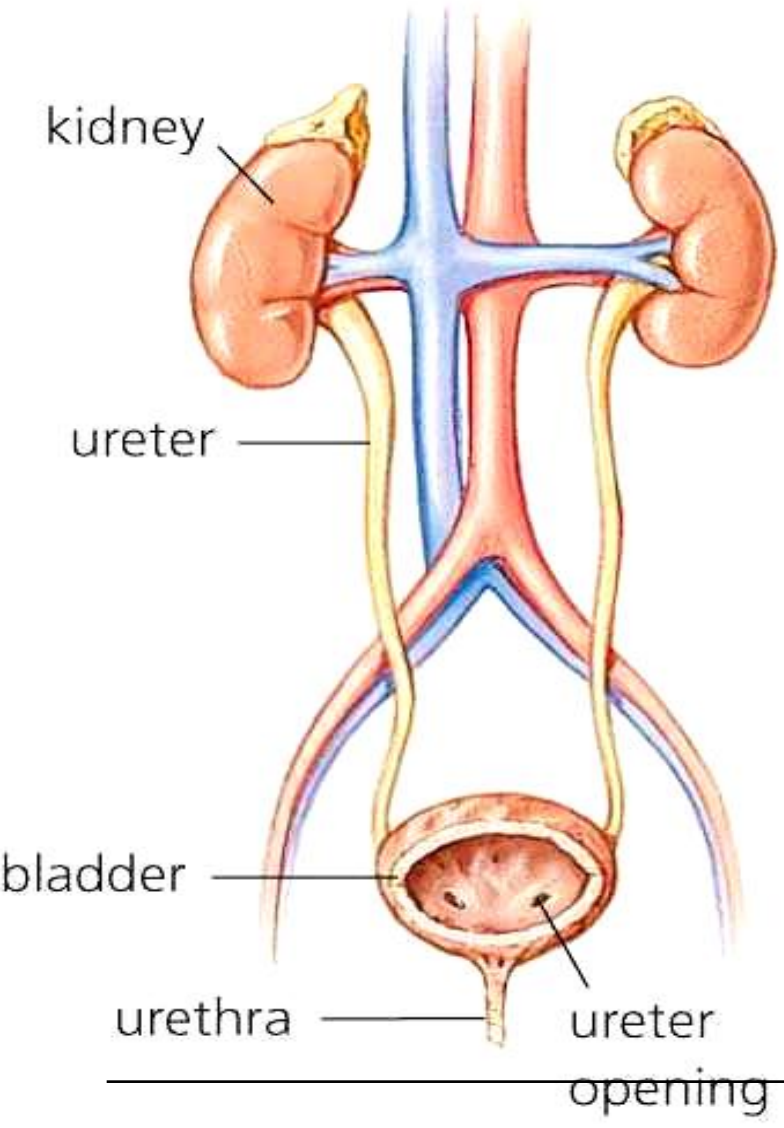
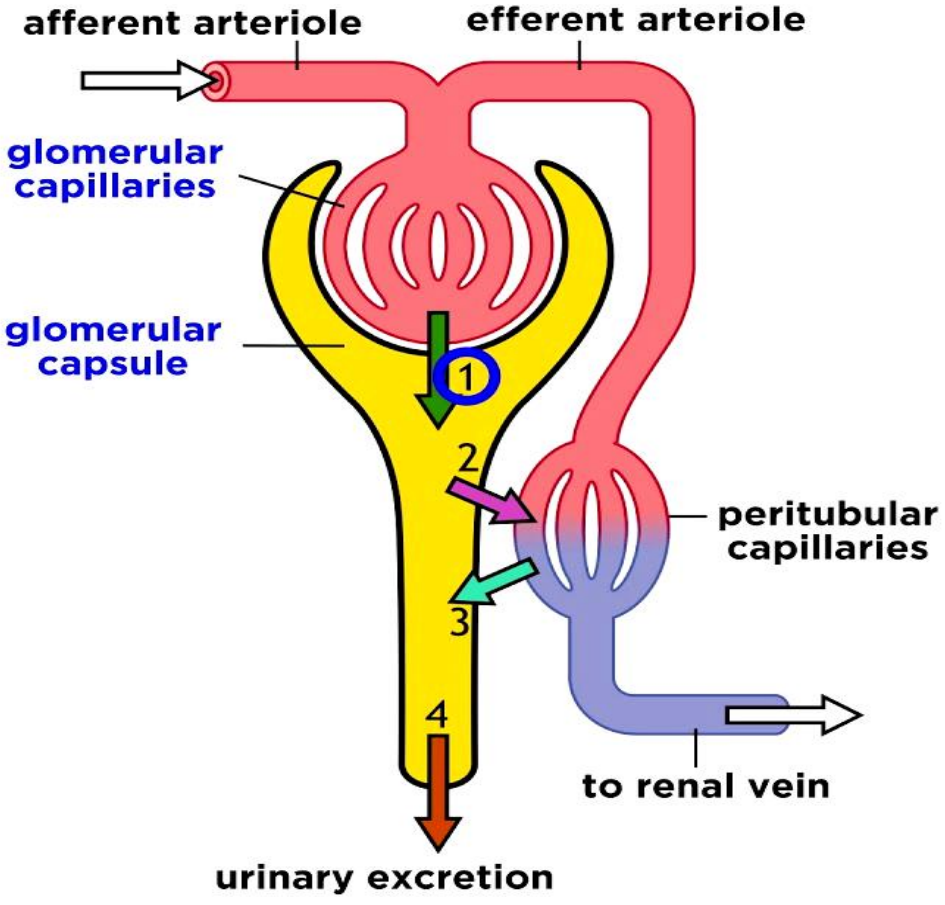
# RENAL FUNCTION TEST




DR. S. SHEKHAR
ASSOC. PROFESSOR
DEPT. OF BIOCHEMISTRY

# FUNCTIONS OF KIDNEY

1. **Maintenance of homeostasis**:

   The kidneys are responsible for the regulation of water, electrolyte & acid-base balance in the body.

2. **Excretion of metabolic waste products**:

   The end products of protein & nucleic acid metabolism are eliminated from the body. These include urea, creatinine, creatine, uric acid, sulfate & phosphate

3. **Retention of substances vital to body:**

   The kidneys reabsorb & retain several substances of biochemical importance in the body e.g. glucose, amino acids etc

## 4. Hormonal functions:

### A. Erythropoietin:

A peptide hormone, stimulates haemoglobin synthesis and formation of erythrocytes.

### B. 1,25-Dihydroxycholecalciferol (calcitriol):

The active form of vitamin D is finally produced in the kidney. It regulates calcium absorption from the gut.

### C. Renin:

A proteolytic enzyme liberated by kidney, stimulates the formation of angiotensin II which, in turn, leads to aldosterone production.

Angiotensin II & aldosterone hormones involved in the regulation of electrolyte balance.

# URINE FORMATION

- Nephron is the functional unit of kidney.
- Each kidney is composed of approximately one million nephrons.
- Nephron, consists of a Bowman's capsule (with blood capillaries), proximal convoluted tubule (PCT), loop of Henle, distal convoluted tubule (DCT) & collecting tubule.
- About 1200 ml of blood (650 ml plasma) passes through the kidneys, every minute.

- About 120-125 ml is filtered per minute by the kidneys & this is referred to as glomerular filtration rate (GFR).
- With a normal GFR (120-125 ml/min), the glomerular filtrate formed in an adult is about 175-180 litres/day, out of which only 1.5 litres is excreted as urine.
- More than 99% of the glomerular filtrate is reabsorbed by the kidneys.
- Urine formation basically involves two steps glomerular filtration & tubular reabsorption.

# Glomerular filtration

- This is a passive process that results in the formation of ultra filtrate of blood.
- All the (unbound) constituents of plasma, with a molecular weight < 68,000, are passed into the filtrate.
- The glomerular filtrate is almost similar in composition to plasma except proteins and cell.

# Tubular reabsorption

- The renal tubules (PCT, DCT & collecting tubules) retain water & most of the soluble constituents of the glomerular filtrate by reabsorption.
- This may occur either by passive or active process.

# Renal threshold

- There are certain substances in the blood whose excretion in urine is dependent on their concentration.
- Such substances are referred to as threshold substances.
- At the normal concentration in the blood, they are completely reabsorbed by the kidneys.
- But when their blood levels are elevated beyond the normal range exceeding the tubular reabsorption capacity the excesss is excreted in urine.
- The renal threshold of a substance is defined as its concentration in blood (or plasma) beyond which it is excreted into urine.

❖ The renal threshold for

Glucose --------180 mg/dl

Ketone bodies--- -3 mg/dl

Calcium ----------10 mg/dl

Bicarbonate ------ 30 mEq/l

❖Tubular maximum (Tm):

- The maximum reabsorptive capacity of the renal tubules to absorb a particular substance.
- Tubular maximum for glucose is 375 mg/min

# INDICATION

1. Detection of renal damage
2. Assessment of extent of renal damage
3. Monitoring the progression of renal damage / disease
4. Monitoring and adjusting the dose of potentially renal toxic drugs
5. Before giving renal excretory contrast in some diagnostic procedure

# CLASSIFICATION

## I. GLOMERULAR FILTRATION CAPACITY TEST

I. Clearance tests

II. Serum urea

III. Serum creatinine

## II. GLOMERULAR FILTRATION BARRIER INTEGRITY TEST

A. Proteinuria

B. Hematuria

C. Urine protein electrophoresis

## III. TUBULAR FUNCTION TESTS

A. Reabsorption test

B. Renal concentration test/ water deprivation test

C. Osmolality

D. Specific Gravity

E. Urine dilution test

F. Sodium Excretion Test

G. Ammonium Chloride loading test or urinary acidification test

## IV. COMPLETE URINE ANALYSIS

# I. GLOMERULAR FILTRATION CAPACITY TEST

- It measures the total filtration occuring at glomerulus.
- These indicates the percentage, or fraction of the total nephron that are functioning.

A decrease in 50% filtration capacity

indicates that about half of the total nephrons have lost their function.

# 1. RENAL CLEARANCE TESTS

- To assess the rate of glomerular filtration & renal blood flow.
- "The renal clearance of a substance is defined as the volume of plasma from which the substance is completely cleared by the kidneys per minute."
- This depend on
  - plasma conc. of the substance & it's excretary rate
  - GFR
  - Renal plasma flow

# Renal Clearance Tests

- The GFR (Normal = 120 ml/minute )
- Usually equal to clearance of that substance and is calculated by the following equation

$$C = \frac{U \times V}{P}$$

Where, C = Clearance of the substance (ml/mt)

U = Conc.of substance in urine (mg/L)

P = Conc.of substance in plasma (mg/L)

V = Vol.of the urine passed per minute

| Renal metabolic change | Clearance Value Vs GFR | Example |
|---|---|---|
| Substance only filtered ( No reabsorbtion No secretion) | Clearance = GFR | Inulin |
| Substance filtered and reabsorbed | Clearance < GFR | Urea |
| Substance filtered and secreted | Clearance > GFR | Creatinine |

# A. Urea clearance test

- Urea is the end product of protein metabolism.
- After filtered by the glomeruli, it is partially reabsorbed by the renal tubules.
- So, Urea clearance is less than the GFR & it is influenced by the protein content of the diet.
- Urea clearance is not as sensitive as creatinine clearance.

# Urea clearance test

- Urea clearance is defined as the volume (ml) of plasma that would be completely cleared of urea per minute.
- If the output of urine is more than 2 ml per minute.
  [ V > 2ml/min]

This is referred to as maximum urea clearance & the normal value is around 75 ml/min

- It is calculated by the formula:

$$Cm = \frac{U \times V}{P}$$

- Cm=Maximum urea clearance.
- U = Urea concentration in urine (mg/dl).
- V = Urine excreted per minute in ml.
- P = Urea concentration in plasma.

# Standard urea clearance

- The urea clearance drastically changes when the volume of urine is less than 2 ml/min.

  [ V < 2ml/min]

- This is known as standard urea clearance (C) & the normal value is around 54 ml/min
- Standard urea clearance is calculated by a modified formula

$$Cs = \frac{U \times \sqrt{V}}{P}$$

- A urea clearance value below 75 % of the normal indicates renal damage.

- Usually blood urea level start rising only when the clearance value falls below 50% of the normal.
- Urea clearance values may not always coincide with blood urea level.

- Urea is reabsorbed by the renal tubule and hence tubular function affects urea clearance.
- Normal level of blood urea: 20-40 mg/dl

# B. Creatinine clearance test

- Creatinine is an excretory product derived from creatine phosphate in muscle.
- The excretion of creatinine is rather constant & is not influenced by body metabolism or dietary factors.
- Creatinine is filtered by the glomeruli & only marginally secreted by the tubules.
- Creatinine clearance may be defined as the volume (ml) of plasma that would be completely cleared of creatinine per minute.

# Procedure:

- In the traditional method, creatinine content of a 24 hr urine collection & the plasma concentration in this period are estimated.
- The creatinine clearance (C) can be calculated as follows:

$$Ccr = \frac{U \times V}{P}$$

U = Urine concentration of creatinine.

V = Urine output in ml/min (24 hr urine volume divided by 24 x 60)

P = Concentration of creatinine.

- The normal range of creatinine clearance is around 120-145 ml/min.
- A decrease in creatinine clearance ( <75% of normal ) indicate a decrease GFR reflecting renal damage.
- It is helpful in the early detection of functional impairment of kidney and also for monitoring the patients with renal insufficiency
- In older people, the clearance is decreased.

# Creatinine clearance test as a GFR marker

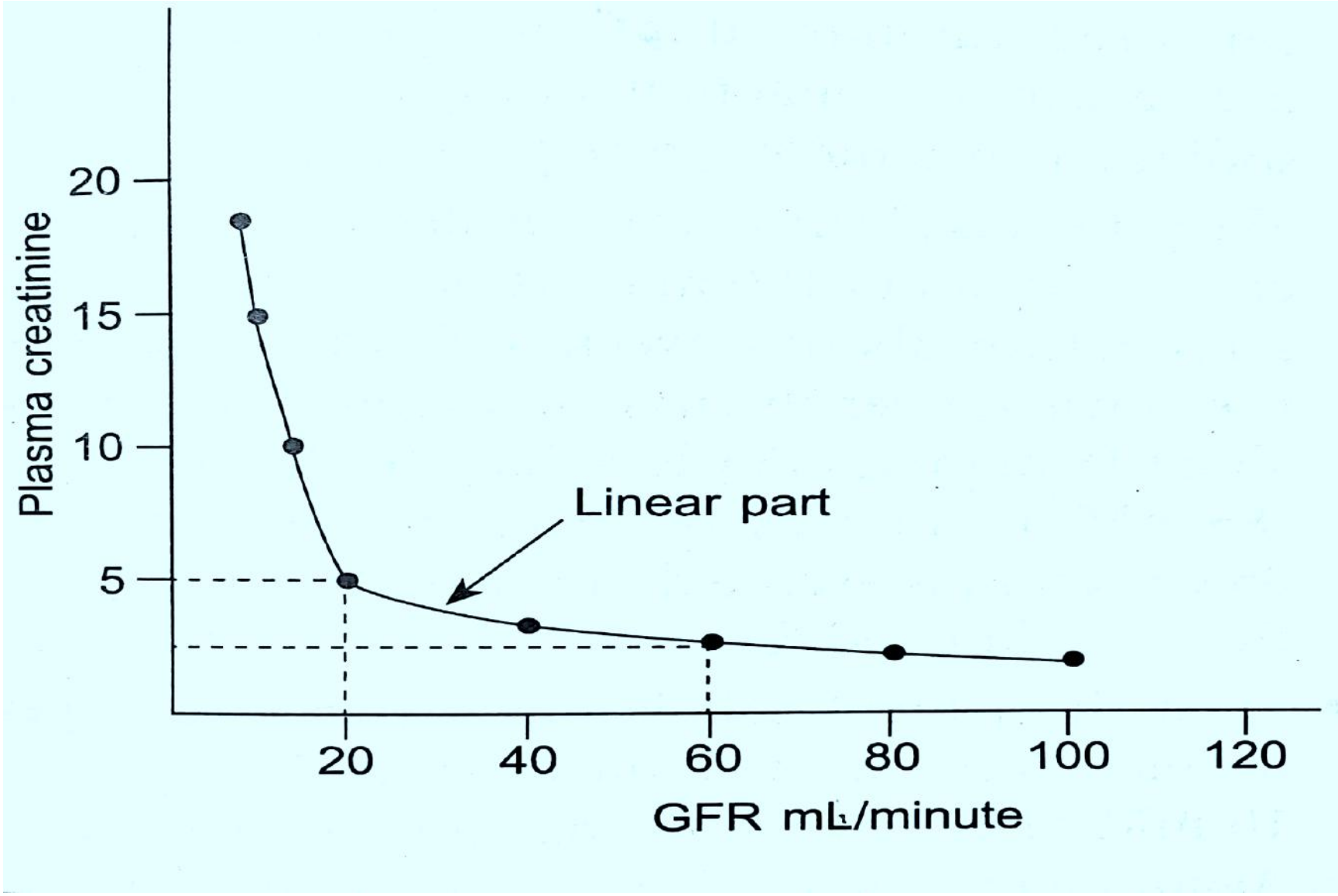
## Advantages

1. It is not affected by diet or exercise
2. Extrarenal factors will rarely interfere.
3. Conversion of creatine phosphate to creatinine is spontaneous, non-enzymatic.
4. As the production is continuous, the blood level will not fluctuate. Blood may be collected at any time.

## Disdvantages

1. Secreted by tubule, so clearance > GFR
2. Overestimate GFR by 10-20 ml/min
3. Very early stages of decrease in GFR (50-70 ml/min) may not be identified by creatinine clearance (**creatinine blind area**).

# Relation between GFR AND Plasma Creatinine



# C. Inulin Clearance Test

- Method of choice when accurate determination of GFR is required.
- Inulin is polysacharide of Fructose.
- Freely filtered by glomerulus not reabsorbed not secreted or metabolically altered by the renal tubule.
- Normal value : 120 ml/mt.
- Disadvantages :need for its IV adminstration technically difficulty of analysis

# D. Cystatin C as a filtration marker

- It is a LMW nonglycosylated protein produced at a constant rate by all nucleated cells in the body,
- Freely filtered by the glomeruli, and totally reabsorbed by the renal tubules.
- It is not secreted, by the tubules. Thus, its plasma level is determined by GFR.
- Normal adults have circulating level of approx. 1mg/l.
- This is better indicator of renal function as compared to creatinine in early stages of GFR impairment as it is independent of age,gender,body composition & muscle mass.

# E. Estimated GFR (eGFR)

- A simpler technique of estimating creatinine clearance and there by GFR is by using serum creatinine level.
- This would eliminate the need for timed urine collections.
- A commonly used formula is Cockcroft-Gault equation.
- Ccr = (140 – age in years) × weight in Kg (0.85 in females)/72 × Pcr in mg/dL
- The factor 0.85 is used in females assuming that they have 15% less muscle mass.

# MDRD (Modification of Diet in Renal Disease) Formula

- This equation directly estimates GFR.
- The estimated GFR (eGFR) (mL/min/1.73m$^2$)

**eGFR = 186 × (Creatinine/88.4) -1.154 × (Age) –0.203 × 0.742 (if female)**

- Estimates GFR adjusted for body surface area.
- Designed for use with laboratory creatinine test.
- Is more accurate than creatinine clearance measured from 24-hour urine collections or estimated by the Cockcroft-Gault formula.

*Cockcroft-Gault* GFR equation:

$$\text{GFR} = \frac{(140 - \text{age}) \times (\text{wt. in kg}) \times (0.85 \text{ if female})}{72 \times \text{serum creatinine}}$$

MDRD GFR equation:

$$\text{GFR} = 186.3 \times \left[\frac{\text{Serum creatinine } (\mu\text{mol/L})}{88.4}\right]^{-1.154}$$

$$\times \text{ age (years)}^{-0.203} \times 0.742 \text{ (}\textit{if female}\text{)}$$

$$\times \textit{ 1.212 (if African American)}$$

# GRADING OF CHRONIC KIDNEY DISEASE

| State | Grade | GFR ml/mt/1.73m² |
|---|---|---|
| Minima damage with normal GFR | 1 | >90 |
| Mild damage with slightly low GFR | 2 | 60-89 |
| Moderately low GFR | 3 | 30-59 |
| Severely low GFR | 4 | 15-29 |
| Kidney failure | 5 | <15 |

# 2. Serum urea

- Urea is major nitrogenous end product of protein and amino acid catabolism, produced by liver and distributed throughout intracellular and extracellular fluid.
- Urea is freely filtered by the glomeruli
- 40-70% of it is passively absorbed by diffusion into renal tubules depending upon urine flow rate.
- The reference interval - 10-40 mg/dl
- High protein diet causes significant increases in plasma urea concentrations

- Many renal diseases with various glomerular, tubular, interstitial or vascular damage can cause an increase in plasma urea concentration

- Nonrenal factors can affect the urea level
    a) Mild dehydration,
    b) high protein diet,
    c) increased protein catabolism,
    d) muscle wasting as in starvation,
    e) reabsorption of blood proteins after a GIT haemorrhage,
    f) treatment with cortisol or its synthetic analogous

- States associated with elevated levels of urea in blood are referred to as **uremia or azotemia**
- Causes of plasma urea elevations:

  Prerenal: renal hypoperfusion

  Renal: acute tubular necrosis

  Postrenal: obstruction of urinary flow
- Parallel determination of urea and creatinine is performed to differentiate between pre-renal and post-renal azotemia
- Pre-renal azotemia leads to increased urea levels, while creatinine values remain within the reference range.
- In post-renal azotemias both urea and creatinine levels rise, but creatinine in a smaller extent

# BLOOD UREA NITROGEN (BUN)

- Sometimes, blood urea level is expressed as blood urea nitrogen (BUN)
- BUN is the nitrogen content of urea present in blood
- Molecular weight of urea is 60 in which the contribution of nitrogen atoms is 28
- Therefore, BUN equals blood urea multiplied by 28/60 i.e. nearly 0.47
- The normal range of BUN is 10-20 mg/dl
- A rise in nitrogen content of blood is called azotaemia

# Serum creatinine

- Creatinine is a breakdown product of creatine phosphate in muscle, and is usually produced at a fairly constant rate by the body depending on muscle mass
- Creatinine is filtered but not reabsorbed in kidney.
- A small amount of creatinine is secreted into tubules and its secretion is increased with the increasing level of plasma creatinine.
- Normal range is 0.8-1.3 mg/dl in men and 0.6-1 mg/dl in women
- Not increased above normal until GFR<50 ml/min .

# Increased serum creatinine

- Impaired renal function
- Anabolic steroid users
- Vary large muscle mass: body builders, acromegaly patients
- Rhabdomyolysis/crush injury
- Athletes taking oral creatine

❖ Drugs:

- Probenecid
- Cimetidine
- Triamterene
- Trimethoprim
- Amiloride

Determination of serum creatinine gives a useful indication of the degree of renal failure

# II. GLOMERULAR FILTRATION BARRIER INTEGRITY TEST

Glomerulus acts as a selective filter of the blood passing through its capillaries

## A. PROTEINURIA

- Proteinuria is the first sign of glomerular injury before any decrease in GFR.
- The glomeruli of kidney are not permeable to substances with molecular weight more than 69,000 & plasma proteins are absent in normal urine

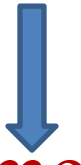
# PROTEINURIA

- When glomeruli are damaged or diseased, they become more permeable & plasma proteins may appear in urine.
- The smaller molecules of albumin pass through damaged glomeruli more readily.
- Albuminuria is always pathological

If total protein excretion > 150 mg/day

Or

Albumin excretion > 30 mg/day



Indicative of glomerular damage

# MICRO-ALBUMINURIA

- It is also called minimal albuminuria or paucialbuminuria.
- It is an early indicator of onset of nephropathy due to microvascular glomerular damage.
- Micro albuminuria is an early indication of nephropathy in patients with diabetes mellitus & hypertension.
- It is identified, when small quantity of albumin (30-300 mg/day) is seen in urine.
- It is also expressed as albumin creatinine ratio. Albumin creatinine ratio – 30-300mg albumin /gm of creatinine

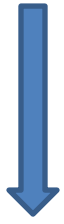
# URINARY PROTEIN ELECTROPHORESIS

- Urine protein electrophoresis separates the proteins according to charge and allows classification of the type of renal injury.
- Protein patterns are interpreted and classified as glomerular, tubular, or mixed patterns

In minimal glomerular damage

Low molecular weight protein such as albumin, α1- AT, and transferrin are excreted

In severe glomerular damage



other proteins having high molecular weight like immunoglobulin are excreted into urine

# TUBULAR PROTEINURIA occurs when the renal tubules cannot reabsorb low molecular weight proteins

Tubular proteinuria is associated with

- Drug toxicity
  i. aminoglycosides,
  ii. cephalosporins,
  iii. Cyclosporine
- Pyelonephritis,
- Interstitial nephritis,
- Renal vascular disease, and
- Transplant rejection.

- This protein pattern reveals protein bands in the
- alpha 2 region (alpha 2 microglobulin, retinol binding protein), and
- one band in the beta 2 region (beta 2 microglobulin)

# Overflow proteinuria

- It is caused by high plasma protein concentrations that exceed the reabsorptive capacity of the tubules.
- Common examples include Bence-Jones Proteins (multiple myeloma) and myoglobinuria.
- In hemolytic conditions, hemoglobin can appear in urine (hemoglobinuria)

# B. HEMATURIA

- An earliest sign of glomerular damage before the overt decrease in GFR.

- Intact glomerulus does not allow the passage of RBC.

When severe glomerular damage

RBC leakage occurs

- Detection of microscopic hematuria or RBC casts confirm glomerular damage

# TUBULAR FUNCTION TEST

- These tests are based on tubular reabsorption and secretion function of kidneys.
- The tubular epithelial cells of nephrons are highly specialized structure, which selectively reabsorb water and some substances and secrete other.

# 1. Specific gravity of urine

- The simplest test of tubular function is the measurement of specific gravity of urine by a urinometer.
- Normal 1.015-1.025
- This is an indication of osmolality.
- Incase of proteinuria S.G. elevated.
- Earliest manifestation of renal disease may be difficulty in concentrating the urine.
- ↓ Sp.gr.— excessive water intake, ch.nephritis, Diabetes Insipidus
- ↑Sp.gr.— diabetes mellitus, nephrosis, Ch.Renal failure.
- Fixed sp.gr. at 1.010 ⟶ isosthenuria — earliest manifestation of tubular disease / chronic renal failure.

- A defect in concentrating capacity is termed hyposthenuria.
- The inability to excrete the waste products may be counterbalanced by large urine output.
- Thus the earliest manifestation of the renal disease may be difficulty in concentrating urine.

# 2. Urine Osmolality

- Osmolality of urine varies from 50 mosm/kg in condition of excessive fluid intake to 1200 mosm/kg in low fluid intake.
- Random urine sample = 850-900 mosm/kg.
- It is found that the urine (without any protein or high molecular weight substance) with an osmolality of 800 mosm/kg has a specific gravity of 1.020
- Therefore, measurement of urine osmolality will also help to assess tubular function
- Plasma osmolality is 285-295 mOsm/kg.

# 3. WATER DEPRIVATION TEST OR URINE CONCENTRATION TEST

- 99% of water of glomerular filtrate is reabsorbed during its passasge through different segment of renal tubule.

Fluid intake is withheld overnight preferably 18 hours

↓

osmolality of 1st urine sample in the morning

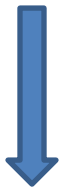
↓

If osmolality > 850 mosmol/kg , Sp.Gv- 1.022

↓

Renal concentrating ability is normal/good

- In low ADH activity ( hypothalmic / pituitary disorder) or nephrogenic diabetes insipidus (lack of response to ADH)



Osmolality is low and rarely exceeds 300 mosm/kg (Sp. Gr. 1.010)

# 4. Urine dilution test

- A normal kidney produce a dilute urine following excessive water intake.

After an overnight fast and fluid deprivation

↓

Bladder is emptied at 7 am and water load (1200 ml over the next 30 min) is given

↓

Hourly urine samples are collected for next four hours

↓

The specific gravity of at least one sample should fall to 1.003 and osmolality to 50 mOsm/kg

- Normal person will excrete all the water load with in 4 hours
- Kidneys which are severely damaged cannot excrete a urine of lower specific gravity than 1.010 or a volume above 400 ml in this time.
- This test is more sensitive and less harmful than concentration test
- The test should not be done if there is oedema or renal failure; water intoxication may result

# 5. Ammonium chloride loading test or Urinary Acidification test

- It is indicated in unexplained hyperchloremic metabolic acidosis
- Acidification defects may occur due to generalized tubular defects or due to genetically determined defects in ion pumps
- Enteric coated capsules containing ammonium chloride at a dose of 0.1 g / kg body wt is given
- In the liver, NH3 is converted to urea and HCl is produced which is excreted by kidney.

- Urine is collected hourly from 2 to 8 hours after ingestion.
- At least one sample should have a pH of 5.3
- In type I distal renal tubular acidosis, urinary pH rarely falls below 6 and never falls below 5.3.
- Liver disease is a contraindication to perform this test.

# FRACTIONAL EXCRETION OF SODIUM

It is a measure of the percentage of sodium that gets excreted in the urine over the total filtered sodium by the kidney

$$FENa = \frac{UNa \times PCr}{PNa \times UCr} \times 100$$

- Interpretation
  - <1% – prerenal, glomerulonephritis, obstruction
  - >2% – ATN
  - 1-2% - either prerenal or ATN
- Not accurate before diuretics or IVF

# URINE ANALYSIS

## A. Physical Examination

Includes:

1. Volume.
2. Color.
3. Odor.
4. Reaction (pH).
5. Specific gravity.

## C. Microscopic Examination

Include:

1. Cells.
2. Crystals.
3. Casts.
4. Microorganism
5. Parasites.
6. Contamination

## B. Biochemical Examination

Includes:

1. Proteins.
2. Sugers.
3. Ketone bodies.
4. Bile salts.
5. Bile Pigments.
6. Blood.

# URINE ANALYSIS