QUANTITATIVE PROTEIN ESTIMATION OF URINE

Introduction

- 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**.

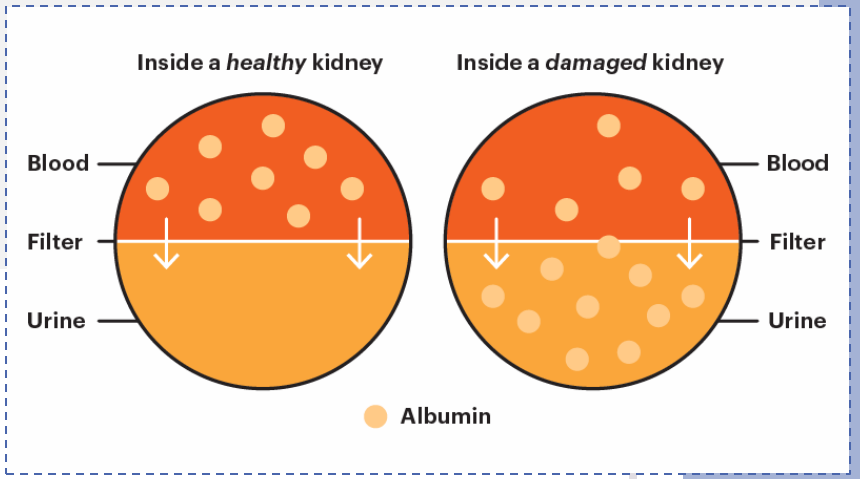


Figure 1. Illustration of proteinuria

Proteinuria

- Proteinuria means protein in urine.
- 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).
- High concentrations of protein cause frothy or sudsy urine.
- Although proteinuria is usually benign, the condition can be a marker for a serious underlying renal disease or systemic disorder.

Notes:

1. **Dipsticks** (is the most common initial screening test for proteinuria) can only detect around 150 mg/L of albumin.

→ The dipstick will not detect non-albumin proteins.

1. /L = /24-hour = /day [for normal, because the average normal output per day is 1000 ml or 1L]

Types of Proteinuria as related to the cause:

Some of the causes of proteinuria are:

1. **Primary** kidney disease called nephritis.
2. **Secondary** kidney disease due to:
   - Diabetes
   - Hypertension
   - Cancer
   - Pregnancy
   - Medications

3. **Other:**
   - Acute infection

Mechanisms of proteinuria

Type	
Glomerular proteinuria *(Filtration)*	▪ **Cause:** due to glomerular disease and abnormal permeability of the glomerular capillaries to protein lead to increased filtration of normal plasma protein and because **albumin** has the highest concentration in the plasma it is called **abuminuria** eg. Malignant hypertension, Glomerulonephritis. ▪ *The most common kind of proteinuria.*
Tubular proteinuria *(Reabsorption)*	▪ **Cause:** Defect in the *reabsorption* process eg, **Fanconi Syndrome.** ▪ **Signs:** Low molecular weight protein that is found in urine eg, beta-2 microglobulin. ▪ **Note:** The amount of proteinuria is < 2 g and dipstick may be negative.
Secretory proteinuria *(Secretion)*	▪ **Cause:** Over-secretion of certain proteins in the tubules, most notably the over secretion of Tamm-Horsfall proteins eg, in **interstitial nephritis.**

Mechanisms of proteinuria

Type	
Overflow proteinuria	▪ **Cause:** Commonly associated with <u>increased production</u> of abnormal low molecular weight proteins (eg, **light chains in multiple myeloma**) i.e overflow of high plasma proteins, that exceeds the reabsorption capacity of the tubules. ▪ **Signe:** concentrations of low molecular weight protein found in urine.
Functional proteinuria	▪ **Cause:** Occurs when <u>increased renal blood flow</u> delivers increased amounts of protein to the nephron, eg. **exercise, fever, high-output heart failure,** resulting in increased protein in the urine. ▪ **Note:** usually < 1 g/day and reverses when renal blood flow returns to normal.

Mechanisms of proteinuria

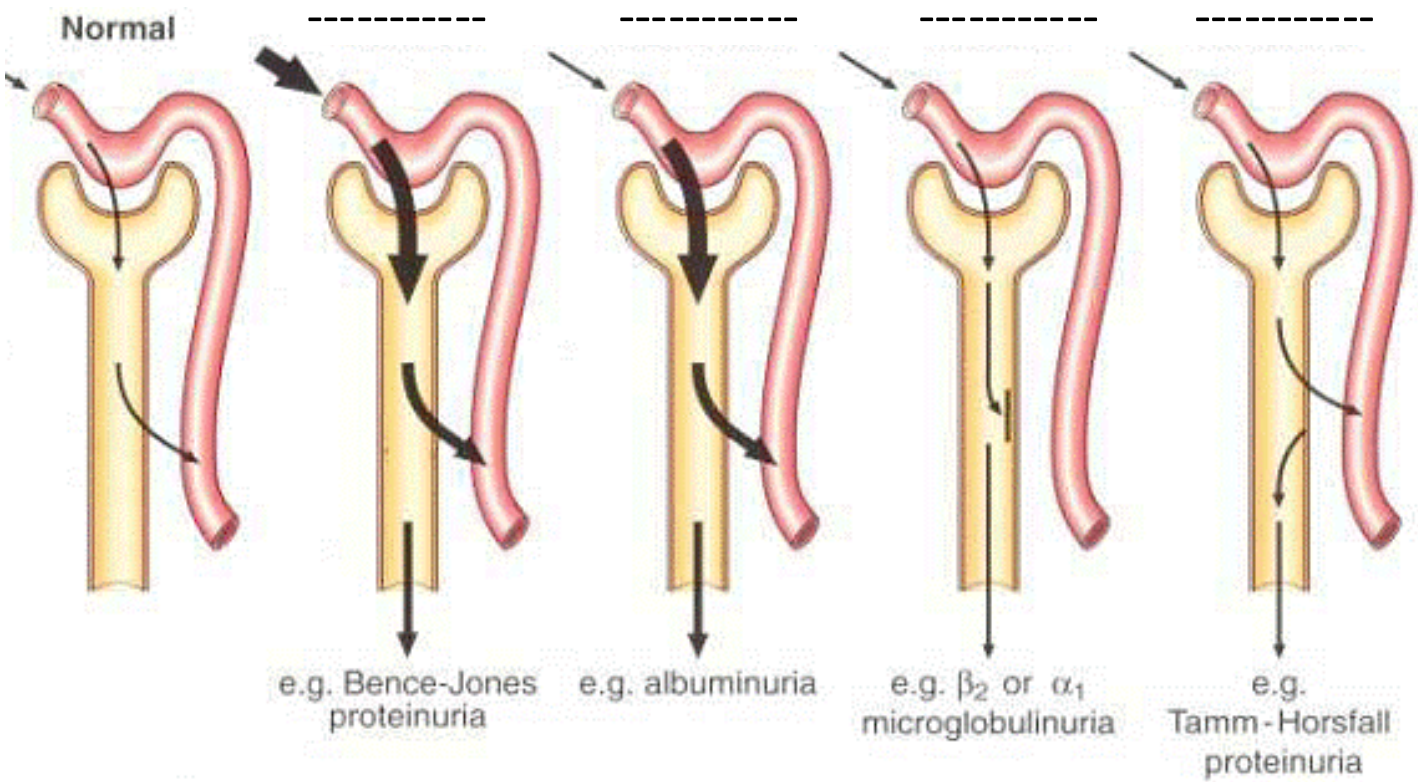


Figure 2. Mechanisms of proteinuria

Types 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*).

Protein determination

- 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 (*HOW*?) its severity and to monitor the results of treatment given.
- The protein content can be determined by numerous methods, 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*).

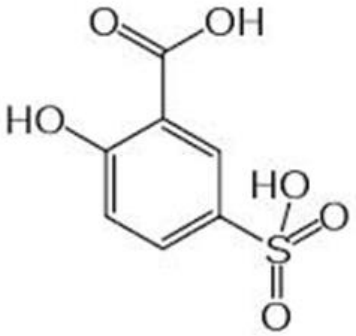


Figure 3. Sulfosalicylic acid structure

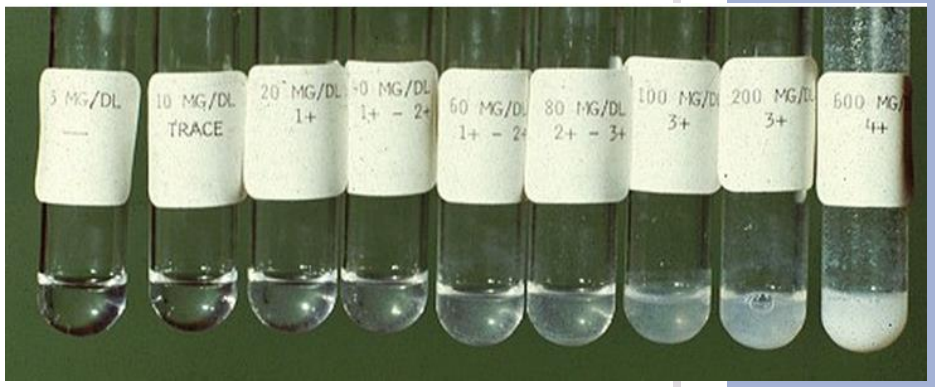


Figure 4. Protein turbidity test results

Sulfosalicylic acid (SSA) test:

Objective:

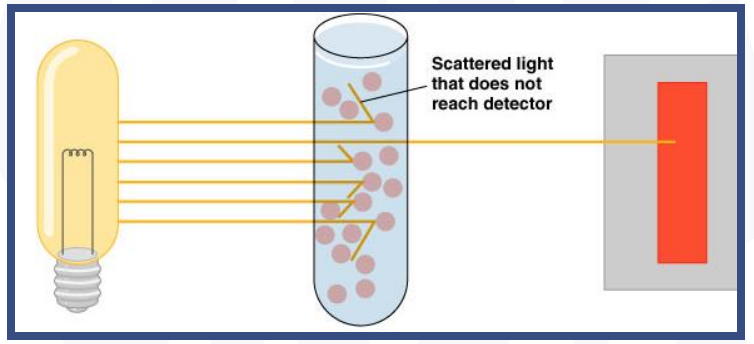
- Quantitative estimation of protein in urine by turbidimetric methods using sulfosalicylic acid.

Principle:

1. 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).
2. 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 higher protein concentration , the lower transmittance value).

Increased concentration
Decreased transmission



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
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 labelled 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 (that you prepared before), 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 100% at 500nm.

6-Then use solutions from 1-6, to recorded respective transmittance of each suspension.

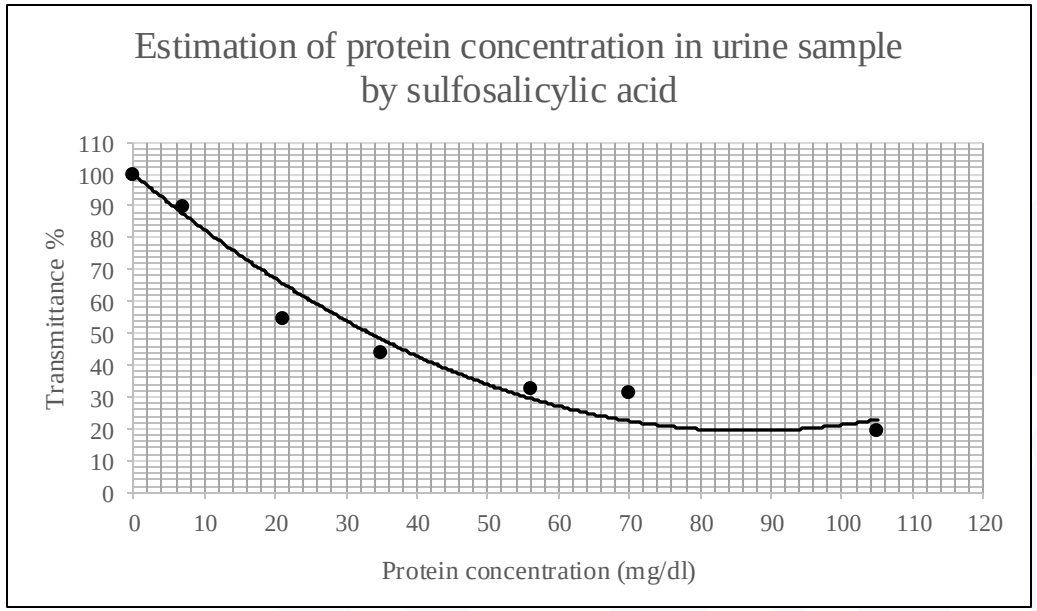
7-Record your results.

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

Results:

- Plot Transmittance against Protein concentration (mg/dl).
- Read 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 if you know that the protein excretion in healthy sample (0- less than 0.150g/24 h).

Note: Assuming that the 24 hour urine sample for the patient = 1000 ml



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- BCH 472 practical note