Experiment 2

Examination of Urine: Detection and Estimation of Some Abnormal Constituents.

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Objectives:

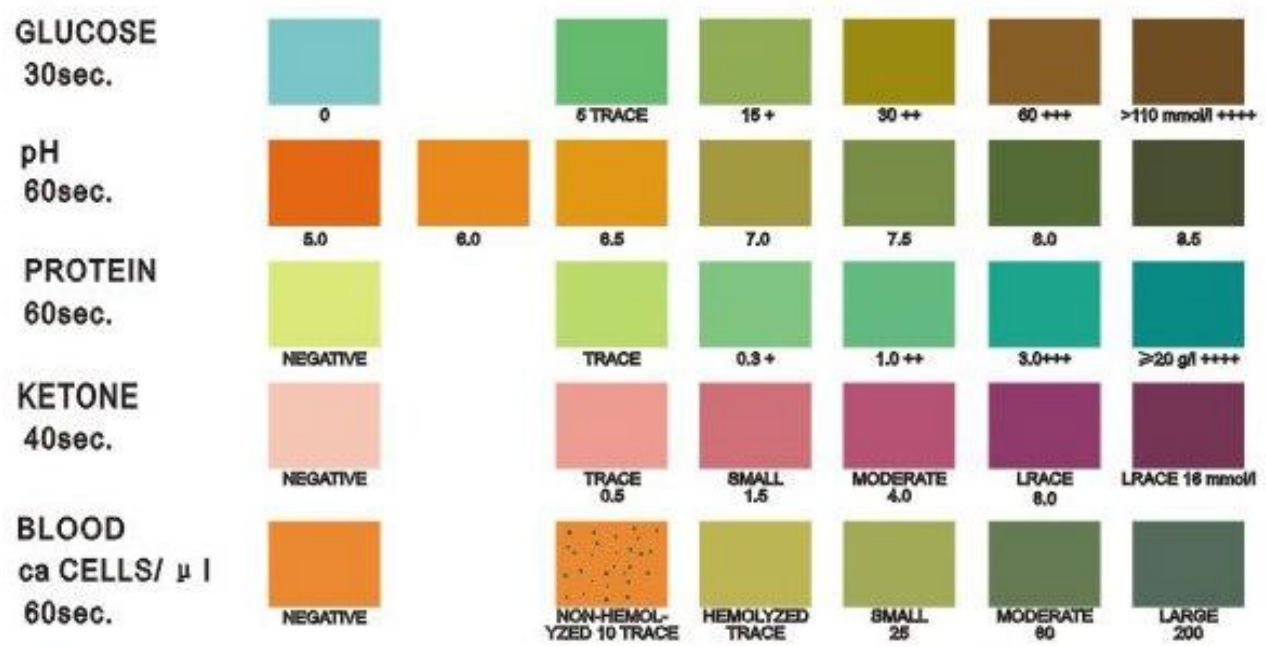
1- The semi-quantitative detection of some abnormal constituents by means of test-strips.

2- The detection of amino-acids in abnormal urine.

3- The quantitative estimation of protein in abnormal urine.

Note:

All the examination in 24 hour collection of urine



Part I: *Detection of Some Abnormal Constituents* using test-strips:

- Test strip (or dipstick) : is a basic instrument (used for **Semi-quantitative** tests on urine specimens) , used to determine *pathological changes in the urine* .
- Test strips' are commercially prepared strips which allow comparisons to be made between colors obtained by the urine sample tested and known standards.
- *Normally, substances such as nitrate, proteins, glucose, ketone bodies, bilirubin, urobilinogen and blood are not present in quantities capable of detection by this method.(the presence of those substance are normally but present in detectable amount are not normally)*
- However, because of disease the concentration of one or more of these substances may be increased to a level which is detectable.

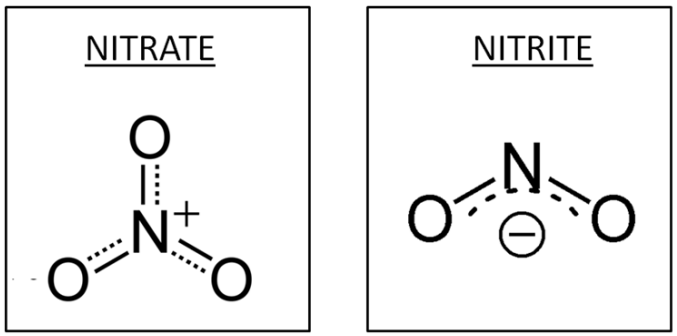
PART I: detection of some abnormal constituents by means of test-strips:

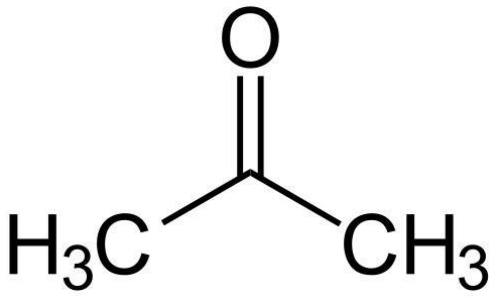
Nitrate: A positive result will occur if bacteria are present in the bladder urine.

PH: Strongly alkaline urine is due to bacteria infection of the urinary tract.

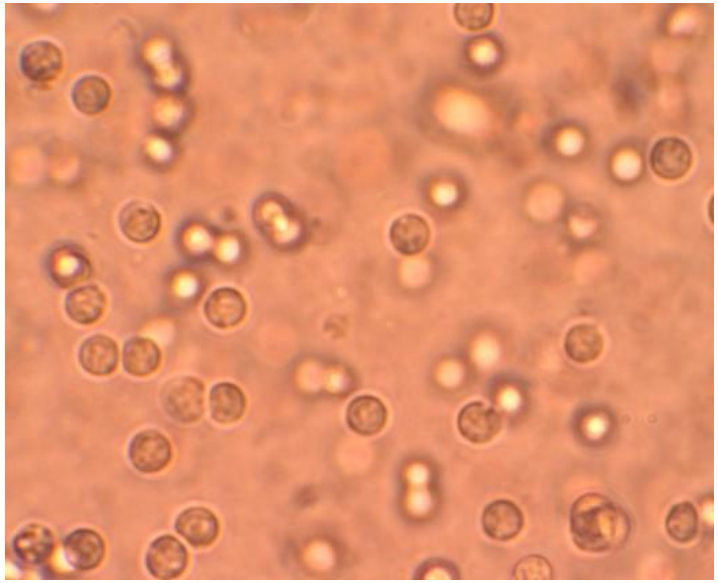
Ketone: is formed with uncontrolled diabetes mellitus and may also occur with starvation and weight reducing diets.

Ascorbic acid: Large urinary concentrations arise from therapeutic doses of vitamin C.





Ketone



Nitrate developing urinary tract infections. Urinary tract infections caused by bacteria are generally treated with correct antibiotics

Protein :

The appearance of protein in the urine gives rise to four different **types of proteinuria , depending on the source of the protein** , these are :

(1)Glomerular proteinuria arising from glomerular disease .

(2)Tubular proteinuria arising from tubular disease

(3)Overflow proteinuria arising from the overflow of high plasma concentrations of low molecular weight protein .

(4) Secretory proteinuria arising from protein secreted by the kidney tubule.

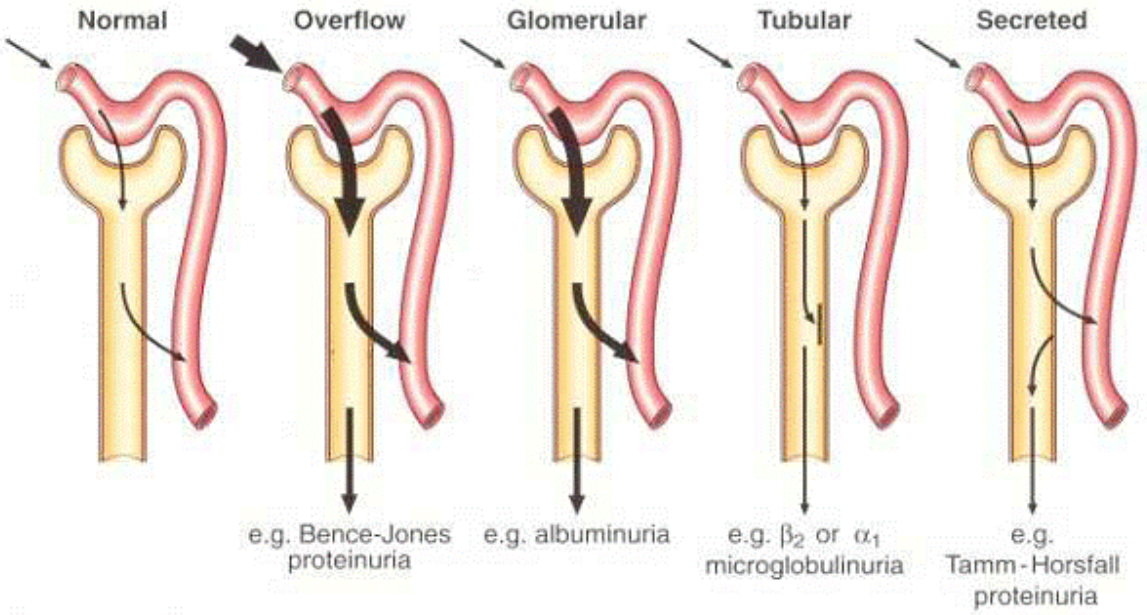


Fig. 1 **The classification of proteinuria.**

✓The presence of protein in urine indicate for not efficient in reabsorbing or/and filtration due to Renal diseases or Nephritic syndrome.
✓The severity of protenuria can categorize depend on Molecular weight of protein in urine or the amount (conc.) Of protein in urine.

(1) Glomerular proteinuria:

Damage to the glomerular basement membrane can result in larger proteins being allowed through into the glomerular filtrate in greater amounts , causing glomerular proteinuria . In this case , protein appears in the urine when the reabsorptive capacity of the tubules is exceeded . Where damage to the glomerulus is limited or slight , an excess **of albumin is found in the urine** (albuminuria) whereas if damage to the basement membrane is more serious , increasingly large proteins are filtered by the glomerulus . In this situation proteins such as **the immunoglobulins appear in the urine**.

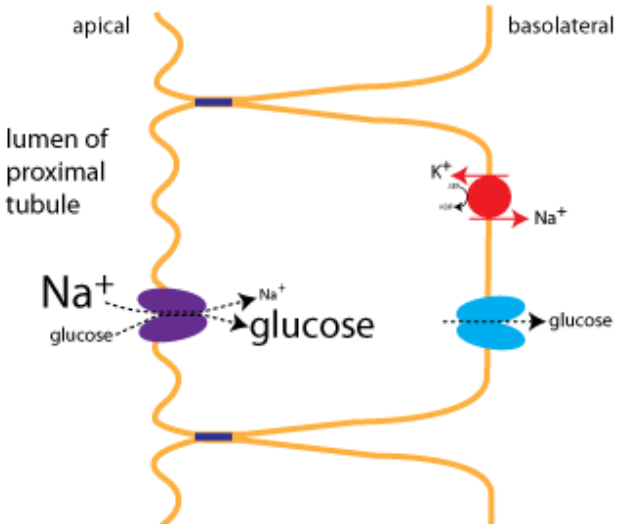
**In health , 95% of the filtered protein is reabsorbed . This involves the small proteins allowed through the glomerular basement membrane : microglobulins , insulin and parathyroid hormone .

Type of Disases	**Damage in**	**Weight of protein excreted**	**Effectence**	**Type of protein that released in urine**
Glomerular proteinuria	glomerular basement membrane	has higher levels of protein , often exceeding 2 g /day ,mainly albumin	limited or slight , an excess of albumin is found in the urine (albuminuria) If damage is more serious, in this situation proteins such as the immunoglobulins appear in the urine.	albumin immunoglobulins
Tubular proteinuria	tubular cells	shows a moderate increase in urinary proteins , usually less than 2 g /day , and show an increased proportion of low molecular weight proteins		Micro immunoglobulins appear in the urine.
Overflow proteinuria	overflow of high plasma concentrations of **low molecular weight protein**			Bence Jones proteins are small proteins (light chains of immunoblobulin) found in the urine. Testing for these proteins is done to diagnose and monitor multiple myeloma and other similar diseases
Secretory proteinuria	arising from protein secreted by the kidney tubule		When this protein is concentrated at low pH, it forms a gel	glycoprotein.

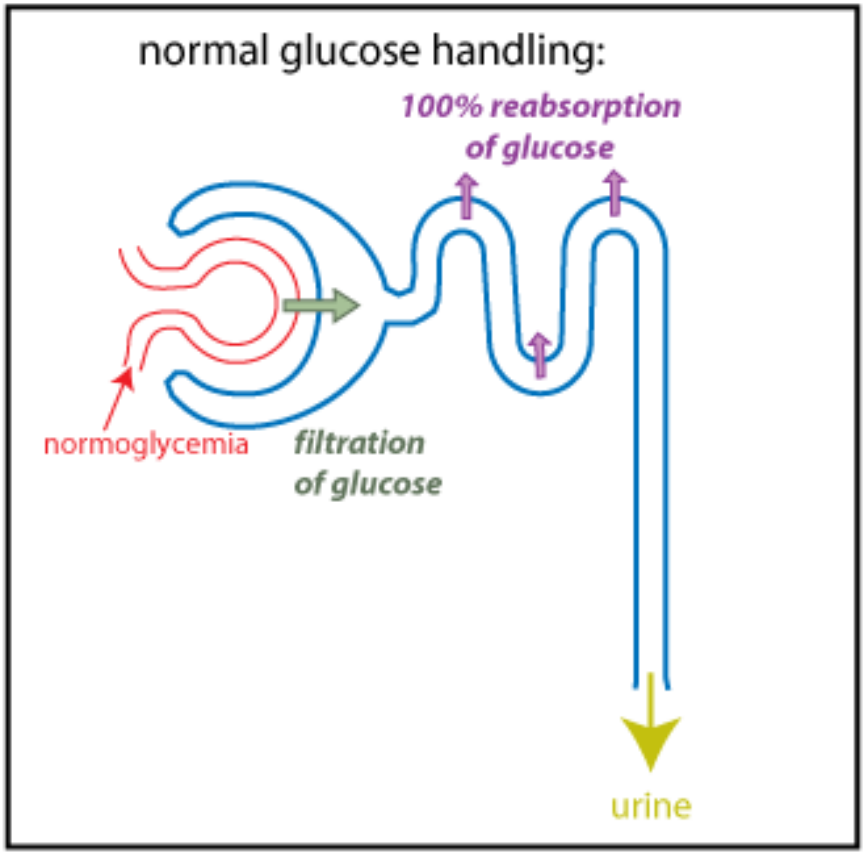
Glucose :

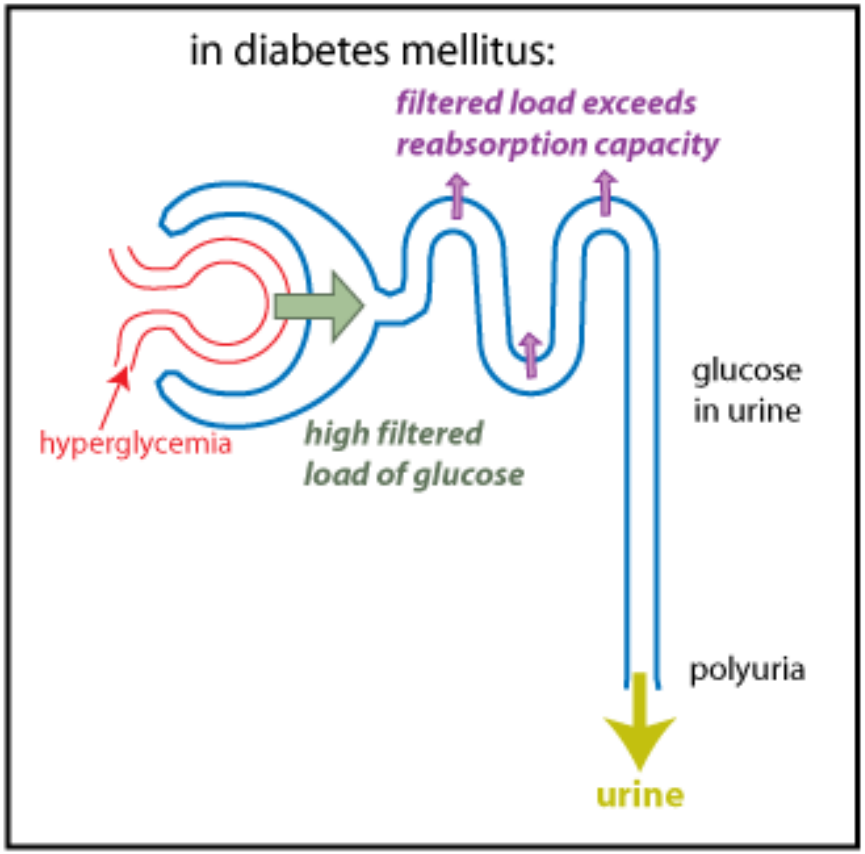
It is filtered by the glomeruli and partly resorbed by the renal tubules. Resorption is an active process and depends on the level of glucose in the filtrate (being that of plasma).

- If the filtered load should exceed capability of filtration the excess glucose will excreated(glucosuria). This will occur normally at a plasma (and filtrate) level of around 180 mg/dl. This is called the “Renal Threshold”.
- **Diabetes mellitus** will give glucouria only when the plasma glucose level exceeds the renal threshold
- Also some of diseases lead to glucouria :**renal glucosuria**, a **harmless proximal tubular defect** and some other **diseases of the proximal tubules** *lower the renal threshold and cause glucosuria*.



Glucose filtration and reabsorbing





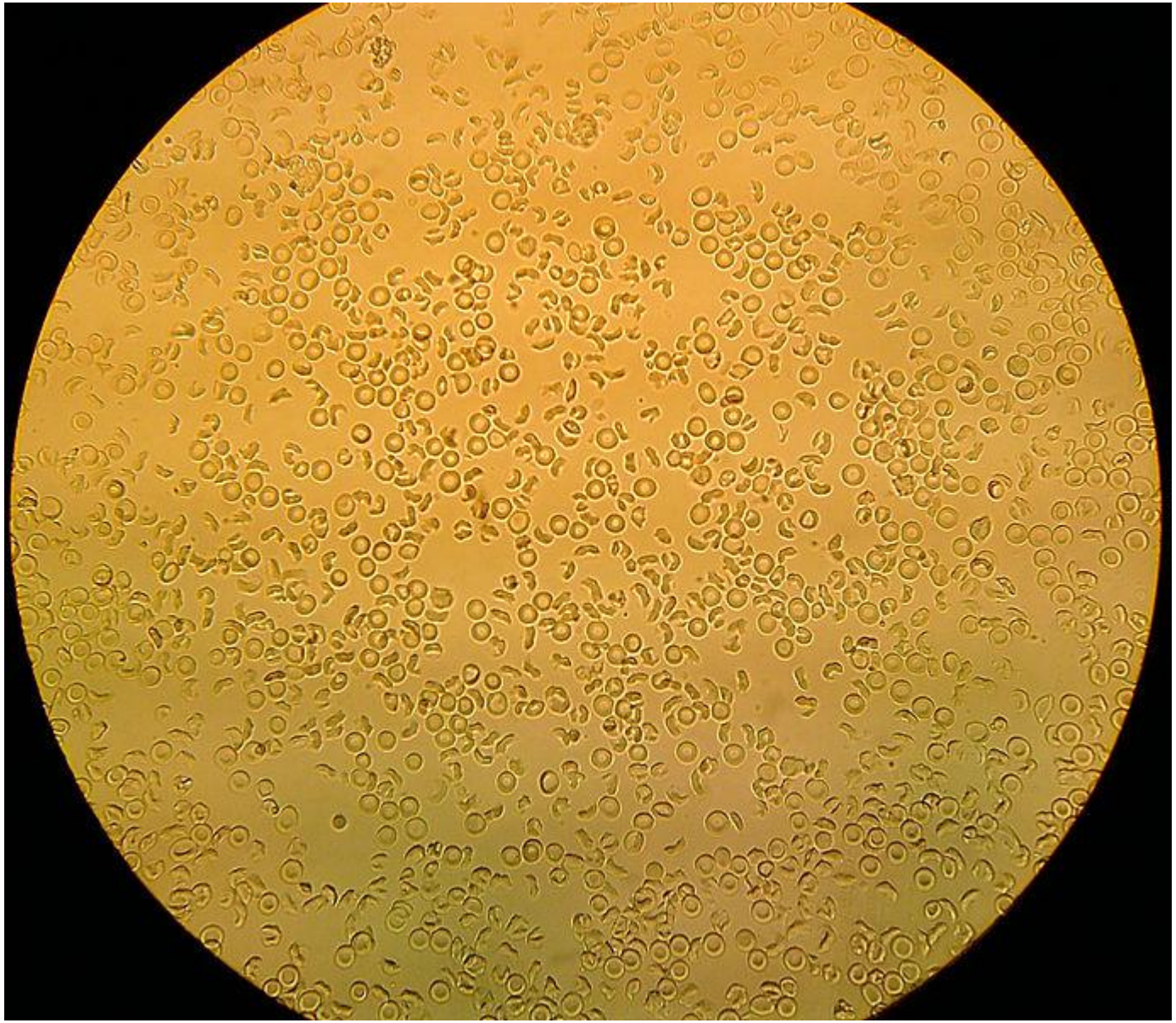
Bilirubin: The urine may be dark with a yellow foam if much is present. It is in the conjugated form and present in urine in **biliary obstruction** and **infective hepatitis**.

Urobilinogen: Its presence does not give a colored foam and occurs in jaundice due to **haemolytic disease**.

Blood:

- **Red blood cells together with casts and proteinuria occur in** acute **glomerulonephritis**, **lupus erythematosus**, **malignant hypertension** or in **lower urinary tract bleeding** (e.g. due to parasites, infection etc).
- **Haemoglobinuria** is due to intravascular haemolysis.
- Any pink, red or brown urine must be considered as bloody until proved otherwise.

hematuria



Part II :Detection of Amino acids

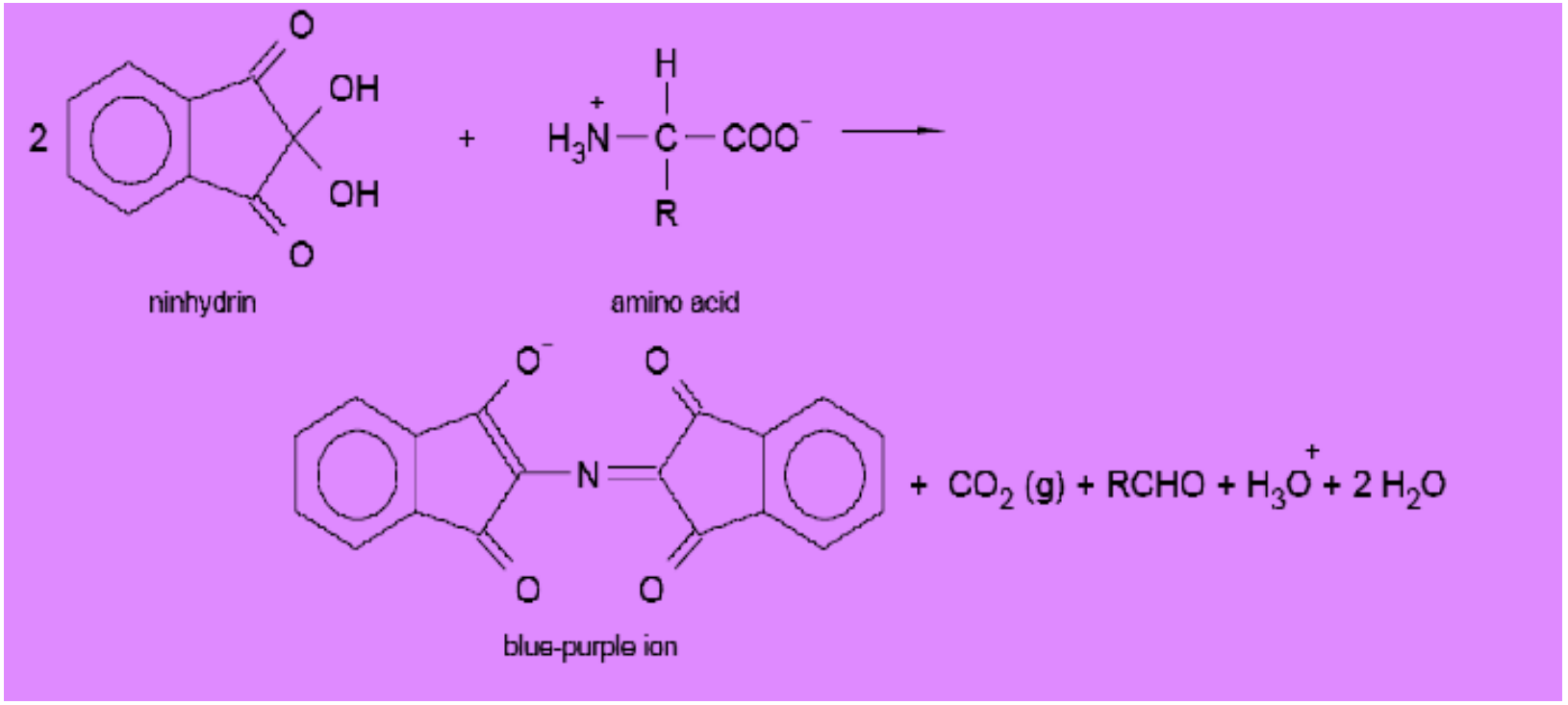
- In general the presence of amino acid in urine indicate for **severe liver disease**, ?? Why??

protein synthesis and deamination of amino acids are reduced leading to increased excretion.

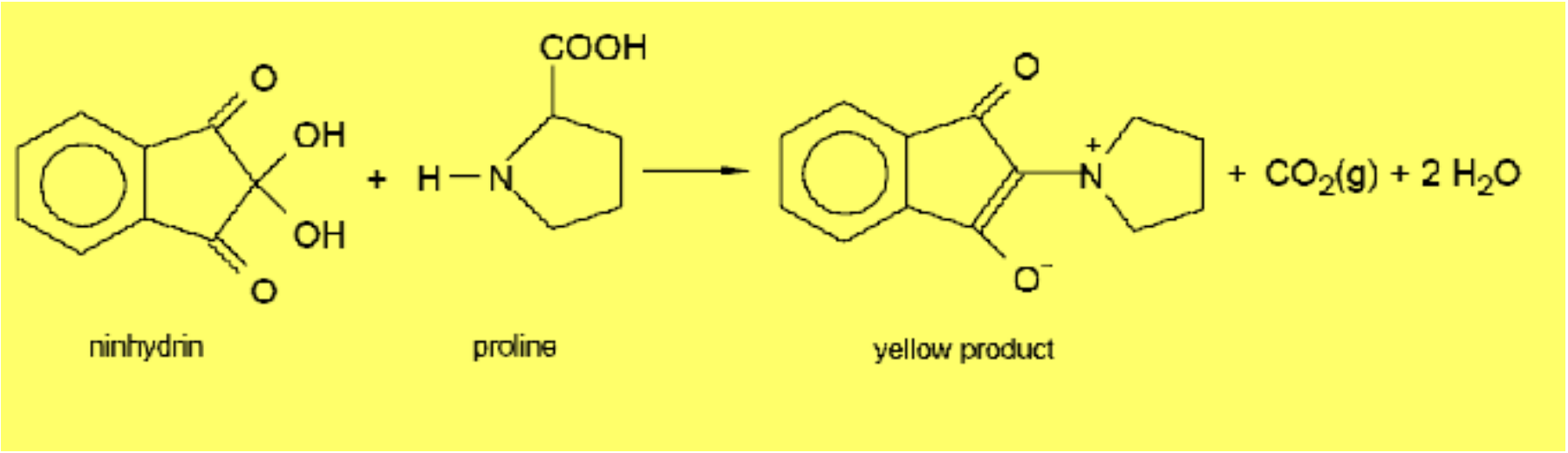
- In **starvation** and **debilitating disease** increased breakdown of plasma and tissue proteins
- the **Fanconi syndrome** a defect in tubular reabsorption lead to increased urine levels.
- In **cystinuria, phenylketonuria and alkaptonuria**, urinary cystine, phenylalanine and tyrosine levels increase respectively.

Principle for detect of amino acid:

Ninhydrin reacts with all amino acids except proline and hydorxyproline at pH 3-4 to give a purple colored compound. 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.



Ninhydrin reacts with all amino acids



Ninhydrin reacts with proline

Part III : Quantitative Estimation of Protein in Urine :

- **Important :** quantitative estimation of the daily excretion of protein to determine the type of renal disease, its severity and to monitor the results of treatment given.

Method	Principle	Comment
(1)Turbidimetric methods (sulfosalicylic acid, TCA or benzethonium chloride)	Proteins are precipitated as fine particles, turbidity is measured spectrophotometrically	Rapid, easy to use; unequal sensitivity for individual proteins
(2)Biuret	Proteins are concentrated by precipitation, redissolved in alkali, then reacted with Cu2+; Cu2+ form colored complex with peptide bonds	Accurate

Method using to estimation of protein (urine protein methods):

Method	Principle	Comment
(3)Folin-Lowry	Initial biuret reaction; oxidation of tyrosine, tryptophan, and histidine residues by Folin phenol reagent (mixture of phosphotungstic and phosphomolybdic acids); measurement of resultant blue color	Very sensitive
(4)Dye-binding (Coomassie blue,ponceau S)	Protein binds to dye, causes shift in absorption maximum	Limited linearity; unequal sensitivity for individual proteins

Part III : Quantitative Estimation of Protein in Urine By Turbidimetric methods using sulfosalicylic acid :

- Because of ease of use , speed , and sensitivity ,the techniques used most frequently today are turbidimetric procedures.

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.

Method:

(1)Detection of Abnormal Constituents Using Test-Strips:

Constituents	Results of Sample 1	Results of Sample 2
Nitrate		
pH		
Ketone bodies		
Ascorbic acid		
Glucose		
Bilirubin		
Urobilinogen		
Blood		

Clinical Diagnosis of sample 1 :.....
Clinical Diagnosis of sample 2 :.....

Part II: Detection of Amino acids

Label 5 test tube A, B, and C

- Place.

  1 ml of urine under test in tube A.

  1 ml of glycine solution in tube B.

  1 ml of proline solution 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.

Tube	A	B	C
	Urine Sample3	glycine solution	proline solution
	few drops of ninhydrin solution		
	Boil the each test tube for 2 minutes		
Results	.....	.....	.....

Part III: Quantitative Estimation of Protein in Urine

Method

1. It is necessary first to prepare a standard curve as follows.
2. Bovine albumin standard. (**50mg/dl)**
3. Set up a series of test tubes as follows**:**

Tube NO.	Protein STD	0.85% salin ml	1.25% HCl	Urine Sample 4	sulphosalicylic acid	Protein (mg/dl)
(Blank)	0.0	2	8 ml	-	-	.....
1	4.5	1.5	-	-	8 ml	.....
2	3	3	-	-	8 ml	.....
3	2.4	3.6	-	-	8 ml	.....
4	1.5	4.5	-	-	8 ml	.....
5	0.9	5.1	-	-	8 ml	.....
6	0.3	5.7	-	-	8 ml	.....
Urine Sample 4	-		-	2 ml	8 ml	.....

- Mix each tube well and allow to stand for 5 minutes.
- Using (blank) in the cuvettes of the spectrophotometer, transmittance at at 500 nm.

Result:

Tube NO.	transmittance at 500 nm	Protein (mg/dl)
(Blank)		
1		
2		
3		
4		
5		
6		
Urine Sample 4		

✓***Record the transmittance of the “unknown”. If it is above 50 mg/dl repeat the estimation after diluting the urine 1:10 with saline solution.(Normal 0-0.150 g)

Part IV: Determination of titrable acidity in urine:

- The titrable acidity in urine is mainly due to phosphates acid

NaH 2 PO 4 and to less extent weak organic acids. It can be determined by titrating urine with a standard alkalin using phenolphalein as the indicator. Calcium should be removed by potassium oxalate as not to interfere with the result .

Titrable acidity of urine is about 200 - 300 ml/day. The value may rise in starvation, diabetic ketosis and acidosis.

METHOD:

1. Pipette 25 ml of urine sample 5 into a 250 ml conical flask, add to spatula
full potassium oxalate powder to precipitate calcium.
2. Add 2 drops of phenolphalein and titrate with 0.1 M NaOH from a burette. Note the titre value (A ml) when a permanent pink color appears.

Note: the volume of urine sample 5 = 1600ml/day

RESULTS:

Volume of 0.1 M NaOH required to neutralize the acidity in 25 ml of urine = A ml

Volume required for 100 ml of urine = A X 4 = 4A

Since 24 h urine output **of urine sample 5 = 1600ml/day ,** titrable acidity of urine 4A X 16 ml/day

Normal

Thank You