Diabetic Ketoacidosis

Alkaline above 8

-due to bacteria infection

Color

Dark yellow , Orange

-Dehydration

-Metabolic disorders

-Medications

Pink or Red color

-Hematuria

-Medications

Specific Gravity

High

-Diarrhea that causes dehydration

-Sugar, or glucose, in the urine

Low

-Diabetes insipidus

Volume

Polyuria

-Diabetes mellitus

Oliguria

-diarrhea or vomiting

Anuria

-Obstruction

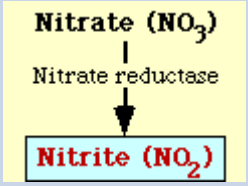
due to a stone or tumor

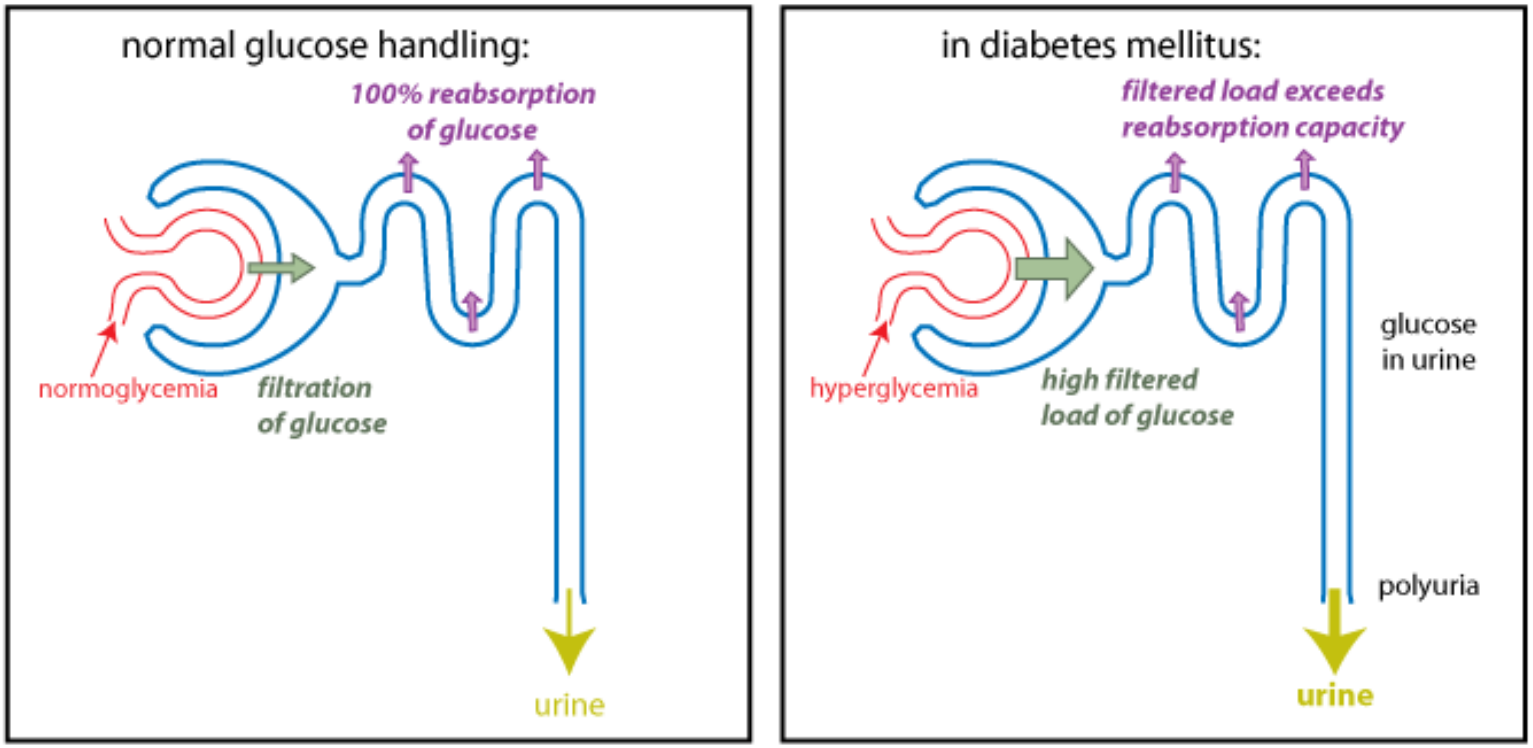
Some abnormal constituents in urine

- **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,Truma to kidneys**	• Any pink, red or brown urine must be considered as bloody until proved otherwise. 
Haemoglobinuria	• Is due to intravascular haemolysis.	
Leukocyte	• Urinary tract infection bacteria	
Ascorbic acid	• Large urinary concentrations arise from therapeutic doses of vitamin C.	

Some abnormal constituents in urine

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**	Glucose is present in the glomeroular filtrate and reabsorbed by the proximal tubules
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



Glucose glucose level exceeds the reabsorption in diabetes

Some Abnormal constituents in urine

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 jaundice**	• Its presence does not give a colored foam
Amino acid (aminoacid uria)	• Blood amino acid level exceeds the reabsorption capacity of the tubules **eg**, **Phenylketonuria**, **Alkaptouria** • Defect in the tubular reabsorption **eg**, **fanconi syndrome**, **cystinuria**	

Test strip (dipstick)

- ***The test strips consist of*** a ribbon made of absorbent microfiber cellulose pads attached to it.
- ***Each pad contains*** the dried reagents needed for a specific test that react with the compounds present in urine producing a characteristic colour. The depth of color produced relates to the concentration of the substance in the urine.
- ***It provides quick*** **Semi-quantitative** determinations of pH, protein, glucose, ketones, bilirubin, hemoglobin (blood), nitrite, leukocyte , urobilinogen, and specific gravity.
- ***Color changes then*** matched to the control chart at the correct time after each stick is dipped into the urine specimen.

! 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 discolored. 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

Test strip (dipstick)

- ***Normally,*** substances such as nitrate, proteins, glucose, ketone bodies, bilirubin, urobilinogen and blood are present in very small quantities that is not capable of detection by this method.
- but present in detectable amount are ***not normal***

False positive and false negative are common when using dipstick

	False-positive	False-negative
protein	Alkaline Urine Ammonia	Dilute Urine
Glucose	Strong Oxidizing agent	Ascorbic acid
Blood	Oxidizing contaminants	High Ascorbic acid
Bilirubin	Pigmented urine	Ascorbic acid,nitrite
Uroblinogen	Alkaline Urine	Nitrite formaline
Nitrite	Pigmented urine	Ascorbic acid
Leukocytes	Oxidizing detergent	

Quantitative protein estimation

- ***In a healthy renal and urinary tract system***, the urine contains no protein or only trace amounts.
- ***The presence of increased amounts of protein*** in the urine can be an important indicator of renal disease. It may be the first sign of a serious problem and may appear before any other clinical symptoms.
- ***However,*** there are other physiologic conditions (eg, exercise, fever) that can lead to increased protein excretion in urine. Also, there are some renal disorders in which proteinuria is absent.
- ***The quantitative estimation*** of the daily excretion of protein is of value to the clinician in order to determine the type of renal disease, its severity and to monitor the results of treatment given.

Types of proteinuria

Type	Cause
Glomerular proteinuria	• Damage to the glomerular which increased filtration of normal plasma protein and because albumin has the highest concentrtion in the plasma it is called abuminuria eg. Malignant hypertention
Tubular proteinuria	• Defect in the reabsorption(low molecular wight protein) eg, Fanconi Syndrom
Overflow proteinuria	overflow of high plasma concentrations of **low molecular weight protein** • eg, Multiple myloma
Secretory proteinuria	• over secretion of certain proteins in the tubules, most notably the over secretion of Tamm-Horsfall proteins in interstitial nephritis

Types of proteinuria

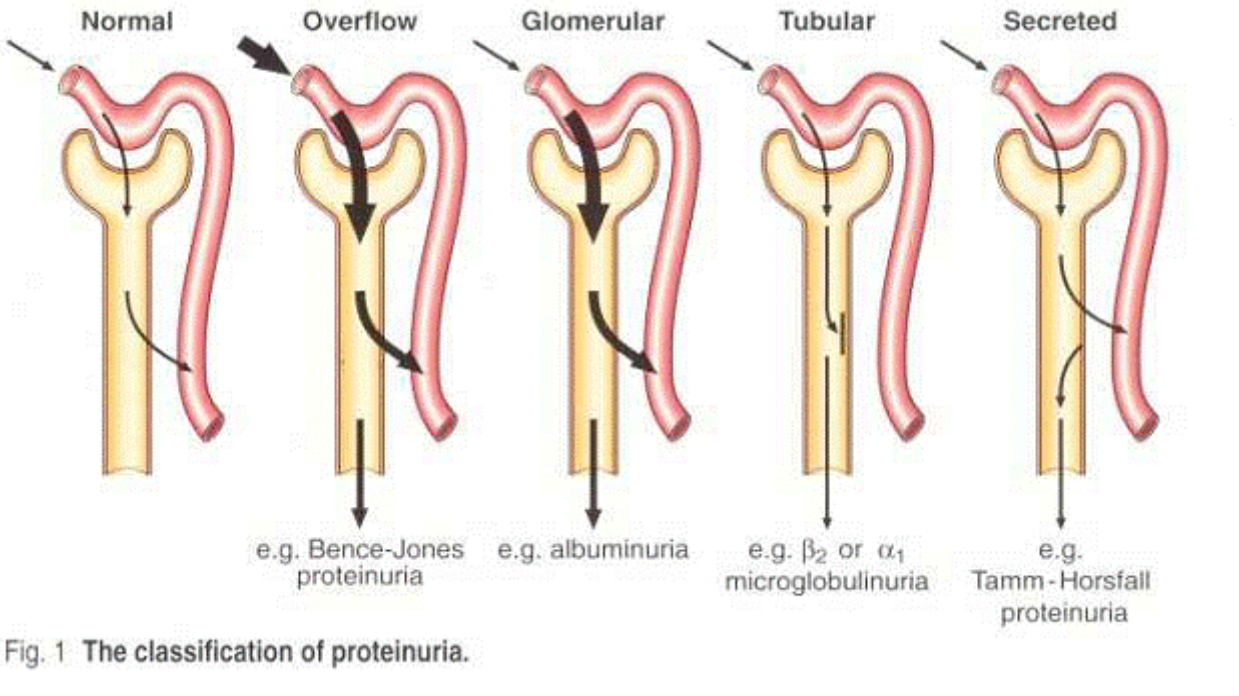


Fig. 1 **The classification of proteinuria.**

- **Glomerular proteinuria**
- has higher levels of protein , often exceeding 2 g /day ,mainly albumin
- **Tubular proteinuria**
- shows a moderate increase in urinary proteins , usually less than 2 g /day , and show an increased proportion of low molecular weight proteins

Practical Part

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

2-The effect of the type of urine collection on the detection of Urine constituents.

3-Detection of amino acid using ninhydrin

4-Quantitative Estimation of Protein in Urine By Turbidimetric methods using sulfosalicylic acid

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

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

Test	Sample 1	Sample 2
Volume	1000 ml	3000 ml
Color		
pH		
Blood		
Bilirubin		
Uroblinogen		
Glucose		
Ketone		
Clinical Diagnosis:		

2-Detection of amino acid using ninhydrin

- **Principle of amino acid detection using ninhydrin**
- Ninhydrin reacts with all amino acids except proline and hydorxyproline at pH 3-4 to give a purple colored compound. Proline will give a yellow color
- 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 :
- Label three test tubes, A, B, C then add the following:
- 1 ml of glycine solution in tube A
- 1 ml of proline solution in tube B
- 1 ml of Abnormal urine in tube C
- 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 3	

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

- 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		

4-Quantitative Estimation of Protein in Urine By Turbidimetric methods using sulfosalicylic acid

- Principle :
- Sulphosalicylic acid is used in this experiment to precipitate the protein in a 24 hour sample of urine. The turbidity is proportional to the concentration of the protein, and may be measured with a spectrophotometer.

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