Quantitative estimation of protein in urine

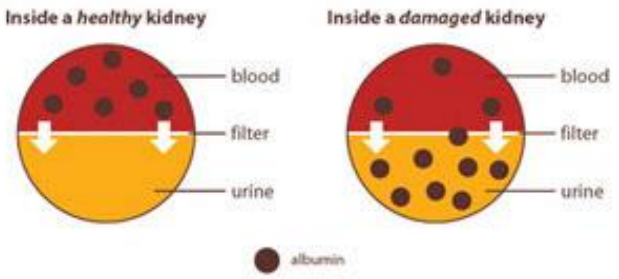
By sulphosalicalic acid Method

BCH 472

- **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.

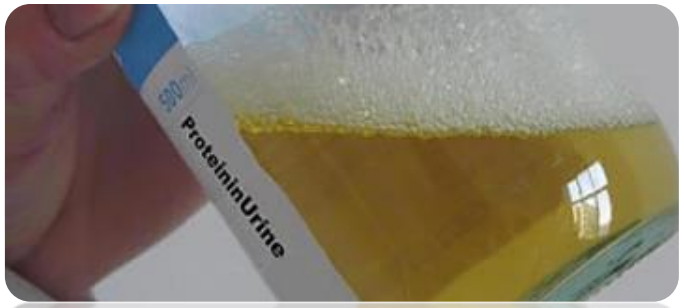
- However, there are other physiologic conditions (eg, exercise, fever) that can lead to increased protein excretion in urine. Also, there are some renal disorders in which proteinuria is absent.



Proteinuria:

- Protein in **normal** urine should be less than 150 mg/L
- **Proteinuria** is defined as urinary protein excretion of greater than 150 mg per day(per one liter).

- **Note**: **Dipsticks** (is the most common initial screening test for proteinuria) can only detect around 150 mg/L of albumin(The dipstick will not detect nonalbumin proteins).
- **Note:** /L = /24-hour = /day.



Types of Proteinuria:

Type	Cause
Glomerular proteinuria	• Results from a **disruption of the glomerular filtration barrier** which increased filtration of normal plasma protein and because albumin has the highest concentration in the plasma it is called albuminuria eg. Malignant hypertention
Tubular proteinuria	• **Defect in the reabsorption** eg, Fanconi Syndrom - low molecular weight protein that is found in urine
Overflow proteinuria	• **Overflow of high plasma** - high concentrations of low molecular weight protein found in urine eg, Multiple myloma. In multiple myeloma excessive amounts of immunoglobulin light chains are produced.
Secretory proteinuria	• **Over secretion** of certain proteins in the tubules, most notably the over secretion of Tamm-Horsfall proteins eg, in interstitial nephritis

Types of Proteinuria:

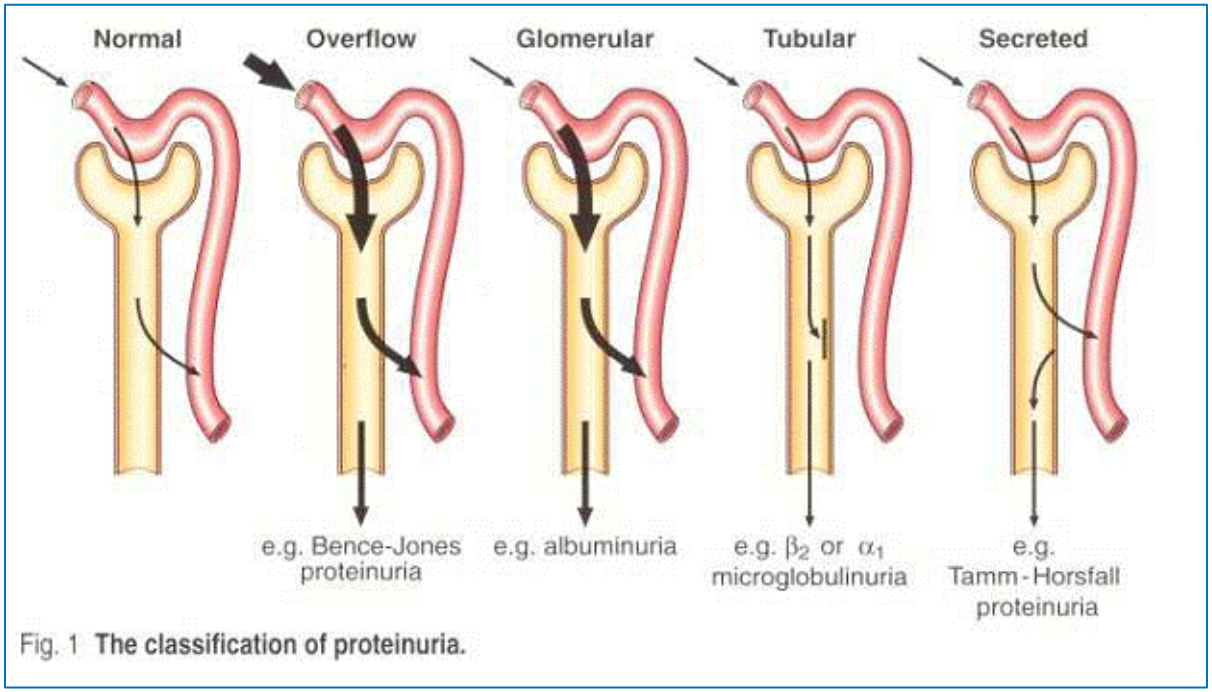


Fig. 1 **The classification of proteinuria.**

Cause of Proteinuria as Related to Quantity

Protein amount per 24-hour	Type of proteinuria
0.15 to 2.0 g	• Tubular proteinuria • Overflow proteinuria (an increased proportion of low molecular weight proteins)
2.0 to 4.0 g	• Usually glomerular
➢ > 4.0 g	• Always glomerular (mainly albumin)

- **The quantitative estimation** of the daily excretion of protein is of value to the clinician in order to give a general idea of the type of renal disease, its severity and to monitor the results of treatment given.

- The protein content can be determined by numerous methods eg, Biuret, Lowry, Bradford.
- In this lab **turbidimetric method** will be used.

- Determination of total protein by measurement of protein **turbidity** produce by mixed with an anionic organic acid such as sulfosalicylic acid , TCA , or benzethonium chloride.

- 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 at **500 nm**.

- # Practical Part

Sulfosalicylic acid (SSA) test :

- The sulfosalicylic acid (SSA) turbidity test quantitatively screens for proteinuria. The advantage of this **easily** performed test is its greater **sensitivity** for proteins such as Bence Jones.

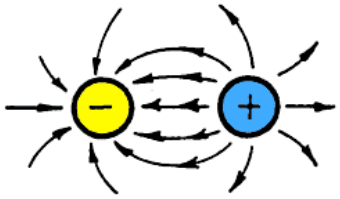
- The SSA reaction will detect globulin and Bence-Jones proteins, in addition to albumin (although it is more sensitive to albumin).

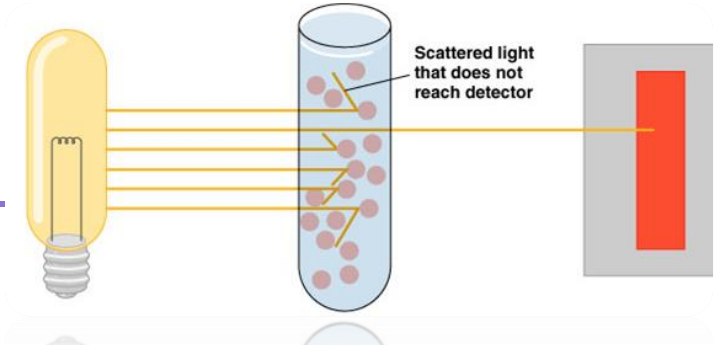
Principle:

- Sulfosalsalyic acid is an **anion(-)** which neutralizes the protein **cations(+)** leading to its precipitation (pH in highly **acidic** media, the protein will be positively charged, which is attracted to the acid anions that cause them to precipitate.)

- Then the radiation of a wavelength which is not absorbed by the solution is made to pass through the suspension and the apparent absorption will be solely because of the **scattering** by the particles.

- The transmission decrease with increasing protein concentration.





Method:

1-Set up a series of test tube as follows, label from 1- 7

Tube	Protein Stock Solution(140 mg/dl)	0.85% Saline	Protein concentration mg/dl
1	4.5	1.5	
2	3	3	
3	2.4	3.6	
4	1.5	4.5	
5	0.9	5.1	
6	0.3	5.7	
7(Blank)	0	6	
Urine Sample	-	-	

2-Set another 8 test tube labeled 1-7 and pipette in each one Add 8 ml of sulfosalicylic acid

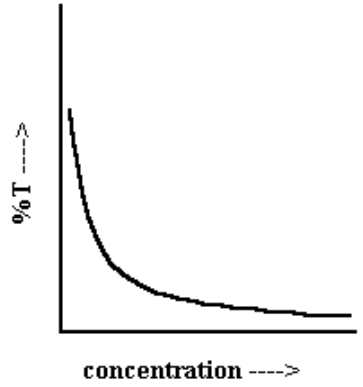
Tube	sulfosalicylic acid
1	8 ml
2	8 ml
3	8 ml
4	8 ml
5	8 ml
6	8 ml
7(Blank)	8 ml
Urine Sample	8 ml

- 3-Into tube 1 pipette 2 ml of protein solution 1, into tube 2 pipette 2 ml of protein solution 2 etc. For the Urine Sample pipette 2ml of the Sample.
- 4-**Mix** contents of each tube well and allow standing for 5 minutes.
- 5-Using solution 7 (Blank) to set transmittance at 100 at 500nm.
- 6-Then use solutions from 1-6, to recorded respective **transmittance** of each suspension.

Results:

Tube	Transmittance at 500 nm	Protein concentration mg/dl
7(Blank)	100 %	
1		
2		
3		
4		
5		
6		
Urine Sample		

- Plot Transmittance against Protein concentration mg/dl.
- Determine the Protein concentration of Urine Sample from the standard curve.
- Compare the result you got with the normal range of protein execration in 24 h urine specimen (you must convert the unit to g/L) if you know that the protein execration in **healthy sample (0- less than 0.150g/24 h).**
- Comment on the clinical conditions of the patient if it is present.
- Assuming that the **24 hour urine sample for the patient = 1000 ml**.



Questions :

Which protein can be used as a marker of Multiple myloma ? And how?

Explain how total protein can be determined by measuring protein turbidity ?

Why the resulting graph is a descending curve ?